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Transformation and fate of neonicotinoid insecticides during drinking water treatment

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TRANSFORMATION AND FATE OF NEONICOTINOID INSECTICIDES DURING DRINKING WATER TREATMENT

by

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee for the thesis requirement for the Master of Science degree in Civil and Environmental Engineering at the December 2017 graduation.

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ABSTRACT

Neonicotinoid insecticides are widespread in surface waters across the agriculturally-intensive Midwestern US. We report for the first time the presence of three neonicotinoids in finished drinking water and demonstrate their general persistence during conventional water treatment. Periodic tap water grab samples were collected at the University of Iowa over seven weeks in 2016 (May-July) after maize/soy planting. Clothianidin, imidacloprid, and thiamethoxam were ubiquitously detected in finished water samples and ranged from 0.24-57.3 ng/L. Samples collected along the University of Iowa treatment train indicate no apparent removal of clothianidin and imidacloprid, with modest thiamethoxam removal (~50%). In contrast, the concentrations of all neonicotinoids were substantially lower in the Iowa City treatment facility finished water using granular activated carbon (GAC) filtration. Batch experiments investigated potential losses. Thiamethoxam losses are due to base-catalyzed hydrolysis at high pH conditions during lime softening. GAC rapidly and nearly completely removed all three neonicotinoids. Clothianidin, hydrolysis products of thiamethoxam and known metabolites of imidacloprid are susceptible to reaction with free chlorine and may undergo transformation during chemical disinfection via chlorination or during distribution with chlorine residual. We identify several transformation products resulting from these oxidation and hydrolysis reactions, and discuss implications for human health. Our work provides new insights into the persistence of neonicotinoids and their potential for transformation during water treatment and distribution, while also identifying GAC as a potentially effective management tool to lower neonicotinoid concentrations in finished drinking water.

PUBLIC ABSTRACT

Neonicotinoids are the most widely used class of insecticides in the world, and as a result of their widespread use and chemical properties, they are commonly found in surface waters across the United States. Many communities across the U.S. rely on surface water as a source of drinking water, however, whether neonicotinoids are removed by drinking water treatment is unknown. We report, for the first time, the presence of three neonicotinoids in finished drinking water. Tap water samples were collected from two water treatment plants, the University of Iowa water treatment plant that serves the University and the Iowa City water treatment plant, which serves the community of Iowa City, IA. Neonicotinoids were present in all samples of drinking water collected over the course of seven weeks in 2016 (May-July) from the University of Iowa. In contrast, the concentration of all neonicotinoids was much lower in the Iowa City drinking water, though source water concentrations were similar. We hypothesize that this difference is due to the use of granular activated carbon filtration, a more advanced type of treatment, at the Iowa City drinking water treatment plant. Although neonicotinoids are more toxic to insects than mammals, our research shows that neonicotinoids may undergo chemical reactions during drinking water treatment, leading to the formation of new compounds with unknown toxicity to humans and other vertebrates. Finally, we demonstrate that granular activated carbon filtration may be used as a method to remove neonicotinoid active ingredients from drinking water.

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1.1. Pesticide Usage in the US

The development and use of pesticides largely began after World War II, reducing the need for tillage and increasing crop production efficiency.[1] In the decades following, the application of pesticides grew rapidly, from 196 million pounds of pesticides applied in 1960 to 632 million pounds in 1981.[1] Since 1981, pesticides use has decreased slightly to 516 million pounds in 2008. These trends are driven by many factors including technological advances, the emergence of weed and pest resistance, economic factors and regulations.[1]

Pesticides are primarily comprised of three main classes: insecticides (targeting insects), herbicides (targeting weeds) and fungicides (targeting fungi). Herbicides are the most widely used pesticides; in 2008 they accounted for 76% of pesticides applied to crops, while insecticides accounted for 6% and fungicides accounted for 7%.[1] Row crops, such as maize, soy, potatoes and cotton account for the vast majority of pesticide use (corn: 39%, soybeans: 22%, potatoes: 10%, cotton: 6%, 80% combined).[1]

Though pesticides have been a boon to agriculture, humans, pollinators and other non-target organisms are exposed to pesticides through contaminated, food, soil, water and air, or by direct contact during application. This exposure presents potential human and environmental health risks, largely resulting from the toxicological nature of pesticides.[1] Surface water contamination by pesticides has been linked to runoff from non-point agricultural sources,[2–5] and is of increasing concern, particularly for those who rely on agriculturally contaminated surface water for drinking water. Herein we discuss a widely used class of insecticide, called neonicotinoids, whose presence in surface waters is well documented in the US[3,6–8] and elsewhere.[9,10]

1.2. Neonicotinoids: Background

For centuries prior to the development of neonicotinoids, nicotine was used to manage sucking insects despite several draw backs, including lack of effectiveness toward insects and high toxicity toward mammals.[11] Neonicotinoids are derived from nicotine and were developed as a more effective method for managing pests. Though structurally similar to nicotine, neonicotinoids have far superior properties as insecticides.[12,13]

First developed by Shell and Bayer in the 1980s,[14] use of neonicotinoids has grown rapidly since their commercialization in the 1990s and early 2000s (**Figure 1-4**, **Figure 1-5**, and **Figure 1-6**).[15,16] Imidacloprid was the first neonicotinoid on the market, commercialized in 1991, with thiamethoxam and clothianidin to follow in 1998 and 2002, respectively.[15] The total neonicotinoid market was approximately $2.4 billion in 2009 (the most recent figures available).[17] In that year, imidacloprid was the most widely used insecticide in the world, accounting for 41.5% ($1.1 billion) of sales. That same year, thiamethoxam accounted for 27% of sales ($627 million) and clothianidin for 19% of sales ($439 million).[17] Neonicotinoids are primarily comprised of eight compounds: imidacloprid, nitenpyram, nithiazine, acetamiprid, thiamethoxam, thiacloprid, clothianidin and dinotefuran.[15] Of these compounds, clothianidin, imidacloprid and thiamethoxam are the most widely used,[17] and are the focus of this thesis.

Neonicotinoids are systemic, insect-targeting neurotoxins that have gained popularity due to their broad spectrum of control, high potency and insect selectivity.[11,18,19] They enjoy a wide range of uses in agriculture and provide defense against many common pests including aphids, whiteflies, thrips, rice hoppers, Colorado potato beetles, flea beetles, wireworms, leaf miners and lepidopterous species. [15] Neonicotinoids are applied to vegetables, stone fruits, citrus, rice, cotton, corn, potato, sugar beet, oilseed rape and soybean crops.[16,17] Neonicotinoids are heavily

used in agricultural regions throughout the U.S. (**Figure 1-1**, **Figure 1-2**, and **Figure 1-3**).[8,20] Imidacloprid is applied to a diverse range of crops, while thiamethoxam is primarily used on corn, soybeans and cotton and clothianidin is primarily used on corn (**Figure 1-4**, **Figure 1-5**, and **Figure 1-6**).[20] Neonicotinoids are applied to crops through a variety of methods including sprays, seed treatments, drip irrigation and soil treatments.[15,17] The extensive adoption of seed treatments in agriculture is an important driver of neonicotinoid use,[21] as nearly all (80%) of treated seeds are coated with neonicotinoids.[17]

Beyond crop protection, neonicotinoids have several other applications including livestock protection, aquaculture, household and urban uses. They are applied to livestock and pets to repel fleas and ticks, and used in fish farming to control water weevil infestations.[12,16,22–24] Around the home, neonicotinoids provide domestic pest control from cockroaches, ants, termites, wasps and flies,[16] and are used in lawn, landscaping and garden care products.[25]

Table 1-1: Neonicotinoids enjoy a wide range of uses in agriculture and provide defense against many common pests.[15-16,17]

Compounds	**Targets**	**Crops**
imidacloprid, nitenpyram, nithiazine, acetamiprid, thiamethoxam, thiacloprid, clothianidin and dinotefuran	aphids, whiteflies, thrips, rice hoppers, Colorado potato beetles, flea beetles, wireworms, leaf miners and lepidopterous species	vegetables, stone fruits, citrus, rice, cotton, corn, potato, sugar beet, oilseed rape and soybean crops

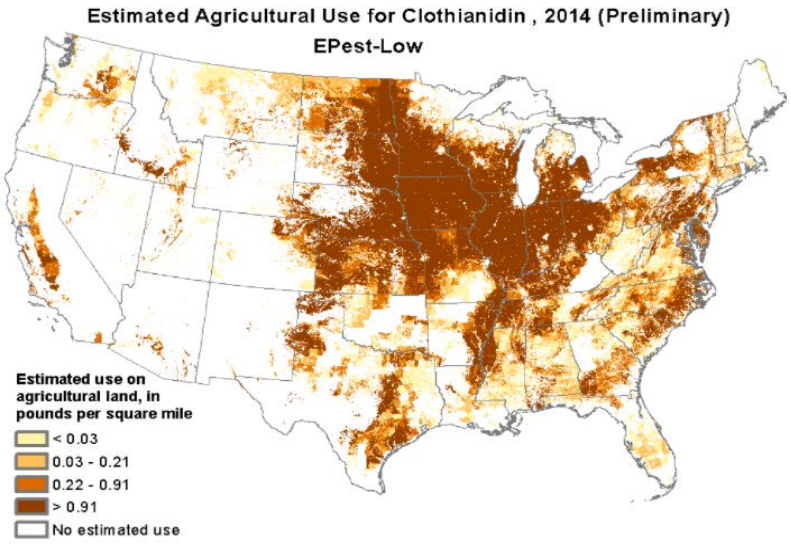


Figure 1-1: Estimated application of clothianidin[20] in the United States, 2014.

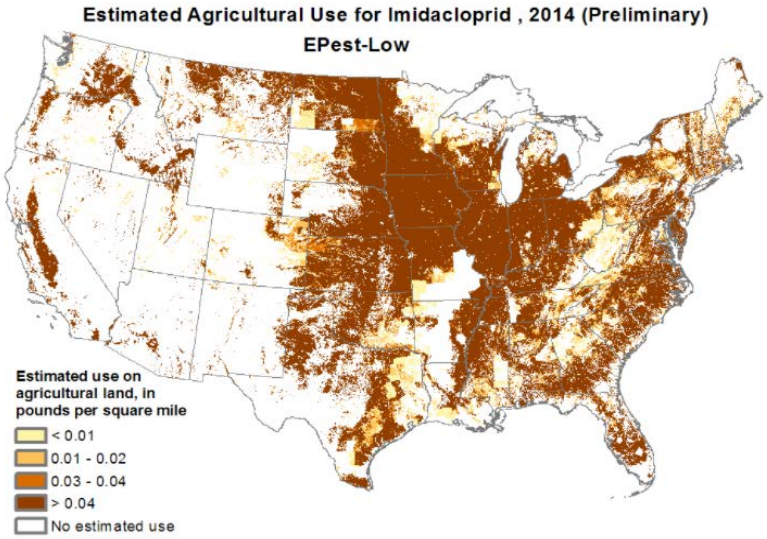


Figure 1-2: Estimated application of imidacloprid[20] in the United States, 2014.

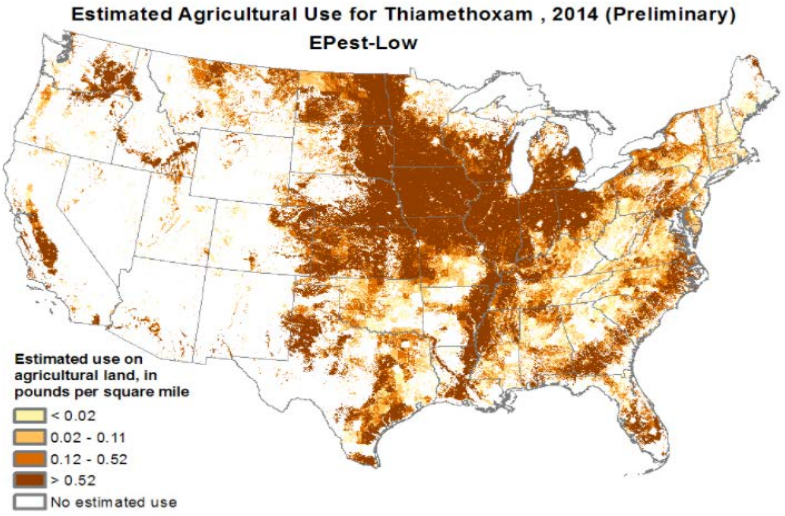


Figure 1-3: Estimated application of thiamethoxam[20] in the United States, 2014.

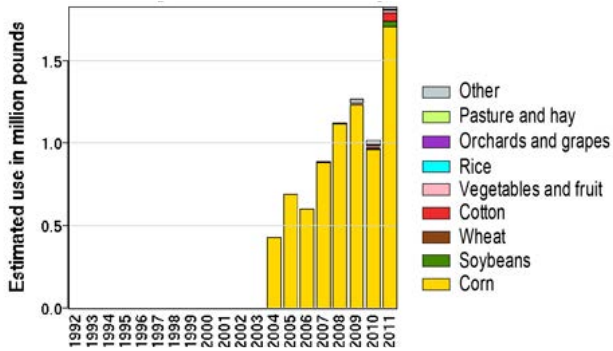


Figure 1-4: Estimated use of clothianidin[20] by crop (1992 – 2011).

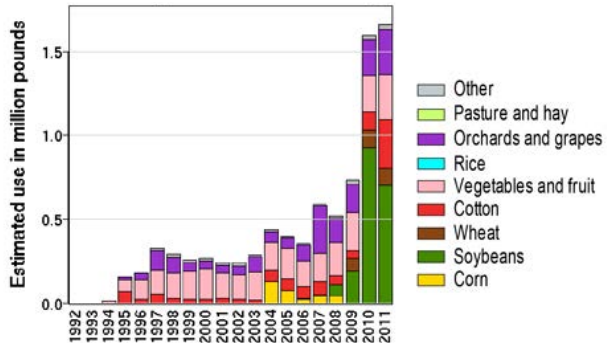


Figure 1-5: Estimated use of imidacloprid[20] by crop (1992 - 2011).

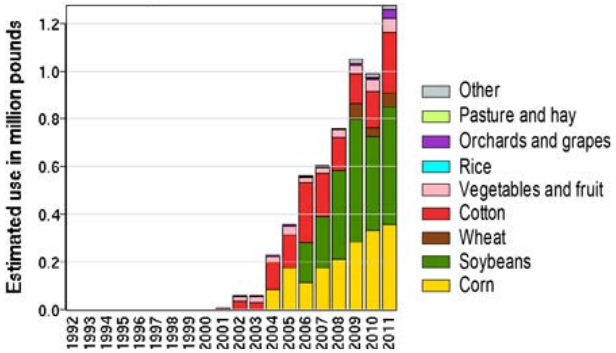


Figure 1-6: Estimated use of thiamethoxam[20] by crop (1992 - 2011).

1.3. Modes of Action and Toxicity Toward Insects and Mammals

Neonicotinoids are systemic neurotoxins (i.e., they are translocated throughout the entire plant structure),[16,18] and work by binding irreversibly to the insect's nicotinic acetylcholine receptors (nAChR, **Figure 1-7**). Neonicotinoids disrupt neural transmission by mimicking the activity of neurotransmitters, leading to rapid firing of the neuron, causing overstimulation and death.[16,18] They are highly potent and can be lethal to insects at nanomolar (< 1 ng/L) doses.[16] They are broadly toxic to many different types of pests due to the similarity of the invertebrate nAChR across species.[18]

Selective toxicity is a necessary requirement for safe and effective pesticides.[11] All neonicotinoids have selectivity ratios greater than one,[12] indicating selectivity toward insects (**Table 1-2**). To cultivate this selectivity, neonicotinoids take advantage of differences between nAChR receptors in vertebrates and invertebrates.[11,26] The insect nAChR is cationic, or positively charged, while the vertebrate nAChR is anionic, or negatively charged.[11] Neonicotinoids share important functional groups (nitroimines, cyanoimines or nitromethylenes) that carry negative electrostatic potential **(Figure 1-8**). The negatively charged tip of the neonicotinoid is rejected by the negatively charged mammalian nAChR, and readily accepted by

the positively charged insect nAChR (**Figure 1-7**).[11] Clothianidin, imidacloprid and thiamethoxam are all nitroimines as shown in **Figure 1-8**. In these molecules, the oxygens in the nitro (NO_2^-) group confer a negative electrostatic potential to the tip of the molecule.[26,27]

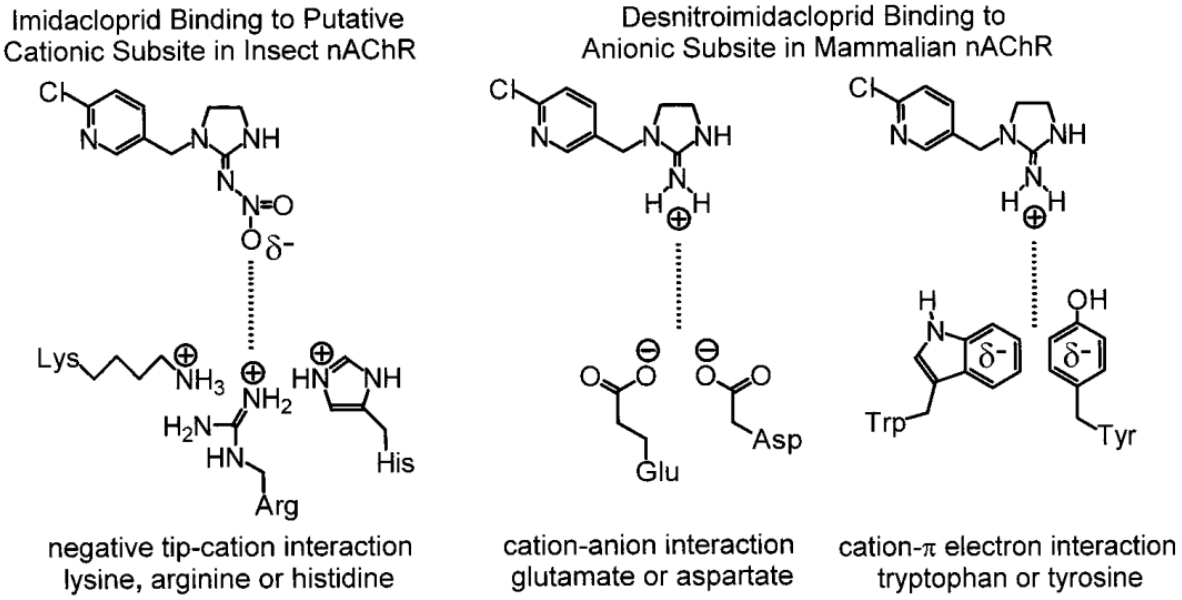


Figure 1-7: Interaction of imidacloprid binding with the anionic insect receptor (left) and desnitro binding to the anionic mammalian nAChR (right).[1]

Table 1-2: The half-maximal inhibitory concentration (IC_{50}) and selectivity ratios of neonicotinoids toward insects and vertebrates.[12] The IC_{50} is a measure of how much of a substance is required to inhibit a specified biological function. The selectivity ratio is computed as the vertebrate IC_{50} / invertebrate IC_{50}. A selectivity ratio greater than one implies selective toxicity toward insects while a selectivity ratio less than one implies selective toxicity toward vertebrates.

Compound	Insect (nM)	Vertebrate (nM)	Selectivity Ratio
Acetamiprid	8.3	700	84
Clothianidin	2.2	3500	1591
Dinotefuran	900	>100,000	>111
Imidacloprid	4.6	2600	565
Nitenpyram	14	49,000	3500
Nithiazine	4800	26,000	5.4
Thiacloprid	2.7	860	319
Thiamethoxam	5000	>100,000	>20

[1] Reprinted (adapted) with permission from Tomizawa, M.; Lee, D. L.; Casida, J. E. Neonicotinoid Insecticides: Molecular Features Conferring Selectivity for Insect versus Mammalian Nicotinic Receptors. *J. Agric. Food Chem.* **2000**, *48*, 6016–6024.[11] Copyright 2000 American Chemical Society.

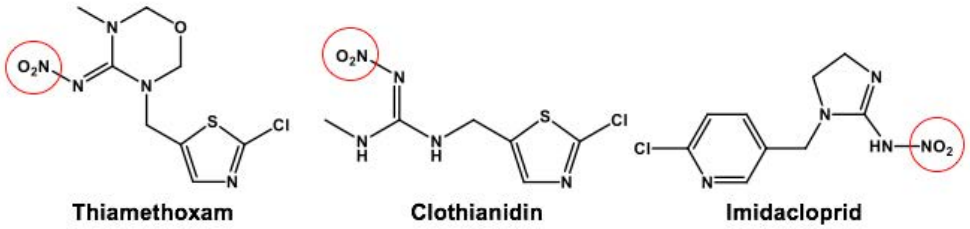


Figure 1-8: Nitro groups conferring insect selectivity to clothianidin, imidacloprid and thiamethoxam.

1.4. Environmental Regulations

The environmental effects of neonicotinoids are largely unregulated in the US and Canada, though some states and provinces have developed their own regulations. Motivated by pollinator concerns, Maryland and Connecticut banned neonicotinoids in 2016, with exceptions for licensed applicators such as farmers and veterinarians.[28–31] Ontario implemented new regulations of neonicotinoids in 2015, the goal of which was to reduce the number of acres of corn and soybeans grown with neonicotinoid-treated seeds by 80% in 2017. [32] The Ontario regulations reduce the use of neonicotinoids by allowing their application only when there is a demonstrated pest problem.[32] The EU is also reportedly considering a permanent ban on neonicotinoids, though regulations have not been officially released.[33,34]

1.5. Environmental Fate

Chemical properties of neonicotinoids govern their fate and transport in the environment. Neonicotinoids are soluble[35] (340, 610, and 4100 mg/L for clothianidin, imidacloprid, and thiamethoxam, respectively), polar[6] (log K_{ow} values of 0.91, 0.57, and -0.13 for clothianidin, imidacloprid, and thiamethoxam, respectively), and mobile in the environment.[35] Soil half-lives

are highly dependent on location and field conditions (such as soil texture, pH, sunlight exposure and sunlight intensity), but half-lives measured in the field for imidacloprid range between 100-1230 days[35] and major degradation pathways are photo degradation (where conditions permit) and microbial degradation.[36,37]

As a result of their extensive use, recalcitrance and mobility, neonicotinoids are found throughout the aquatic environment.[23] They are pervasive in surface waters throughout the US at concentrations ranging from 0-6900 ng/L.[3,6–8,38] Nineteen percent of samples tested for imidacloprid in an agricultural region in California were over the US EPA's chronic invertebrate aquatic benchmark[39] of 1.05 μg/L. In a nationwide study in the US, at least one neonicotinoid was detected in 63% of the 48 streams monitored.[8] Similarly, in a study of streams in Iowa, at least one neonicotinoid compound was detected in all samples.[6] These detections include clothianidin (3.5-79 ng/L), imidacloprid (nd-15 ng/L) and thiamethoxam (nd-43 ng/L) as measured in the Iowa River at Wapello, IA (approximately 45 miles downstream of Iowa City),[6] and imidacloprid measured in Old Man's Creek near Iowa City (4.5-35 ng/L).[6] In a study of drained wetlands in Iowa's prairie pothole region, clothianidin (nd-3500 ng/L) was detected in 98% of samples, thiamethoxam (nd – 6900 ng/L) in 54% of samples and imidacloprid (nd – 120 ng/L) in 48% of samples.[40] Agricultural uses, primarily though the planting of neonicotinoid treated seeds, are thought to be the primary source of neonicotinoids in Iowa surface waters, as 65% (23,421,255 acres) of Iowa's total land area (35,002,874 acres) is comprised of cultivated row crops, while only 7.4% (2,667,701 acres) of Iowa's land area is in urban development.[41]

In other studies, imidacloprid was measured in a stream (3.4-10 ng/L) in Georgia,[7] as well as in other small streams throughout the Midwest with concentrations ranging[3] from 0-2900 ng/L. Neonicotinoids are frequently detected in wetlands in Canada's prairie pothole region, with

the highest frequency of detections during spring snowmelt and the highest concentrations measured during summer.[9]

Neonicotinoids have also been detected in groundwater in North America and Asia. Imidacloprid was detected in groundwater near paddy rice cultivation in Vietnam, with a maximum concentration of 220 ng/L. Imidacloprid has occasionally been detected in Canadian ground waters, with maximum concentrations near 300 ng/L.[42] Clothianidin, imidacloprid and thiamethoxam were also detected in groundwater in Wisconsin.[43]

1.6. Fate During Water and Wastewater Treatment

To date, the fate of neonicotinoids during drinking water treatment processes has not been investigated. Some limited work has considered their fate during wastewater treatment. Neonicotinoids were present in wastewater treatment due to household uses and urban runoff from lawns, golf courses, gardens, turf and pavement.[35,12,24,44] They appear poorly removed via engineered treatment systems, such as in wastewater plants. Sadaria et al[45] examined six neonicotinoids at 13 wastewater treatment plants and one engineered treatment wetland. Results demonstrated that neonicotinoids are persistent in engineered systems, with insignificant or marginal removal observed during conventional wastewater treatment, and no removal observed in the constructed treatment wetland. Imidacloprid was also monitored at eight San Francisco area wastewater treatment plants; influent and effluent detections were ubiquitous with concentrations ranging from 58-306 ng/L and no significant removal.[44]

1.7. Environmental Neonicotinoid Transformation Products

Transformation products from neonicotinoids may be formed via physical, chemical and biological degradation processes.[12,46] Importantly, the toxicological profiles of transformation products may be different from that of the parent compound. Thus, understanding the formation of transformation products is critical to understanding the impact of neonicotinoids on ecosystem and human health. Presently, the identity and toxicity of many transformation products is unknown.[16] Among those that have been studied, most are biological metabolites, and some exhibit increased potency toward non-target organisms, including honey bees and mammals.[11,12,16,26,47]

The metabolism of neonicotinoids has been extensively studied in plants and animals as part of the United States Environmental Protection Agency (EPA) approval process.[12] In mice, most clothianidin is excreted unchanged, indicating that little to no metabolism of clothianidin occurs in mammals.[12] Comparatively, only 22% of imidacloprid and 1.3% of thiacloprid are excreted unchanged in the urine of mice.[47] There are many known biotransformation products of neonicotinoids, some of which retain insect selectivity, others of which are bioactive in mammals.[16] Desnitro and descyano products, such as desnitro imidacloprid and descyano thiacloprid, are of primary interest due to their increased toxicity toward mammals (**Table 1-3**).[16,47] These products have been measured in the brains, livers and urine of mice exposed to imidacloprid and thiacloprid.[16,47]

Neonicotinoids also undergo transformation in plants, resulting in a wide range of metabolites present throughout the life of the plant and at harvest.[16] Thiamethoxam is rapidly metabolized to clothianidin in plants, clothianidin is then further metabolized to a broad range of transformation products including desnitro clothianidin.[16] Because clothianidin is a degradate of

thiamethoxam, their metabolites are nearly identical.[16] Imidacloprid follows similar metabolic pathways in both plants and animals, leading to the formation of many transformation products, and including desnitro imidacloprid.[16] Thus, plant transformation presents a potential exposure route for humans and animals to toxic metabolites of neonicotinoids.[16] Indeed, seven metabolites were found in the urine of patients with suspected subacute exposure to neonicotinoids through contaminated foods.[48]

Neonicotinoids are also degraded and transformed via several non-biological processes, including direct and indirect photodegradation, hydrolysis and chlorination. Neonicotinoids (including clothianidin, imidacloprid and thiamethoxam) readily undergo photodegradation,[17,46,49–54] with estimated half-lives for exposure to direct sunlight between 0.2-1.5 days for thiamethoxam, 0.5-3.31 days for clothianidin and 0.36 – 2.22 days for imidacloprid (reaction rates are dependent on surface water temperature).[46] However, photoattenuation at depths greater than 8 cm was negligible, which the authors attribute to their environmental persistence.[46] In natural waters, the proportion of sunlight transmitted through the water column is dependent on the turbidity of the water and wavelength of the light.[35,55] Photodegradation rates are also heavily influenced by latitude and season, but are less temperature dependent than most reaction kinetics.[46] Thus, photodegradation rates measured in the field vary widely, with neonicotinoids being more persistent in turbid waters and other low light conditions.[46,56] Thiamethoxam also readily undergoes based-induced hydrolysis, with decreasing half-lives for increasing pH. Reported half-lives range from 2.1 days (pH 9.2)[57] to 6.1 days (pH 9)[15] at alkaline pH, and 29.2 days[58] to 152 days[15] at neutral pH (7). This behavior appears to be unique to thiamethoxam; there are no reports of significant base-catalyzed hydrolysis for clothianidin and imidacloprid over the timescales and conditions relevant to water treatment.[17] Studies have

shown that photodegradation of neonicotinoids and thiamethoxam hydrolysis result in removal of the nitrogroup,[15,46,59] which could lead to increased toxicity toward aquatic vertebrates, and mammals.[11]

Finally, neonicotinoids may undergo oxidation/hydrolysis in the presence of chlorine. Nitenpyram undergoes rapid degradation when added to finished drinking water containing chlorine residual.[60] Noestheden et al. hypothesize that degradation products may be present in water treatment facilities and the environment due to the widespread use of chlorine in water treatment.[60] As a result, non-target organisms may be exposed to these products via irrigation with chlorinated water, or during the wastewater treatment process.[60] Drinking water treatment offers another potential exposure route of exposure, though the authors do not specifically mention this in their research.[60] These results carry implications for pollinators and other non-target organisms who may be exposed to transformation products of unknown toxicity.[60]

1.8. Toxicity of Transformation Products Toward Insects and Mammals

Insect selectivity of neonicotinoids may be lost in metabolites and transformation products, particularly upon removal of the nitro or cyano groups.[11,16,18,19,26,51,61,62] The toxicity of most transformation products and metabolites to humans and insects in unknown.[16] Nevertheless, desnitro imidacloprid and descyano thiacloprid have been toxicologically evaluated to fulfill requirements of the EPA approval process.[12] In contrast to the parent compounds, desnitro imidacloprid and descyano thiacloprid are selective toward vertebrates as indicated by their selectivity ratios of $<< 1$.[12] In fact, the toxicological profiles of desnitro imidacloprid and descyano thiacloprid are considered comparable[12] to that of nicotine (**Table 1-3**).

Increased toxicity toward vertebrates results from loss of the nitro and cyano groups,

causing the compounds to become partially positively charged at physiological pH.[11,61] The partial positive charge allows them to bind with the mammalian nAChR, which carries negative electrostatic potential (**Figure 1-7**).[11,61] Similarly, other transformation products may lose insect selectivity as removal of the groups conferring this selectivity occurs during photo degradation,[46] base-induced hydrolysis,[15,59] biotransformation[16,47] and chlorination processes.[47]

Table 1-3: IC_{50} and selectivity ratios of desnitro-imidacloprid, descyano-thiacloprid and nicotine.[12]

Compound	Insect (nM)	Vertebrate (nM)	Selectivity ratio
Desnitroimidacloprid	1530	8.2	0.005
Descyanothiacloprid	200	4.4	0.022
Nicotine	4000	7.0	0.002

1.9. Ecosystem and Human Health Effects

Much attention has been given to the effects of neonicotinoids toward non-target organisms, particularly honey bees, but also insectivorous birds, aquatic invertebrates and humans. Neonicotinoids are suspected of contributing to colony collapse disorder in honey bees,[63] as bees are exposed to neonicotinoids by contaminated soil and/or pollen while foraging.[64] Neonicotinoids may also slow the growth rate of bee colonies, and decrease the formation of new queen bees.[65] Chronic exposure to neonicotinoids may also limit survival and growth of a wide range of aquatic invertebrates and other organisms.[66,67] Imidacloprid is shown to have adverse effects on feeding and survivorship in mayflies,[68] and may also reduce invertebrate abundance and diversity in streams.[69]

Humans may be exposed to neonicotinoids through the consumption of food treated with neonicotinoids, by contact with pets and livestock, in the process of handling treated seeds or by their presence in air, water and soil. Neonicotinoids are frequently detected in fresh fruits and vegetables for human consumption:[70,71] according to the most recent USDA Pesticide Data

Program Annual Report, clothianidin was detected in 31.1% of spinach samples, 23.2% of potato samples and 10% of tomato samples.[71] Thiamethoxam was detected in 30.5% of frozen cherries, 14.1% of watermelons, 22% of lettuce.[71] Imidacloprid was detected in 43% of spinach, 46% of potatoes, 34.7 % of frozen cherries.[71] Furthermore, neonicotinoids are absorbed with high efficiency in the human intestinal cell model,[72,73] and cannot be easily washed from produce due to their systemic nature.[70]

Although chronic, low-level exposure to humans is near certain, there is little data on the chronic impacts of neonicotinoids to human health.[74] A 2016 review of neonicotinoids identified eight studies relating to health effects on humans.[74] Of those studies, four examined acute exposure and those studying chronic exposure were considered methodologically weak.[74] The authors of the review conclude that more studies are necessary to fully understand chronic impacts of neonicotinoids to human health.[74] A more recent review of the chronic effects on humans concluded that there is evidence of harm to human health from neonicotinoids, however, the toxicological tools to measure these effects are still in development.[75] The groups most at risk include certain occupational groups (e.g. farm workers), pregnant women and children.[75]

1.10. Objectives of Study

Due to their widespread detection in surface water, neonicotinoids are likely to be present in drinking water, unless removed during water treatment. Although neonicotinoids have been shown to persist through wastewater treatment, there are no known studies to date examining their removal or transformation during drinking water treatment. Moreover, neonicotinoids may form transformation products during drinking water treatment due to various physical/chemical treatment processes such as lime softening and chlorination, which vary solution pH and redox

conditions, respectively.

The overall goal of this study is to address extensive gaps in our current understanding of neonicotinoid fate in drinking water treatment. Specific objectives and associated hypotheses include:

- **Objective 1:** Evaluate removal of neonicotinoids via processes relying on their partitioning (e.g., sorption onto activated carbon). Hypothesis: Neonicotinoid removal will be limited because of their high polarity and water solubility ($\log K_{ow}$: -0.55-1.26)
- **Objective 2:** Determine rates and products of neonicotinoid transformation during chemical disinfection processes (e.g., chlorination) and during the alkaline conditions of lime-soda softening. Hypothesis: Electron-rich functional groups (e.g., π-bonds) in neonicotinoids will promote their oxidation and generate novel chlorinated byproducts. Alkaline conditions will accelerate hydrolysis, particularly for those compounds known to undergo hydrolysis at neutral pH conditions (e.g., thiamethoxam).
- **Objective 3:** Quantify the occurrence and removal of neonicotinoids in a full-scale water treatment plant. Hypothesis: Removal will mirror expectations from laboratory studies; due to their high solubility, limited removal will be observed prior to chemical disinfection, and their major byproducts of disinfection will be identifiable in the treated drinking water.

2. OCCURRENCE OF NEONICOTINOID INSECTICIDES IN FINISHED DRINKING WATER AND FATE DURING DRINKING WATER TREATMENT[2]

2.1. Introduction

Neonicotinoid pesticides have become the most widely-used insecticides in the world.[16,17] Neonicotinoids are systemic, insect-targeting,[11,18,19] potent neurotoxins that are often applied as seed treatments to crops in the United States and in urban pest control applications.[16,35] Neonicotinoids have also been implicated in a variety of ecosystem effects,[76] including decline of pollinators[77,78] (e.g., honeybees) and effects to non-target organisms.[79–84] They are substantially more toxic to insects than vertebrates;[35] however, most vertebrate toxicity research has focused on acute exposure and chronic exposure remains a concern.[82] Several studies report associations between chronic exposure to neonicotinoids and adverse developmental or neurological outcomes.[74] Other studies highlight potential concerns including inflammation of the liver and central nervous system due to chronic exposure to neonicotinoids,[85] loss of insect selectivity in biological and abiotic metabolites,[18,61,59] and negative effects to non-target species in aquatic ecosystems.[79]

High use and chemical properties have caused proliferation of neonicotinoids in surface waters.[6,8–10] In a nationwide study of streams in the US, at least one neonicotinoid compound was detected in 63% of the 48 streams measured.[8] Neonicotinoids were ubiquitously detected at all streams sampled that drain intensively row-cropped areas of the Midwestern US,[6] with

[2] Reprinted with permission from Kathryn L. Klarich, Nicholas C. Pflug, Eden M. DeWald, Michelle L. Hladik, Dana W. Kolpin, David M. Cwiertny, and Gregory H. LeFevre**.** Occurrence of Neonicotinoid Insecticides in Finished Drinking Water and Fate during Drinking Water Treatment**.** *Environmental Science & Technology Letters* **2017** *4* (5), 168-173**.** DOI: 10.1021/acs.estlett.7b00081.[98] Copyright 2017 American Chemical Society.

maximum concentrations of 260, 43, and 190 ng/L for clothianidin, imidacloprid, and thiamethoxam, respectively, which represent the most widely used and commonly observed compounds in this class of insecticides. Neonicotinoids are water soluble[35] (340, 610, and 4100 mg/L for clothianidin, imidacloprid, and thiamethoxam, respectively) and polar[6] (log Kow= 0.91, 0.57, and -0.13 for clothianidin, imidacloprid, and thiamethoxam, respectively). Research to date suggests general neonicotinoid persistence in the environment[86] (e.g., imidacloprid and clothianidin were documented to have conservative transport through a study stream reach[8]), although photolysis can occur to various extents among the different neonicotinoids.[46,59]

Based on limited data, neonicotinoids appear poorly removed via treatment systems, with insignificant or very marginal removal observed during conventional wastewater treatment and no removal in a constructed treatment wetland.[44,45] To date, no known research has examined neonicotinoid presence in finished drinking water, particularly for communities relying on agriculturally-impacted surface water sources. Here, we present results of field analyses and laboratory experiments measuring neonicotinoid fate during drinking water treatment. Our objectives were to: 1) quantify neonicotinoid residues in two public drinking water facilities that derive their water from a agriculturally-impacted sources, and 2) determine the efficacy of drinking water treatment operations to remove neonicotinoids.

2.2. Chemicals

Important Chemicals. Important chemicals include: clothianidin (99.9%, CAS 210880-92-5), imidacloprid (99.9%, CAS 138261-41-3), imidacloprid-d_4 (99.9%, CAS 1015855-75-0), and thiamethoxam (99.6%, CAS 153719-23-4). All neonicotinoids were manufactured by Fluka and used as received. All solvents used for LC-MS analysis were of LC-MS grade.

Solvents. Solvents used include: acetonitrile (optima grade, HPLC grade), acetone (optima grade) and dichloromethane (>99%).

Other Chemicals. Other chemicals include sodium hypochlorite solution 5.65-6% (Fisher Scientific), granular activated carbon (Calgon Centaur 12X40), 5 mM potassium phosphate buffer (made in lab) and sodium sulfite (Fisher).

2.3. Materials and Methods

Between May and July 2016 following maize/soy planting, finished drinking water samples were collected from a tap at the University of Iowa and at three locations in Iowa City, IA, USA. The University of Iowa drinking water treatment plant ('UI DWTP') serves the University of Iowa ('UI') while the Iowa City water treatment plant ('City DWTP') serves Iowa City ('City'). The UI DWTP (**Figure 2-1** and **Table 2-1**) uses the Iowa River for source water and uses screening, chemical pretreatment, sedimentation, lime softening, recarbonation, chlorination, and sand filtration for treatment. The City DWTP (**Figure 2-1**) uses water from alluvial wells fed by the Iowa River (*i.e.*, groundwater under the influence of surface water), and provides treatment via aeration, lime softening, recarbonation, granular activated carbon (GAC) filtration, and chlorination. The Iowa River drains a watershed that is 8,150 km^2 in a heavily row-cropped agoecosystem[41,87] where prior work has demonstrated frequent detection of neonicotinoid pesticides.[6] The river flow is composed of overland flow and tile drainage (from rainfall, no snowmelt during the study period) and groundwater. The City alluvial wells and UI DWTP intakes are located approximately 10 and 15 km downstream of the Coralville reservoir, respectively. University drinking water samples were collected periodically from a sink in the laboratory located in the Seamans Center at UI. Samples of the City drinking water were

collected from three residential taps at separate locations in Iowa City. To assess neonicotinoid fate during treatment, the raw source water, sedimentation basin effluent, recarbonation effluent (pre-chlorination), recarbonation effluent (post-chlorination), filtration effluent, and finished water were sampled at the UI DWTP, and the source and finished water were sampled at the City DWTP (**Figure 2-1**). Water samples were enriched via solid phase extraction (SPE), analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) and quantified according to established USGS methods.[7] Fate during unit processes was tested in laboratory batch systems using free chlorine, GAC, and pH adjustment, with neonicotinoid concentrations measured by LC with diode array detector and mass spectrometry (LC-DAD/MS). Field and laboratory QA/QC samples were analyzed throughout the study.

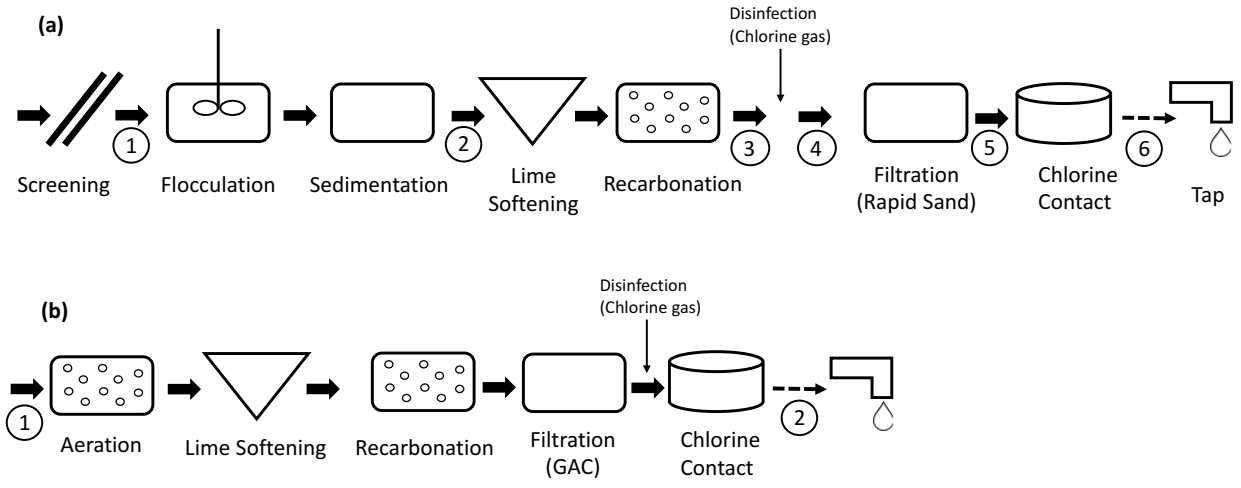


Figure 2-1: Schematic of sampling locations (circled) at the two drinking water treatment plant (DWTP) systems studied. **a.** University of Iowa DWTP schematic. Samples: (1) Raw source water, (2) Sedimentation basin effluent (3) Recarbonation effluent – pre-chlorination, (4) Recarbonation effluent – post chlorination (5) Filtration effluent (6) Finished water. **b**. Iowa City DWTP Schematic. Samples (1) Source water (2) finished water.

Table 2-1: Hydraulic residence times for the University of Iowa Water Treatment Plant unit operations. Ranges based on minimum expected flow (2.0 MGD) and maximum expected flow (4.25 MGD).

Operation	**Residence time (h)**
Flocculation and Sedimentation	2.7-5.7
Softening	1.5-3.2
Filtration	1.1-2.3
Total	5.3-11.2

Sorption of Neonicotinoids to Granular Activated Carbon. Batch experiments measured the extent and timescale of neonicotinoid sorption onto granular active carbon (GAC). Reactors were assembled in clear, crimp-top glass vials (10-40 mL) and contained 5 g/L of GAC (Calgon) and 100 μg/L of an individual neonicotinoid (clothianidin, imidacloprid, or thiamethoxam) in deionized water. A second set of experiments was conducted in pH 7-phosphate buffer. Once assembled, reactors were mixed by an end-over-end rotator for up to 4 h. Periodically, samples (0.5 mL) of the suspension supernatant were collected at specified time intervals for LC-DAD/MS analysis.

Chlorination of Neonicotinoids. Bench scale chlorination experiments were conducted to assess the potential for neonicotinoid transformation during chemical disinfection and distribution in the presence of residual disinfectant. To initiate reaction, hypochlorous acid (HOCl) was added to a closed reactor (10 – 50 mL) containing either clothianidin, imidacloprid, or thiamethoxam in 5 mM phosphate buffer at pH 7. A range of neonicotinoid (from 0.34 – 10 μM or 0.10 – 2.9 μg/L) and HOCl (0.0014-1.41 mM or 0.1-100 mg/L as Cl_2) concentrations were tested. Samples (0.5 – 1.0 mL) were collected at defined intervals and transferred to amber glass vials for immediate analysis via high performance liquid chromatography coupled with a diode array detector and single quadrupole mass spectrometer (LC-DAD/MS). Measurements of

solution pH and chlorine concentration (via titration of ferrous ammonium sulfate or FAS[88]) were conducted immediately after chlorine addition and at the conclusion of each experiment. We note that for experiments with clothianidin, which was most reactive toward free chlorine, residual chlorine in samples was quenched with 1.8 mg sodium sulfite (Na2SO3) per mg of chlorine[89] (as Cl2) prior to LC-DAD/MS analysis. Sodium sulfite was not used for reaction samples with imidacloprid and thiamethoxam; both reacted sufficiently slowly such that samples could be immediately analyzed without altering the extent of decay.

Analytical Methods. Water samples collected from the taps and treatment plants were enriched by solid phase extraction (SPE) methods adapted from the USGS.[7] Briefly, DWTP samples were filtered using a 0.7 μm glass filter (GF/F, Whatman) prior to SPE. Tap water samples were not filtered. Samples were then spiked with imidacloprid-d_4 as an internal standard before being loaded onto an Oasis SPE cartridge (500 mg HLB; Waters). Prior to use, cartridges were conditioned with 5 mL of dichloromethane (DCM), 5 mL of acetone, and 10 mL of deionized water. One liter of sample (containing imidacloprid-d_4) was loaded onto the cartridge using negative pressure at a flow rate of ~10 mL/min or less. Sample bottles were washed with 100 mL of DI and the rinsate was also loaded onto the cartridge. Following extraction, the cartridge was dried under vacuum until visibly dry. The sample was then eluted into an acid-washed glass vial using 10 mL of 50/50 DCM:acetone. The solvent was evaporated until just dry using a gentle stream of nitrogen. The sample was then reconstituted into 1 mL of 50/50 acetonitrile: DI water and stored at -20 °C until analysis via LC-MS/MS. Clean water controls indicated a method recovery of 95 ± 0.4% (average ± SD, n = 3). Additional details are included in the quality assurance and control (QA/QC).

Neonicotinoid samples were analyzed via high performance liquid chromatography

(Agilent 1260) coupled to a MS/MS spectrometer (LC-MS/MS; Agilent 6460 Triple Quadrupole MS with MassHunter, version B.07.00) for tap water samples or DAD/MS (Agilent 6140 Quadrupole LC/MS and diode array detector with OpenLab ChemStation C.07.00) for chlorination or GAC experiments. The chromatography column was a C18 Zorbax Eclipse Plus (4.6 mm x 150 mm, 5 μm) held at 50 °C for LC-MS/MS and ambient temperature for LC-DAD/MS. An injection volume of 20 μL was used, and the mobile phases were acetonitrile and water with 0.1% formic acid at 0.8 mL/min. The mobile phase gradient is described in **Table 2-2**.

Samples were quantified using the DAD at a wavelength of 260 nm (clothianidin and thiamethoxam) and 280 nm (imidacloprid) and by mass spectrometry where possible. For detection with mass spectrometer, samples were analyzed on electrospray ionization positive mode, gas temperature 300 °C, gas flow 5 L/min, nebulizer 45 psi, sheath gas temp 250 °C, sheath gas flow 11 L/min, capillary voltage 3500 V. Data were collected in multiple-reaction-monitoring (MRM) mode using two transition ions (quantitation and verification). Optimum MRM parameters were determined using Agilent Optimizer software (version B.07.00) by injecting a 1 mg/L solution of each compound (clothianidin, imidacloprid, thiamethoxam) onto the LC-MS/MS. MRM parameters are provided in **Table 2-3**. The lower level of detection (LLD) on the LC-MS/MS without sample enrichment for clothianidin, imidacloprid and thiamethoxam were 167, 99.7 and 204 ng/L, respectively. The LLD following sample enrichment for clothianidin, imidacloprid and thiamethoxam were 0.167, 0.010 and 0.204 ng/L respectively.

Table 2-2: HPLC mobile phase gradient.

Time (min)	% Acetonitrile	% Deionized Water
0	15	85
11	25	75
13	25	75
15	95	5
15.5	15	85
21	15	85

Table 2-3: Multiple Reaction Monitoring (MRM) Parameters. IMI=Imidacloprid, CLO=clothianidin, THX=Thiamethoxam, IMI-d4=Imidacloprid-d_4.

	Precursor Ion (m/z)	Quantitation Ion (m/z)	Qualitative Ion (m/z)	Fragmentor (V)	Quantitation ion collision energy (V)	Qualitative ion collision energy (V)	Retention time (min)
IMI	256.06	209	175.1	59	12	12	11.2
CLO	250.02	169.1	131.9	67	8	12	10.0
THX	292.03	211	181	63	8	20	7.8
IMI-d4	260.09	213	179.1	59	12	16	11.1

QA/QC Procedure. Deionized water (5 mL) was spiked with clothianidin (1 μM). A sample of the 1 μM solution was run on the LC-MS/MS as a control. Three jars were filled with 1 L of deionized water, and each jar was spiked with 1 mL of the 1 μM clothianidin solution (the concentration in each 1 L jar is 1 nM). The 1 nM samples were each run through the entire SPE process, concentrating the 1 L samples down to 1 mL. The concentrated samples were analyzed by the LC/MS/MS, and peak areas were compared to the control to estimate recovery. Recovery of clothianidin was 95%, 95% and 96% percent (average = 95%, SD=0.4%) for the three samples.

All water samples (*i.e.*, tap water and those from the DWTP process trains) were collected directly into clean 1 L amber glass jars (pre-baked at 550 °C) with minimal headspace. DWTP samples were collected from each unit operation and analyzed within 48 h of collection.

For tap water samples, the faucet was flushed for at least two minutes prior to sample collection, and samples were stored for a maximum of 30 d at 11 °C to analysis.

A five-point internal standard normalized external calibration curve was used to account for surrogate recovery and matrix effects during ionization, and was run with each set of samples. The instrument response was linear throughout the calibration range. Multiple blanks were run with each set of samples and no contamination was observed in the blanks. Lab blanks only were generated (i.e., no "field blanks") because neonicotinoids are non-volatile making cross-contamination unlikely and residential samples were all collected by the authors in their private residences where neonicotinoids were not used.

FAS titration method. Reagents include **(**see full description in standard methods): phosphate buffer solution (169 mM as PO_4), N,N-Diethyl-p-phenylenediamine (DPD) indicator solution (5.72 mM), ferrous ammonium sulfate (FAS) titrant (2.8 mM as FeII).

1. Measure 100 mL of DI water using a volumetric flask
2. Pour DI water into a beaker
3. Add 1 mL of sample to the 100 mL of DI water
4. Add 5 mL of phosphate buffer solution and 5 mL of N,N-Diethyl-p-phenylenediamine (DPD) indicator solution
5. Titrate with Standard ferrous ammonium sulfate (FAS) until the red color is gone
6. Calculate free chlorine concentration: (volume of FAS added)*100=Free chlorine (mg/L as Cl_2)

Lower Level of Detection Calculation. Based on Standard Methods 1030 E Method Detection Level. [90] Method overview:

1. A standard containing 0.1 uM of clothianidin, imidacloprid and thiamethoxam was injected seven times in a row on the LC-MS/MS.
2. The standard deviation (s) of the concentration measured was calculated for each compound
3. To reduce the probability of a type I error, the standard devation was multiplied by two times 1.645 from a cumulative normal probability table: LLD = 2*1.645*s.

2.4. Results and Discussion

Neonicotinoid Occurrence in Drinking Water. Clothianidin, imidacloprid, and thiamethoxam were ubiquitously present (*i.e.*, 100%) in all samples (n=16) collected from University tap water, with concentrations ranging between 3.89-57.3 ng/L, 1.22-39.5 ng/L, and 0.24-4.15 ng/L, respectively (**Table 2-4**). Maximum concentrations of clothianidin and imidacloprid occurred a few days after a runoff event in the Iowa River (**Figure 2-2**), indicating a possible relationship between neonicotinoid concentration and river flow. The delay between maximum river flow and maximum tap water concentration may be due to residence time in the distribution system, which is typically <1 to 3 days but can extend in some locations up to six days, and is complicated by the regulated aspect of this stream system (e.g. the Coralville Reservoir).[91] Samples of City finished tap water collected at private residences (**Table 2-5**) contained up to 0.52 ng/L of thiamethoxam; however, clothianidin and imidacloprid were not present above detection limits.

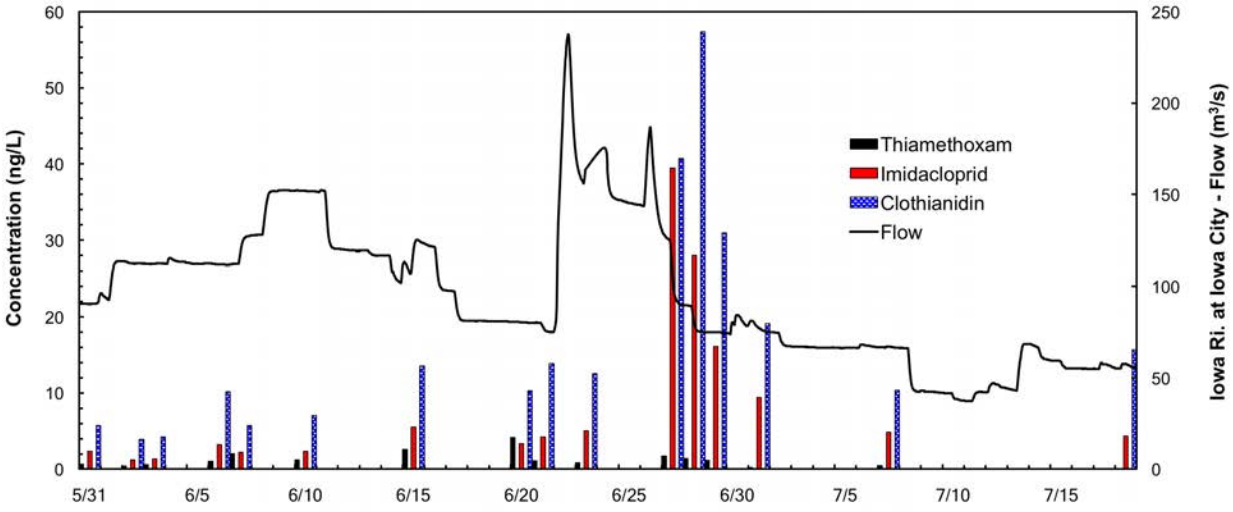


Figure 2-2: Concentration of clothianidin, imidacloprid, and thiamethoxam in samples collected from University of Iowa tap water in 2016. Concurrent streamflow in the Iowa River at Iowa City, IA is shown.

Table 2-4: University of Iowa tap water sample results. Samples collected from the same tap in the laboratory at Seamans Center for Engineering.

Date	Location	Thiamethoxam (ng/L)	Imidacloprid (ng/L)	Clothianidin (ng/L)
5/31	SC 4249	0.65	2.32	5.73
6/2	SC 4249	0.42	1.22	3.89
6/3	SC 4249	0.61	1.38	4.24
6/6	SC 4249	1.04	3.26	10.19
6/7	SC 4249	2.04	2.26	5.73
6/10	SC 4249	1.22	2.33	7.02
6/15	SC 4249	2.61	5.53	13.57
6/20	SC 4249	4.15	3.38	10.29
6/21	SC 4249	1.13	4.24	13.88
6/23	SC 4249	0.84	5.05	12.58
6/27	SC 4249	1.19	26.36	27.27
6/28	SC 4249	0.85	16.30	33.46
6/29	SC 4249	1.19	16.13	30.97
7/1	SC 4249	0.26	10.20	20.51
7/7	SC 4249	0.49	5.27	11.19
7/18	SC 4249	0.77	3.69	13.30

Table 2-5: Iowa City tap water results summary from samples collected from three residential locations in Iowa City.

Date	Location	Clothianidin (ng/L)	Imidacloprid (ng/L)	Thiamethoxam (ng/L)
7/18/16	1	ND	ND	0.34
7/18/16	2	ND	ND	<0.20
7/18/16	3	ND	ND	0.37
7/27/16	1	ND	<0.10	0.47

**ND indicates non-detect, <LLD indicates that compound was detected at concentrations below the LLD

Neonicotinoid Fate during Drinking Water Treatment. Samples collected from the UI DWTP (**Figure 2-3**) suggest that clothianidin and imidacloprid persist throughout conventional water treatment processes, while thiamethoxam is partially removed. Neonicotinoid concentrations on the two different sampling dates (**Figure 2-3**) varied, but trends across the

treatment train were consistent. Raw source water (*i.e.*, Iowa River) concentrations ranged from 10.7-25.9 ng/L for clothianidin, 2.15-13.3 ng/L for imidacloprid, and 1.93-8.23 ng/L for thiamethoxam, whereas finished water concentrations ranged between 10.6-31.2 ng/L for clothianidin, 1.97-13.6 ng/L for imidacloprid and 1.07-3.11 ng/L for thiamethoxam. Although we did not attempt to follow a single parcel of water through the treatment process (*i.e.*, all samples were collected at approximately the same time in a given sampling round), little to no concentration change for clothianidin and imidacloprid was measured. In contrast, thiamethoxam concentrations exhibited a clear drop of ~40-60% after lime softening and recarbonation, but were essentially stable thereafter through the treatment train.

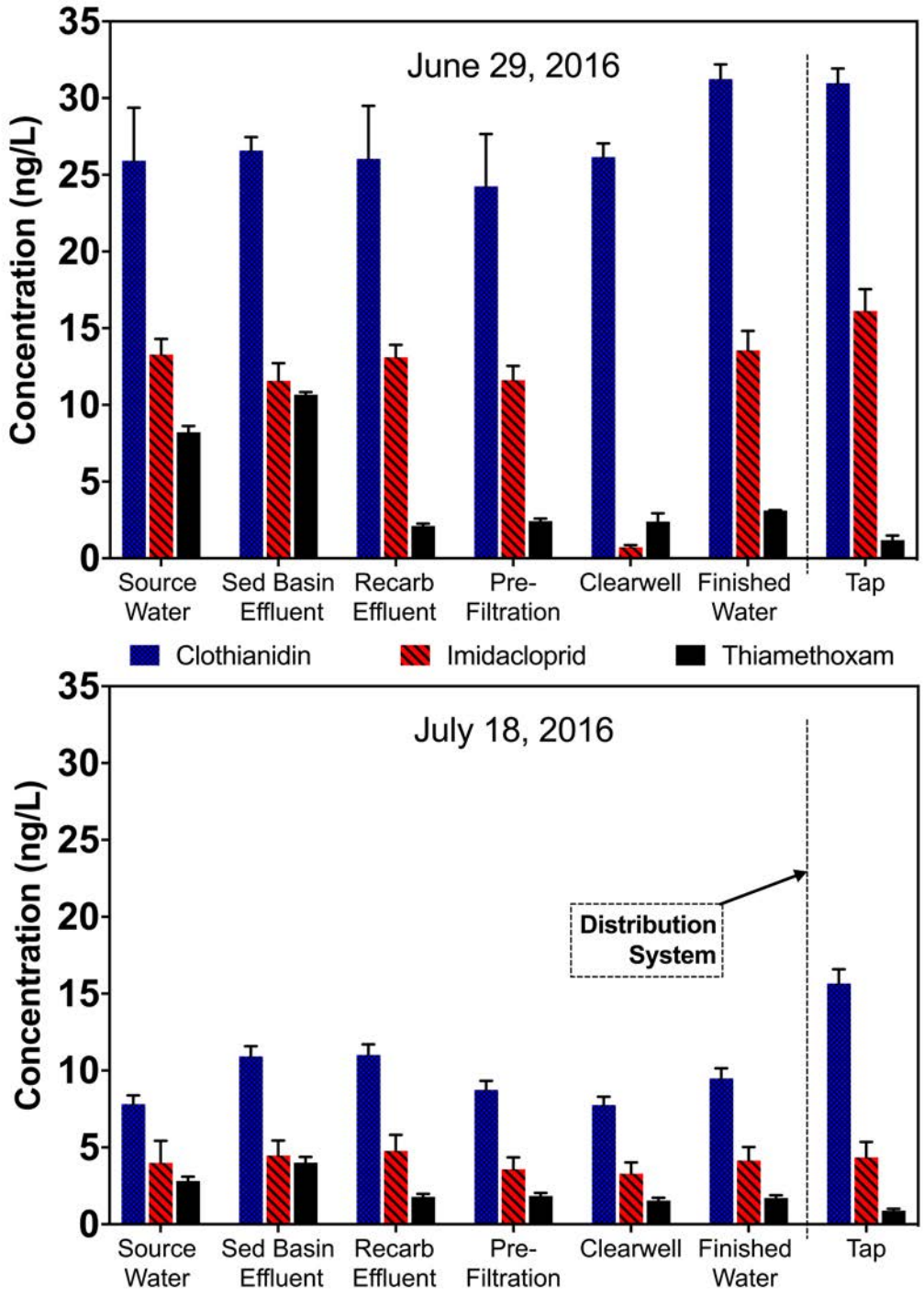


Figure 2-3: Concentrations of clothianidin, imidacloprid, and thiamethoxam measured at different unit operations at the University Water Treatment Plant on the two indicated sampling dates (additional data **Table 2-6**, **Table 2-7**, **Table 2-8**). Neonicotinoid concentrations differed on the two sampling dates, but overall trends across the treatment train were consistent. Error bars represent the standard error of regression associated with the composite enrichment sample extraction and analysis (1 L enriched to 1 mL).

Table 2-6: Clothianidin concentrations in samples from the University of Iowa water treatment plant (concentrations in nanograms per liter).

Date	Source Water (1)	Sedimentation Basin (2)	Recarbonation (Pre-chlorination)(3)	Recarbonation (Post-chlorination) (4)	Filtration effluent (5)	Finished Water (6)
6/29/16	26.0	26.6	26.0	24.3	26.2	31.2
7/18/16	7.82	10.9	11.0	8.75	7.76	9.50

Table 2-7: Imidacloprid concentrations in samples from the University of Iowa water treatment plant (concentrations in nanograms per liter).

Date	Source Water (1)	Sedimentation Basin (2)	Recarbonation (Pre-chlorination) (3)	Recarbonation (Post-chlorination) (4)	Filtration effluent (5)	Finished Water (6)
6/29/16	13.3	11.6	13.1	11.6	0.72	13.6
7/16/16	4.00	4.48	4.78	3.58	3.30	4.14

Table 2-8: Thiamethoxam concentrations in samples from the University of Iowa water treatment plant (concentrations in nanograms per liter).

Date	Source Water (1)	Sedimentation Basin (2)	Recarbonation (Pre-chlorination) (3)	Recarbonation (Post-chlorination) (4)	Filtration effluent (5)	Finished Water (6)
6/29/16	8.23	10.7	2.12	2.43	2.40	3.11
7/18/16	2.81	4.01	1.78	1.84	1.55	1.71

We also collected samples from the City and University DWTP to compare source water and finished water concentrations of clothianidin, imidacloprid, and thiamethoxam (**Figure 2-4**; **Table 2-9**, **Table 2-10**, **Table 2-11**) between the two treatment plants. Samples from the UI DWTP were collected within three hours of City DWTP samples. Source water concentrations of the three compounds were within 30% between sites for a given compound, despite the fact that UI DWTP water originates from the Iowa River and the City DWTP water originates from the shallow alluvial aquifer under the influence of the Iowa River.

Neonicotinoid concentration decreases appeared to be greater at the City DWTP (~100%, 94% and 85% for clothianidin, imidacloprid and thiamethoxam, respectively) than at the

University DWTP (~1%, 8% and 44% respectively). A notable distinction is that the City DWTP uses GAC filtration compared to rapid sand filtration at the UI DWTP; the latter process only removes particles. These analyses were consistent with earlier UI DWTP process train results that indicated no discernable concentration changes for clothianidin or imidacloprid, and modest loss of thiamethoxam. Additionally, finished water concentrations of clothianidin, imidacloprid, and thiamethoxam from each treatment plant were similar to the corresponding measurements from tap water samples.

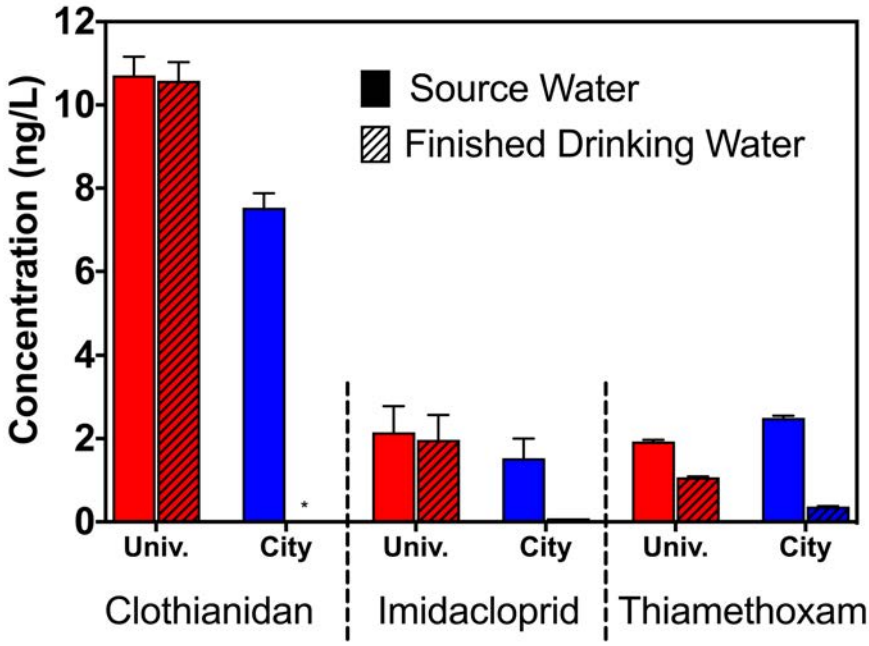


Figure 2-4: Concentrations of the three neonicotinoids measured in the Iowa City and University of Iowa drinking water treatment plant (DWTP) source and finished drinking waters (August 9, 2016). The Iowa City DWTP uses granular activated carbon (GAC) filtration compared to rapid sand filtration at the University DWTP. * Indicates non-detect. Error bars represent the standard error of regression associated with the composite enrichment sample extraction and analysis (1 L enriched to 1 mL).

Table 2-9: Clothianidin concentrations in the University of Iowa and Iowa City Source and Finished waters (August 9, 2016).

WTP	Source Water (ng/L)	Finished Water (ng/L)
UI	10.7	10.6
City	7.53	ND

Table 2-10: Imidacloprid concentrations in the University of Iowa and Iowa City Source and Finished Waters (August 9, 2016).

WTP	Source Water (ng/L)	Finished Water (ng/L)
UI	2.15	1.97
City	1.53	0.09

Table 2-11: Thiamethoxam concentrations in the University of Iowa and Iowa City Source and Finished Waters (August 9, 2016).

WTP	Source Water (ng/L)	Finished Water (ng/L)
UI	1.93	1.07
City	2.50	0.37

Hydrolysis of Thiamethoxam. We attribute thiamethoxam removal to base-catalyzed hydrolysis. Base-catalyzed hydrolysis of thiamethoxam has been reported with half-lives ($t_{1/2}$ values) ranging from 2.1 days[58] at pH 9.2 and 28^{o} C (corresponding to a pseudo-first-order rate constant, k_{obs}, value of 0.33 d^{-1}) to 6.1 days[15] at pH 9.0 and 25^{o}C (k_{obs}=0.11 d^{-1}). Furthermore, the stability of thiamethoxam is known to decrease with increasingly alkaline conditions.[58,59,92]

Batch tests confirmed that thiamethoxam hydrolysis is likely to occur over timescales relevant to treatment and distribution (**Figure 2-5**, **Figure 2-6**, **Figure 2-7**; **Table 2-1**). Using a University DWTP softening basin water sample spiked with 100 μM thiamethoxam, we measured a $t_{1/2}$ of 0.75 d (k_{obs}= 0.9 d^{-1}) at pH 10.4 (the softening basin pH) and 20^{o} C. During the lime softening process at the University DWTP, pH is increased to ≥10.3 with a residence time of 1.5-3.2 h. Accordingly, thiamethoxam removal observed in **Figure 2-2** and **Figure 2-3**

reflects degradation from hydrolysis during treatment and distribution (finished water pH ~9.9), as well as during the handling time between sample collection and processing (typically 24 h). Thiamethoxam hydrolysis is also expected to occur in the City DWTP, which also employs lime softening (finished water pH ~9.2).

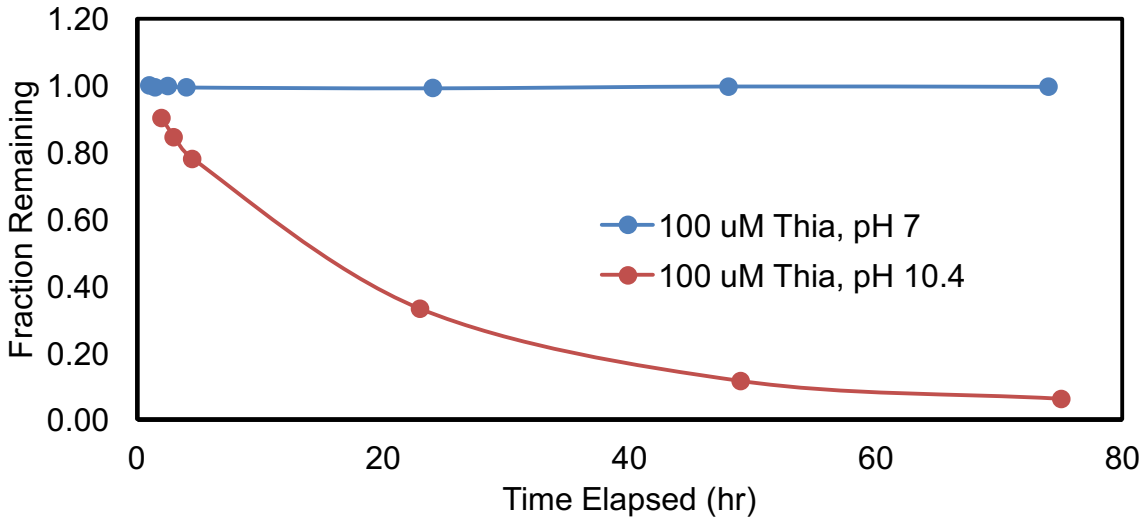


Figure 2-5: Thiamethoxam hydrolysis in ambient pH University DWTP softening basin water (pH 10.4) compared to University DWTP softening basin water adjusted to pH 7.

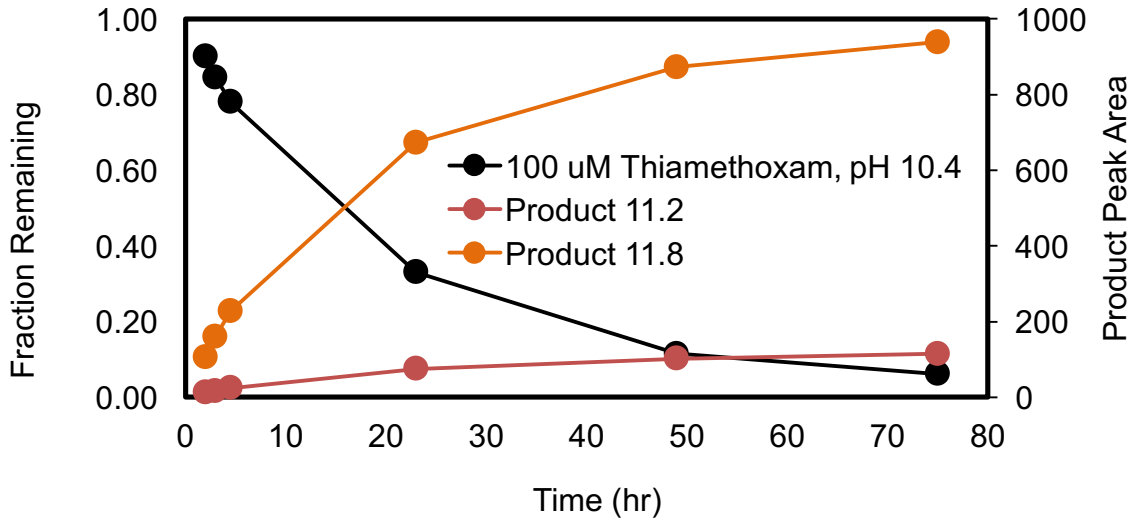


Figure 2-6: Product formation during thiamethoxam hydrolysis in University DWTP softening basin water.

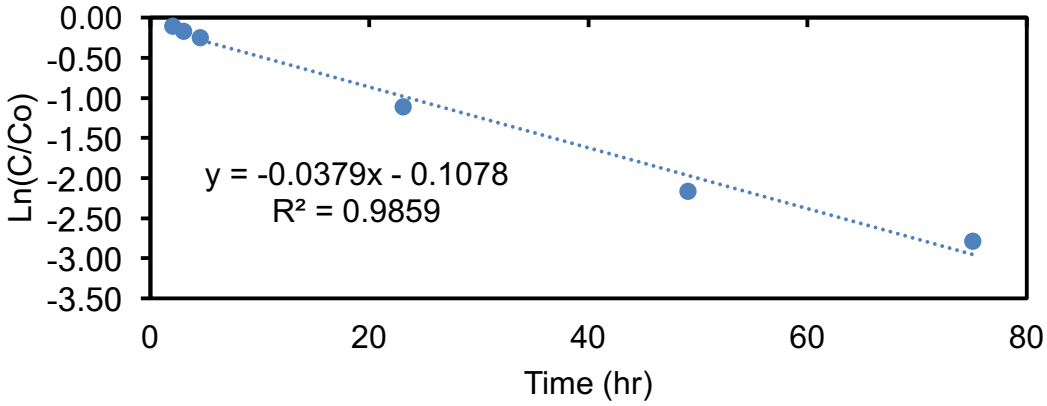


Figure 2-7: Ln(C/C_o) versus time for thiamethoxam hydrolysis in University DWTP softening basin water. K_{obs} = 0.0379 h^{-1}, $t_{1/2} = \ln(0.5)/K_{obs}$ = 18.3 h.

Neonicotinoid Removal via Sorption onto Granular Activated Carbon. All three neonicotinoids studied exhibited relatively rapid removal via sorption onto GAC, with >80% removal in suspensions after 1 h of contact time (**Figure 2-8**). Initial sorption was rapid, followed by stabilized aqueous concentrations consistent with equilibrium by 30 min. Some heterocyclic aromatic nitrogen compounds and protonated bases, such as the neonicotinoids studied herein, have been reported[93] to exhibit greater removal by GAC than would be predicted by K_{ow} values alone. Neonicotinoid removal by GAC is likely attributable to specific binding interactions between surface sites on GAC and specific structural moieties in the neonicotinoids, although additional experimental studies are recommended to evaluate adsorption mechanisms, long-term effectiveness, optimal dosing, and overflow rates.

Neonicotinoid Transformation during Chemical Disinfection with Free Chlorine. Both treatment plants employ chlorination, with typical contact times of 3-4 h (City DWTP) and 20 min–3 h (UI DWTP), and with residuals of 1.8 mg/L Cl_2 (City DWTP) and 2.5 mg/L Cl_2 (UI DWTP). Laboratory batch studies revealed a range of reactivity of neonicotinoids toward free chlorine (HOCl; **Figure 2-8**). Thiamethoxam was generally recalcitrant, exhibiting no significant

loss ($p>0.50$) at even the greatest free chlorine concentrations tested (Cl_2:Thiamethoxam=12,500; M/M) over a prolonged reaction. In contrast, imidacloprid and clothianidin exhibited greater reactivity, with clothianidin being most reactive. Second-order rate coefficients for HOCl reaction with clothianidin (4.7×10^{-2} $M^{-1}s^{-1}$) and imidacloprid (1.6×10^{-3} $M^{-1}s^{-1}$) were calculated from measured pseudo-first-order rate constants (**Figure 2-8**) assuming a constant HOCl concentration ($k_2=k_{obs}/[HOCl]$). At chlorine concentrations more typical for disinfection (*i.e.*, 5 mg/L as Cl_2) and assuming a constant residual, half-lives for clothianidin and imidacloprid would be ~2.5 d and ~70 d, respectively. Although imidacloprid is practically resistant to transformation, a modest degree of clothianidin decay may be expected during chemical disinfection, particularly in distribution systems with longer residence times.[94] We note that using conditions more representative of drinking water treatment (C_0=5 mg/L HOCl as Cl_2; 0.10-1.25 mg/L of clothianidin), extensive transformation of clothianidin occurred (>80% in 1.5h; **Figure 2-9**) at rates greater than expected from estimated k_2 values. We suspect that differences in clothianidin transformation rate across a range of chlorine concentrations reflect the formation of highly reactive intermediates that contribute to chlorine demand, which in turn influences the extent of clothianidin degradation (**Figure 2-11**).

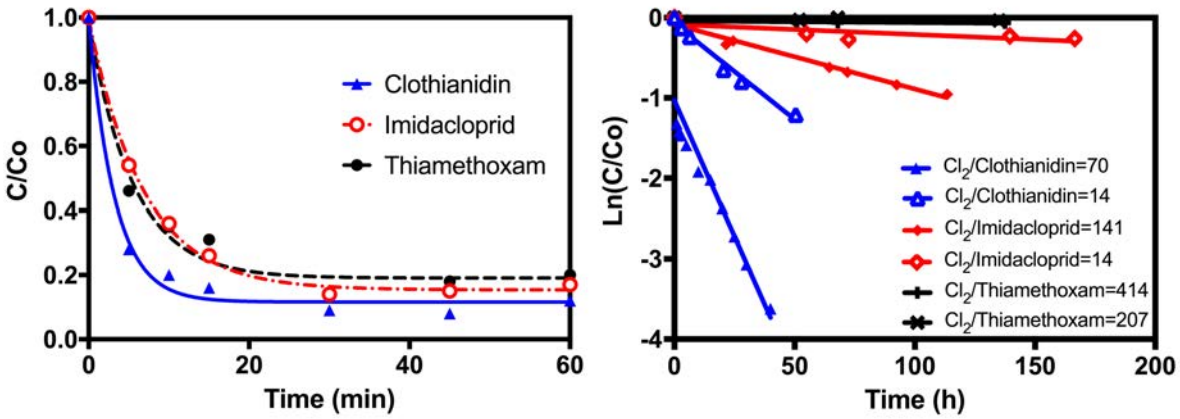


Figure 2-8: Neonicotinoid batch kinetics tests. Left: Change in aqueous neonicotinoid concentration (C_o=100 µg/L) in suspensions of granular activated carbon (5 g/L GAC in pH 7-phosphate buffer). Data fitted to exponential decay model (**Table 2-12**). Right: Chlorination loss kinetics. Cl_2/Neonicotinoid values reported as molar ratio (M/M). Titrations with FAS revealed chlorine concentrations (10 mg/L, 50 mg/L and 100 mg/L as Cl_2) constant over the experiment, allowing calculation of k_{obs} from the slopes of linear regressions.

Table 2-12: Exponential decay parameters for GAC adsorption (**Figure 2-8**).

Compound	**Clothianidin**	**Imidacloprid**	**Thiamethoxam**
K (h^{-1})	0.3098	0.1469	0.1812
R^2	0.9910	0.9974	0.9784

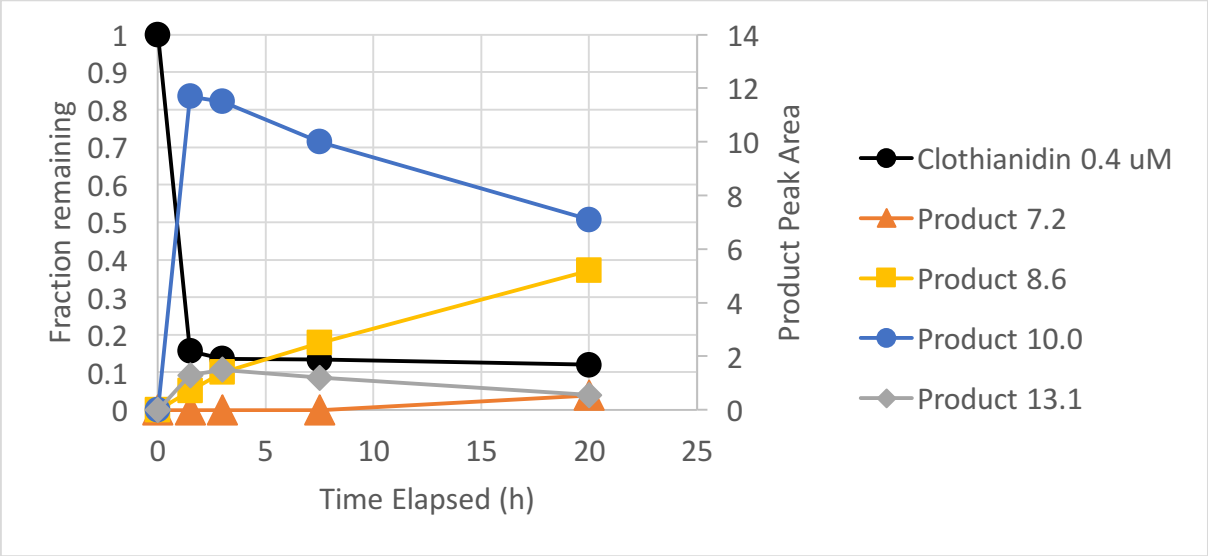


Figure 2-9: Product formation during chlorination of clothianidin. Experimental conditions: Chlorine 10 mg/L as Cl_2, clothianidin 0.4 µM, pH 7. The formation of intermediates (shown in this figure) may explain why we observe initial fast reaction rates followed by slow decay of clothianidin. We hypothesize that the intermediates are more reactive and may outcompete clothianidin for chlorine causing the decay of clothianidin to slow after a fast initial reaction.

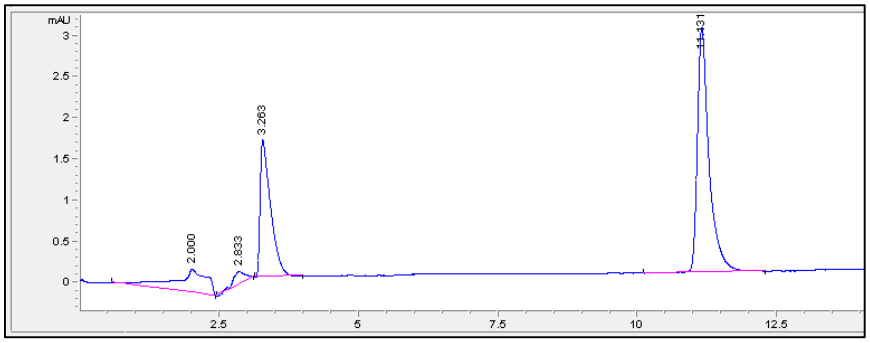


Figure 2-10: Chromatogram of chlorination reaction shown in **Figure 2-9**. Clothianidin concentration 100 µg/L, just prior to adding chlorine (0 mg/L Cl_2, t=0). Clothianidin residence time = 11.13 min, wavelength = 260 nm.

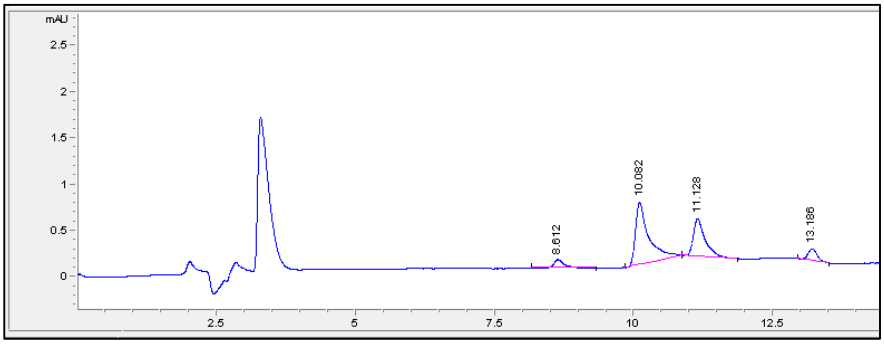


Figure 2-11: Chromatogram of chlorination reaction shown in **Figure 2-9**. Clothianidin concentration 100 µg/L, chlorine concentration 10 mg/L Cl_2, time = 3 h. Clothianidin residence time = 11.13 min, wavelength = 260 nm.

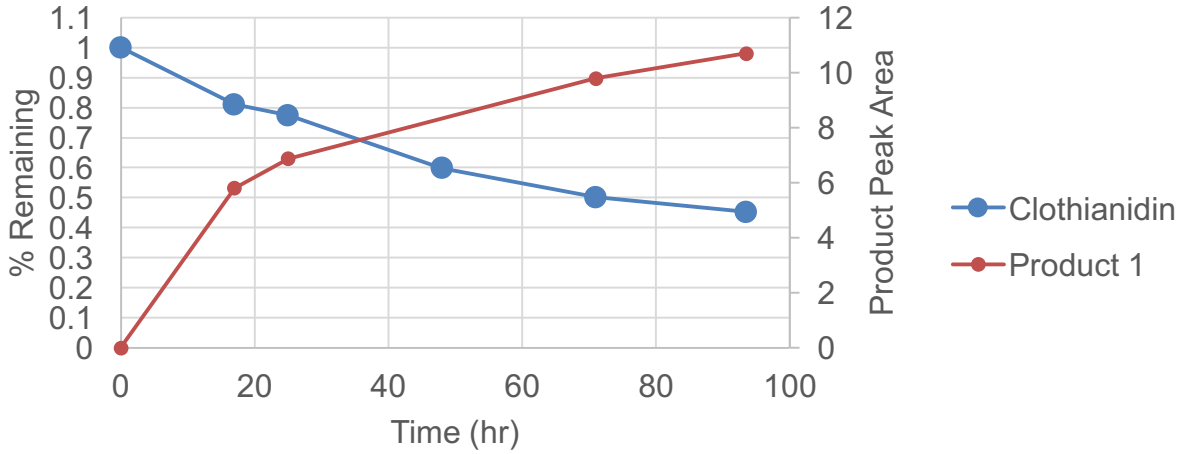


Figure 2-12: Product formation during chlorination of clothianidin. Experimental conditions: Chlorine 5 mg/L as Cl_2, clothianidin 5 µM, pH 7. The formation of intermediates (shown in this figure) may explain why we observe initial fast reaction rates followed by slow decay of clothianidin. We hypothesize that the intermediates are more reactive and may outcompete clothianidin for chlorine causing the decay of clothianidin to slow after a fast initial reaction.

Environmental Implications. To our knowledge, this is the first peer-reviewed study to document the presence of neonicotinoids in finished tap water samples. Conventional water treatment results in no measurable removal of clothianidin or imidacloprid, although the alkaline conditions of lime softening result in the partial transformation of thiamethoxam via base-catalyzed hydrolysis. Due to their pervasiveness in source waters[3,6–8] and persistence through treatment systems,[45] neonicotinoids are likely present in other drinking water systems across the

US. Transformation products formed by chlorination or hydrolysis warrant great consideration due to the potential to form transformation products that have greater mammalian toxicity than their parent compounds (**Figure 2-6**, **Figure 2-9**, and **Figure 2-12**). For example, the metabolite desnitro-imidacloprid exhibits 300 times greater mammalian receptor binding affinity than imidacloprid due to the loss of the nitro group that confers insect specificity.[18] For management, GAC filtration presents a substantially more economical treatment option for removal of neonicotinoids in resource-constrained communities reliant on agriculturally impacted surface waters or point-of-use systems than reverse osmosis or advanced oxidation processes.[95]

3. TRANSFORMATION PRODUCTS

3.1. Introduction

Neonicotinoids are insect selective pesticides, meaning they are more toxic to insects than mammals. Nevertheless, transformation products of neonicotinoids, resulting from processes such as degradation, oxidation, photolysis and metabolism, may lose insect specificity and have increased mammalian toxicity through loss of the functional groups conferring insect selectivity. As demonstrated in Chapter 2, neonicotinoids may be degraded at elevated pH (e.g., thiamethoxam) or during chemical oxidation (e.g., clothianidin) over timescales relevant to water treatment and distribution systems. The objectives of this chapter are to identify transformation products resulting from chlorination of clothianidin and hydrolysis of thiamethoxam, and also to explore whether hydrolysis products of thiamethoxam undergo chlorination under conditions and timescales relevant to water treatment. We also examine the chlorination of known metabolites of imidacloprid (desnitro-imidacloprid and imidacloprid-urea) as these species may be present in surface waters due to physical or biological degradation processes.

3.2. Chemicals

Chemicals used in this work include: clothianidin (99.9%, Fluka, CAS 210880-92-5), imidacloprid (99.9%, Fluka, CAS 138261-41-3), thiamethoxam (99.6%, Fluka, CAS 153719-23-4), imidacloprid-urea (99.0%, Dr. Ehrenstorfer, CAS 120868-66-8) and desnitro-imidacloprid hydrochloride (99.9%, Fluka, CAS 115970-17-7). All neonicotinoids were used as received. HPLC grade acetonitrile was used for LC-MS and LC-DAD analysis. Other chemicals used in experiments and analysis include sodium hypochlorite solution 5.65-6% (Fisher Scientific, used for pH adjustment) and 5 mM potassium phosphate buffer (made in lab, used for chlorination

and hydrolysis reactions).

3.3. Methods

Chlorination of Clothianidin, Imidacloprid, Imidacloprid-urea and Desnitro-imidacloprid. Bench scale chlorination experiments were conducted to assess the kinetics of neonicotinoid transformation and product formation during chemical disinfection and distribution in the presence of residual disinfectant. To initiate reaction, hypochlorous acid (HOCl) was added to a closed reactor (1 - 10 mL) containing either clothianidin, imidacloprid, imidacloprid-urea or desnitro-imidacloprid in 5 mM phosphate buffer at pH 7. A range of neonicotinoid (1 – 50 μM) and HOCl (1 – 50 mg/L as Cl_2) concentrations were tested. Samples (0.5 – 1.0 mL) were collected at defined intervals and transferred to amber glass vials for immediate analysis. Samples were monitored for 24-72 hours via high performance liquid chromatography coupled with a diode array detector (LC-DAD), and then brought to the High Resolution Mass Spectrometry Facility (HRMSF) at the University of Iowa for exact mass identification and fragment analyisis via HR-LC-MS and HR-LC-MS/MS.

Sequential Hydrolysis and Chlorination of Thiamethoxam. Bench scale hydrolysis and chlorination experiments were conducted to assess the kinetics of thiamethoxam transformation and product formation during lime softening, chemical disinfection and distribution in the presence of residual disinfectant and elevated pH. Thiamethoxam hydrolysis products were formed by adding thiamethoxam (50 μM) to a closed reactor containing 10 mL of pH 10 phosphate buffer and allowing the mixture to react for one week. HOCl (50 mg/L) was then added to the reactor containing thiamethoxam and its hydrolysis products. Samples (0.5 – 1.0 mL) were collected at defined intervals and transferred to amber glass vials for immediate

analysis. Samples were monitored for 48 hours via high performance liquid chromatography coupled with a diode array detector, and then brought to the High Resolution Mass Spectrometry Facility (HRMSF) at the University of Iowa for exact mass identification and fragment analysis via TOF-HR-LC-MS and TOF-HR-LC-MS/MS.

Analytical Methods. Neonicotinoid samples were monitored for kinetics via LC-DAD (Agilent 6140 Quadrupole LC-MS and diode array detector with OpenLab ChemStation C.07.00) during chlorination and hydrolysis experiments. The chromatography column was a C18 Zorbax Eclipse Plus (4.6 mm x 150 mm, 5 μm) at ambient temperature. An injection volume of 20 μL was used, and the mobile phases were acetonitrile and water with 0.1% formic acid at 0.8 mL/min. The mobile phase gradient is described in **Table 3-1**. Samples were quantified using the DAD at a wavelength of 260 nm (clothianidin, imidacloprid-urea and thiamethoxam), 280 nm (imidacloprid) and 273 nm (desnitro-imidacloprid).

Samples were also monitored using UV absorbance at a wavelength of 260 nm. The HR-LC-MS was used with an injection volume of 20 μL. The mobile phases were acetonitrile and water with 0.1% formic acid at 0.7 mL/min. Samples were analyzed on electrospray ionization positive mode, desolvation gas temperature was 350 °C, nebulizer gas flow was 700 L/Hr, cone gas flow 30 L/hr, capillary voltage was 2.8k V and cone voltage was 10 V, 20 V, 35V or 40 V. HR-LC-MS data were collected in full scan mode. Fragmentation analysis was conducted by HR-MS/MS. The mobile phase gradient is described in **Table 3-2** and the collision energy was 10-30 eV.

Table 3-1: HPLC mobile phase gradient (LC-DAD).

Time (min)	% Acetonitrile	% Deionized Water
0	25	75
10	25	75

Table 3-2: HPLC mobile phase gradient (HR-MS).

Time (min)	% Acetonitrile	% Deionized Water
0	15	85
11	25	75
13	25	75
18	95	5
20	95	5
20.1	15	85

3.4. Results and Discussion

Desnitro-imidacloprid and Imidacloprid-urea Reactivity with Chlorine. Desnitro-imidacloprid and imidacloprid-urea undergo transformation during chlorination at timescales relevant to water treatment (**Figure 3-1**, **Figure 3-2**). Second order rate coefficients for imidacloprid-urea (2.7 $M^{-1}s^{-1}$) and desnitro-imidacloprid (72 $M^{-1}s^{-1}$) were calculated from measured pseudo-first-order rate constants (**Figure 3-1** and **Figure 3-2**) assuming a constant HOCl concentration ($k_2=k_{obs}$/[HOCl]). At typical chlorine concentrations for disinfection (*i.e.*, 5 mg/L as Cl_2) and assuming a constant residual, half-lives for imidacloprid-urea and desnitro-imidacloprid would be ~1.0 hr and ~2.4 min, respectively. Thus, the metabolites of imidacloprid would be expected to degrade readily in a chlorine contactor and during distribution.

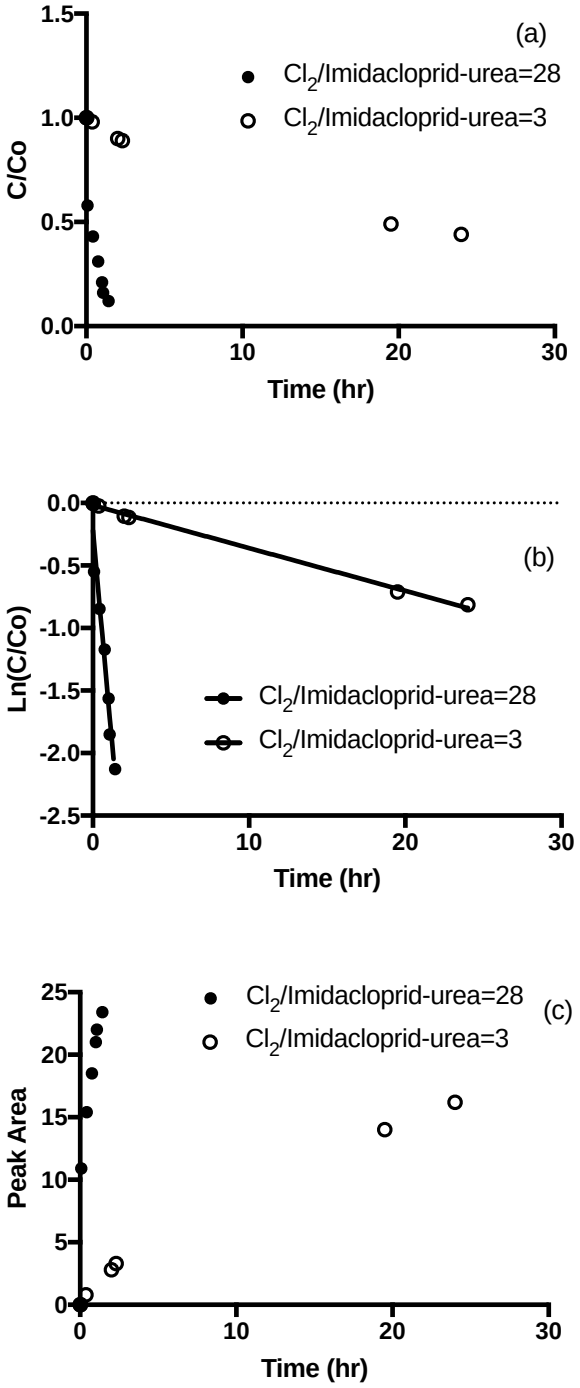


Figure 3-1: (a) Chlorination loss kinetics for imidacloprid urea (5 uM), (b) data fitted to an exponential decay model and (c) product formation. Cl_2/neonicotinoid values reported as molar ratio (M/M). Initial chlorine concentrations were 10 mg/L Cl_2 and 1 mg/L as Cl_2. Titration of the Cl_2/Imidacloprid-urea=28 trial at the beginning and end of the experiment showed that the concentration of chlorine was constant, allowing calculation of k_{obs} from the slope of the linear regression.

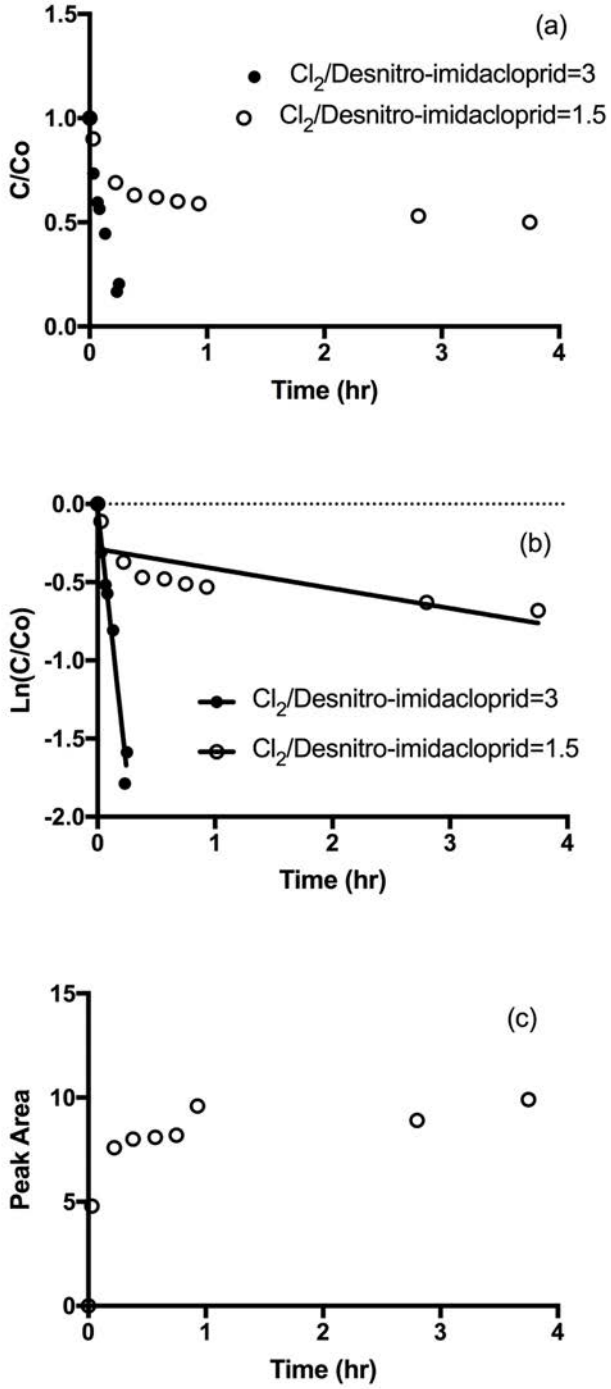


Figure 3-2: (a) Chlorination loss kinetics for desnitro-imidacloprid (10 uM), (b) data fitted to an exponential decay model and (c) product formation. Cl_2/neonicotinoid values reported as molar ratio (M/M). Initial chlorine concentrations were 2 mg/L Cl_2 and 1 mg/L Cl_2. Titration of the Cl_2/Desnitro-imidacloprid=3 trial at the beginning and end of the experiment showed that the concentration of chlorine was constant, allowing calculation of k_{obs} from the slope of the linear regression.

Hydrolysis Products of Thiamethoxam and Reactivity with Chlorine. As shown in **Figure 3-3**, Product 11.2 appears to be recalcitrant, while product 11.8 readily reacts with chlorine to form product 9.2. The second-order rate coefficient for the reaction of HOCl with product 11.8 (-0.6658 $M^{-1}s^{-1}$) was calculated from the measured pseudo-first-order rate constant (**Figure 3-4**). Assuming a constant chlorine residual (5 mg/L Cl_2), the half-life of product 11.8 would be 4.8 hr. Thus, the product 11.8 would be expected to degrade during disinfection and distribution.

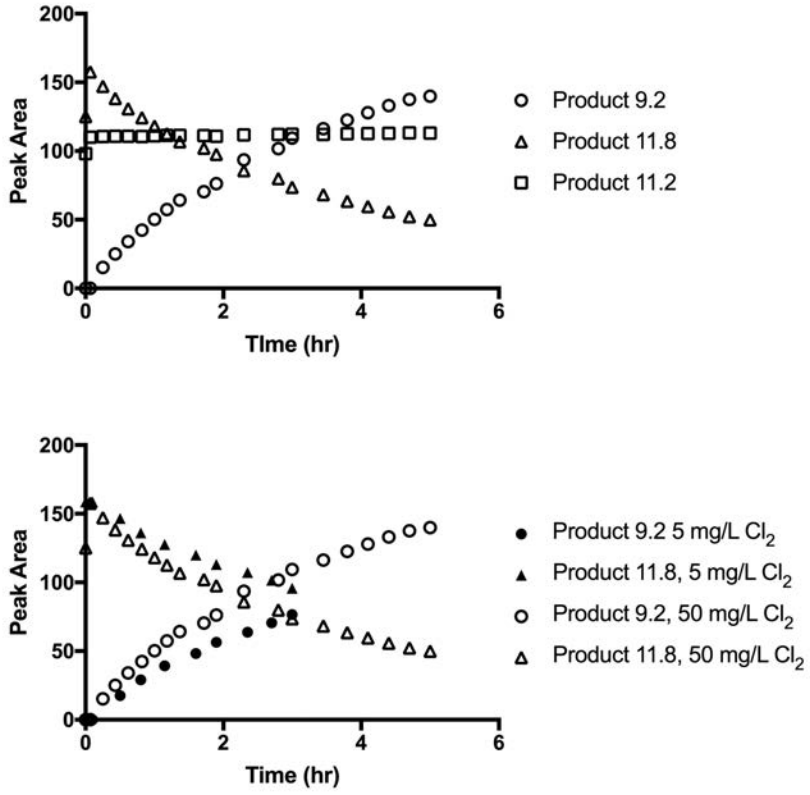


Figure 3-3: Top: Chlorination (50 mg/L Cl_2) of thiamethoxam hydrolysis products (product 11.2 and product 11.8) and formation of product 9.2. Bottom: chlorination of product 11.8 to form product 9.2 at 50 mg/L-Cl_2 and 5 mg/L-Cl_2. Initial thiamethoxam concentration for all experiments was 50 uM, reactions were conducted in pH 10 phosphate buffer.

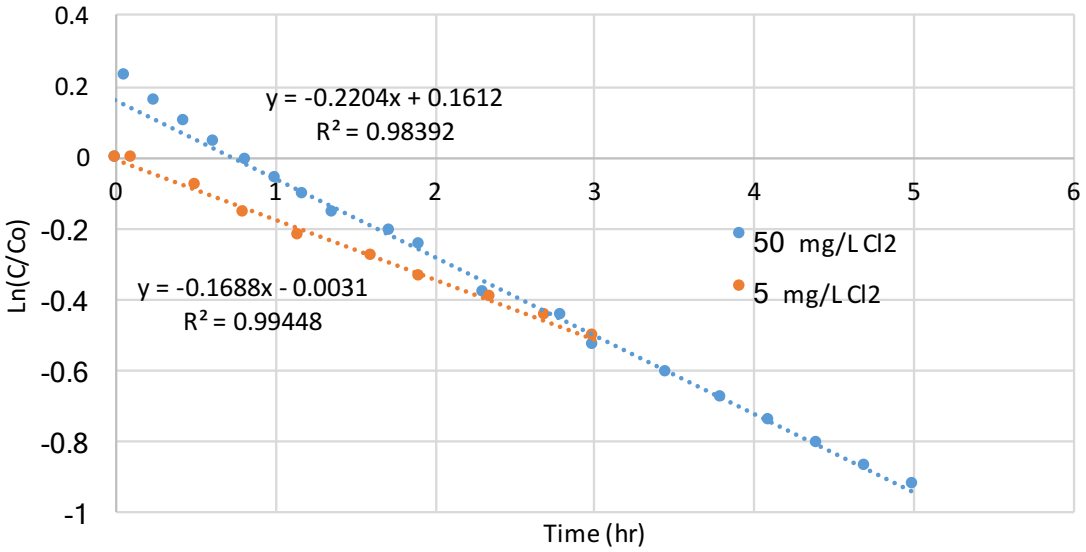


Figure 3-4: Ln(C/C$_o$) for the reaction of chlorine (50 mg/L Cl_2, 5 mg/L Cl_2 in pH 10 phosphate buffer) with product 11.8.

Transformation Product Identification. Identifying the transformation products formed during chlorination and hydrolysis is the first step to evaluating the impact of these products on human health. Using LC-MS/MS, HR-MS and HR-MS/MS we propose product structures resulting from the chlorination of clothianidin, imidacloprid and two metabolites of imidacloprid (imidacloprid-urea and desnitro-imidacloprid), as well as the sequential hydrolysis and chlorination products of thiamethoxam. The results of this analysis are summarized in **Table 3-3**, structural and fragmentation data for the parent compounds (clothianidin, imidacloprid, thiamethoxam, desnitro-imidacloprid and imidacloprid-urea) are provided in **Table 3-4** for comparison. The confidence level of each product and its structure are characterized according to the Schymanski et al. framework (summarized in **Figure 3-5**).

Chlorination of clothianidin results in three major products: CLO-239a, CLO-239b and CLO-THX 270. The location of the chlorine on these products could not be confirmed with certainty, though the presence of an existing chlorine makes chlorination on the ring unlikely

(chlorination is expected to occur at primary, secondary and tertiary aliphatic amines).[96] These products are confirmed to a level 3 confidence level, according to the Schymanski et al. framework.[97]

Chlorination of imidacloprid leads to the formation of three transformation products. One of the products (IMI-246) is chlorinated imidacloprid-urea and was confirmed to level 2b. Unsurprisingly, chlorination of an imidacloprid-urea standard also leads to the formation of IMI-246. IMI-290 is chlorinated imidacloprid (without removal of the nitro group), though the exact location of the chlorine is unknown (level 3 confidence), but most likely not located on the chlorinated ring. One product, IMI-341, could only be confirmed to level 5 confidence, thus no structure is proposed.

Thiamethoxam hydrolysis forms two products (THX-248 and THX-237), both of which were previously identified by Maienfisch et al.[15] Upon addition of chlorine to the mixture, THX-237 appears to react to form CLO-THX 270, while THX-248 is persistent (**Figure 3-3**).

Finally, chlorination of desnitro-imidacloprid results in the formation of two products – one doubly chlorinated and one triply chlorinated product (desnitro-imidacloprid contains one native chlorine, thus one or two chlorines are added to the molecule to form the products). Both products are confirmed to level 2b confidence level according to the Schymanski et. al framework.[97]

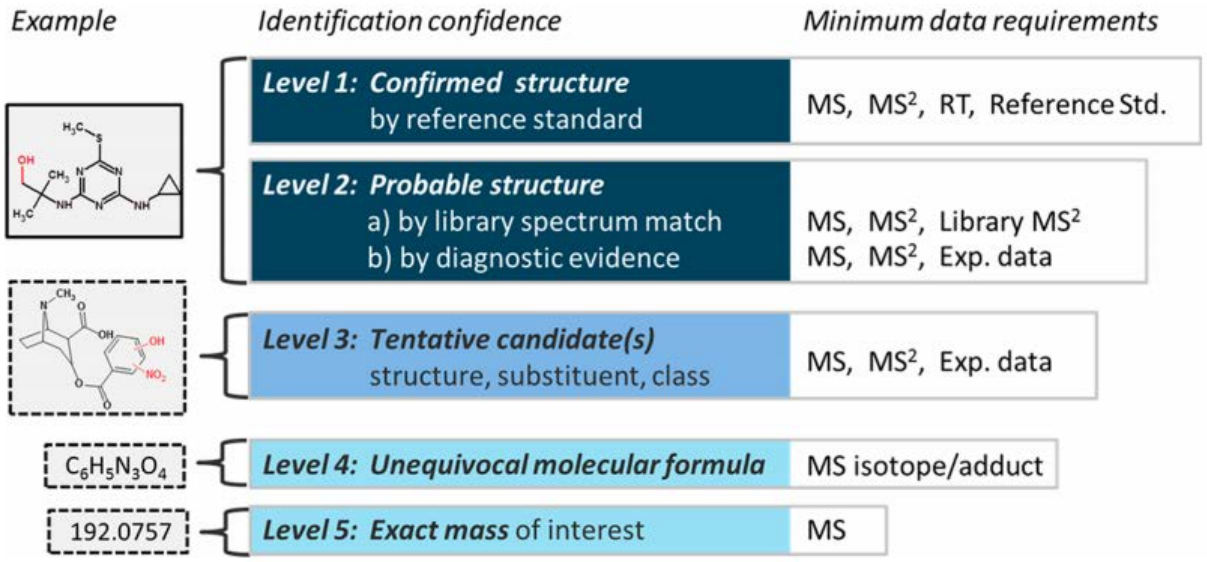


Figure 3-5: Summary of identification confidence levels proposed by Schymanski et. al for high resolution mass spectrometric analysis.[3]

[3] Reprinted with permission from Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097–2098.[sp] Copyright 2014 American Chemical Society.

Table 3-3: Transformation products of clothianidin, imidacloprid, desnitro-imidacloprid, imidacloprid-urea and thiamethoxam.

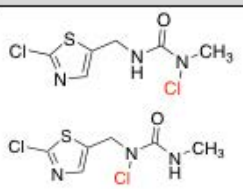
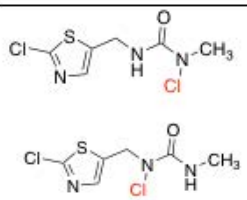
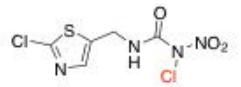
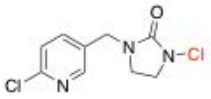
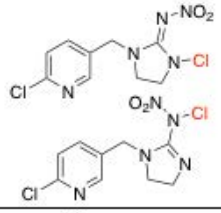
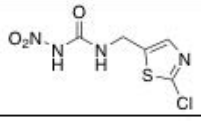
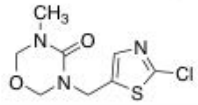
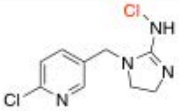
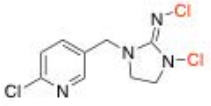
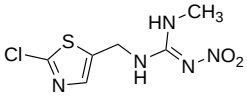
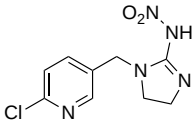
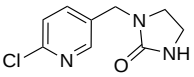
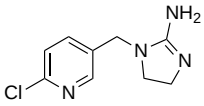
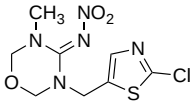
Product	Proposed Structure	Proposed Formula	Confidence Level	RT (min)	ESI Mode (+/-)	Accurate Mass (m/z)	Fragment ions (nominal mass; m/z)	Accurate mass (m/z)	Proposed Molecular Formula
CLO 239 a		$C_6H_7Cl_2N_3OS$	Level 3	16.1	positive	239.9799	168	168.03	$C_6H_7N_3OS$
							85	84.96	C_3H_3NS
CLO 239 b		$C_6H_7Cl_2N_3OS$	Level 2b	16.4	positive	239.9798	175	174.98	$C_5H_4ClN_2OS$
							147	146.98	$C_4H_4ClN_2S$
							133	132.98	C_4H_4ClNS
							119	119.97	C_3H_2ClNS
CLO-THX 270		$C_5H_4Cl_2N_4O_3S$	Level 3	9.2	positive	270.9442	182	181.9439	Unknown
							148	147.9768	$C_4H_5ClN_2S$
							133	132.9717	C_4H_4ClNS
IMI 246		$C_9H_9Cl_2N_3O$	Level 2b	15.9	positive	246.0222	211	211.0487	$C_9H_{10}ClN_3O$
							155	155.0348	$C_7H_8ClN_2$
							141	141.0206	$C_6H_6ClN_2$
							126	126.0097	C_6H_5ClN
IMI 341	Unknown	Ambiguous	Level 5	16.6	positive	341.9938	218	218.0239	Unknown
							155	155.0367	Unknown
							126	126.0104	Unknown
IMI 290		$C_9H_9Cl_2N_5O_2$	Level 3	16.9	positive	290.0222	246	246.0217	$C_9H_9Cl_2N_3O$
							209	209.0617	$C_9H_{10}ClN_4$
							173	173.0839	Unknown
							126	126.0123	C_6H_5ClN
THX 237		$C_5H_5ClN_4O_3S$	Level 2b	11.8	positive	236.9838	175	174.9724	$C_5H_4ClN_2OS$
							148	147.9772	$C_4H_5ClN_2S$
							97	97.0388	Unknown
THX 248		$C_8H_{10}ClN_3O_2S$	Level 2b	11.2	positive	248.0248	175	174.9718	$C_5H_4ClN_2OS$
							132	131.9665	C_4H_3ClNS
DN-IMI 245		$C_9H_{10}Cl_2N_4$	Level 2b	14.7	positive	245.0377	209	209.0622	$C_9H_{10}ClN_4$
							173	173.0848	Unknown
							126	126.0133	C_6H_5ClN
DN-IMI 279		$C_9H_9Cl_3N_4$	Level 2b	18.5	positive	279.0004	209	209.0506	$C_9H_{10}ClN_4$
							173	173.0848	Unknown
							126	126.013	C_6H_5ClN

Table 3-4: Structural and fragmentation data for clothianidin, imidacloprid and thiamethoxam as well as select known metabolites of imidacloprid (desnitro-imidacloprid and imidacloprid-urea) with available reference standards.

Neonicotinoids and Select Metabollites						Fragment Ions		
Compound	Structure	Formula	RT (min)	ESI Mode (+/-)	Accurate Mass (m/z)	Fragment ions (nominal mass; m/z)	Accurate mass (m/z)	Proposed Molecular Formula
Clothianidin		$C_6N_5H_8SO_2Cl$	10.8	+	250.02	169	169.0534	unknown
						132	131.9664	C_4H_3ClNS
						110	110.0699	unknown
Imidacloprid		$C_9H_{10}ClN_5O_2$	12.2	+	256.0607	209	209.0577	$C_9H_{10}ClN_4$
						175	175.096	unknown
Imidacloprid-urea		$C_9H_{10}ClN_3O$	8.5	+	212.0701	128	128.0333	unknown
						99	99.0607	unknown
Desnitro-imidacloprid		$C_9H_{11}ClN_4$	3.5	+	211.0792	unknown	unknown	unknown
Thiamethoxam		$C_8H_{10}ClN_5O_3S$	8.7	+	292.0261	211	211.0637	$C_7H_{10}N_5O_3$
						181	181.0535	unknown
						132	131.9664	C_4H_3ClNS

3.5. Environmental Implications

Neonicotinoids present in water treatment and distribution systems may form transformation products during lime softening and/or disinfection, at time scales relevant to water treatment and distribution. These transformation products are of concern from a human health standpoint as their toxicity toward mammals is unknown. In particular, removal of the nitro group may increase the toxicity of these products toward mammals. Several of the transformation products identified in **Table 3-3** (CLO-239a, CLO-239b, IMI-246, THX-248, DN-IMI245 and DN-IMI 279) appear to lose the nitro group through chlorination or hydrolysis. More work is necessary to understand whether these transformation products are present in finished drinking water, and if so, if there are any implications for human health.

4. CONCLUSIONS

Neonicotinoids are present in surface water used for drinking water. In Chapter 2 we demonstrate that neonicotinoids are not removed by conventional water treatment processes. As a result, neonicotinoids were measured in all tap water samples collected from the University drinking water during the summer of 2016. Furthermore, some neonicotinoids may form transformation products during lime softening (through based induced hydrolysis), or during chemical disinfection (through reaction with chlorine).

Though neonicotinoids are generally considered to be of low toxicity toward mammals, metabolites and transformation products may be more toxic to mammals than their parent compounds through loss of the functional groups conferring insect selectivity. Transformation products of neonicotinoids may be formed during drinking water treatment through hydrolysis and/or chlorination reactions, and these transformation products carry unknown toxicity toward mammals. In Chapter 3 we show that several of the transformation products identified in this study exhibit nitro-group removal, indicating the potential for bio activation in mammals.

Finally, granular activated carbon filtration may present an effective method for removing neonicotinoids from drinking water. In contrast to University drinking water, samples collected from City tap water contained little to no clothianidin, imidacloprid or thiamethoxam. As demonstrated by bench scale studies in Chapter 2, this is likely due to the use of granular activated carbon filtration at the City DWTP. However, the mechanism for this removal is presently unknown.

Future research should focus on transformation product identification and toxicity. This work should include the measurement of transformation products in drinking water as well as

toxicity testing to better understand implications of these transformation products (if present in drinking water) for human health. Lastly, additional research is needed to determine the mechanisms of granular activated carbon removal, optimal dosing, potential for transformation product removal, and the long-term effectiveness of this technology.

APPENDIX

Chlorination of Clothianidin. 10 mg/L Cl_2 was added to 25 uM clothianidin. Samples were run on the HR-MS for product identification.

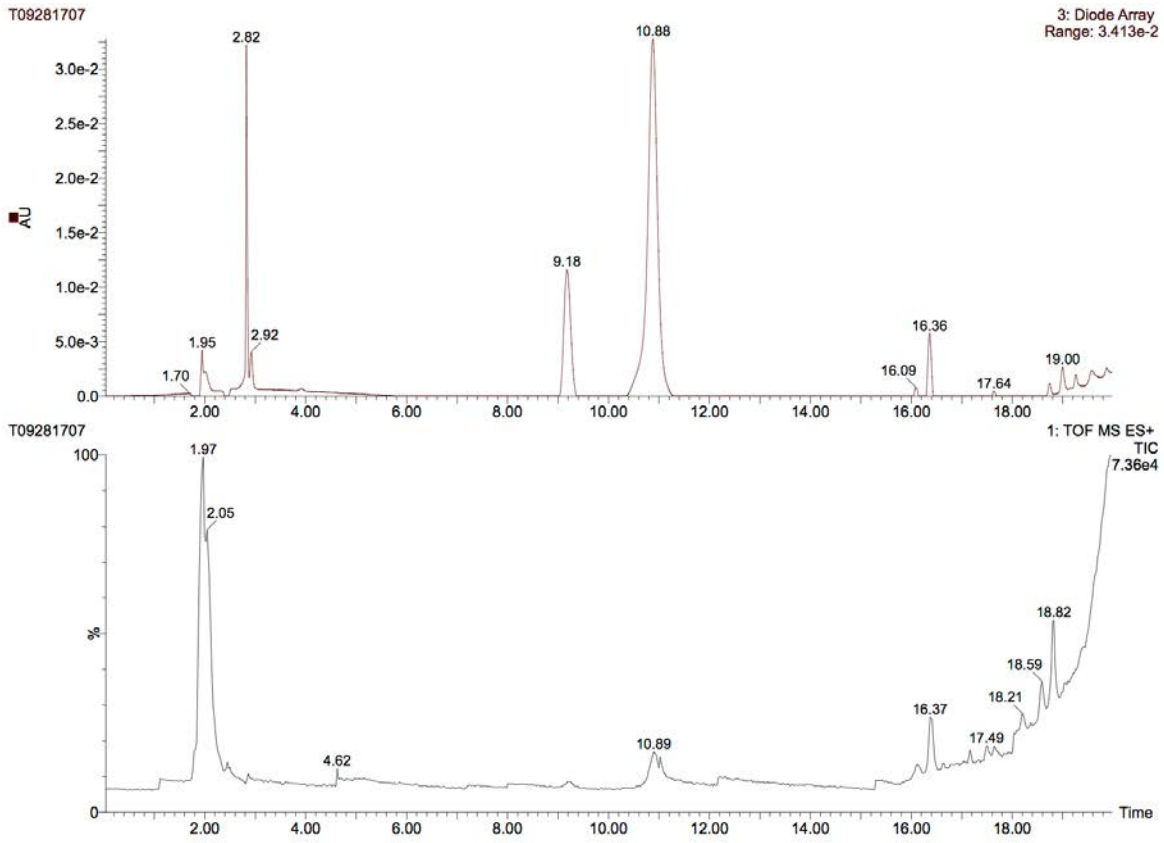


Figure A - 1: (a) UV scan of the reaction of clothianidin with chlorine. (b) TIC scan of the reaction of clothianidin with chlorine.

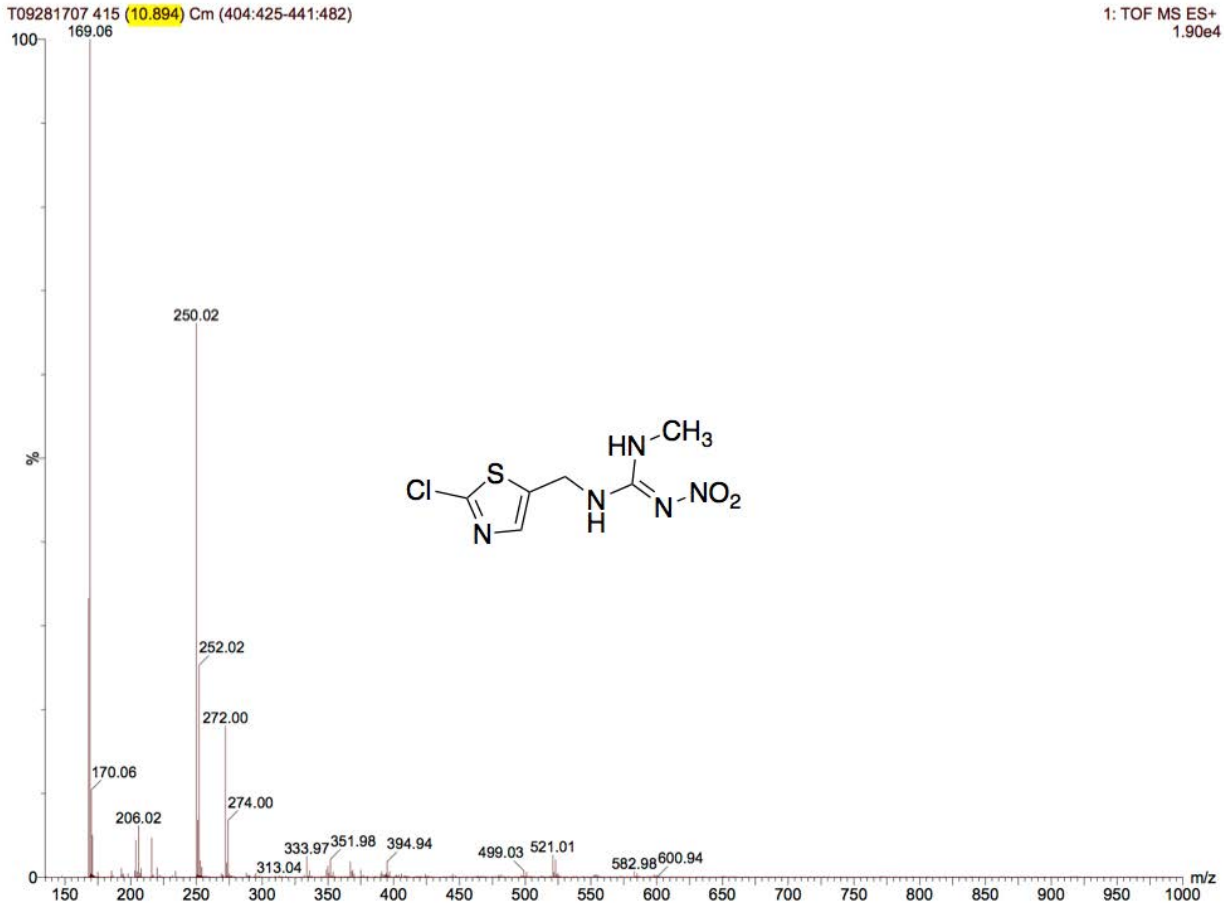


Figure A - 2: Mass spectrum and structure of clothianidin.

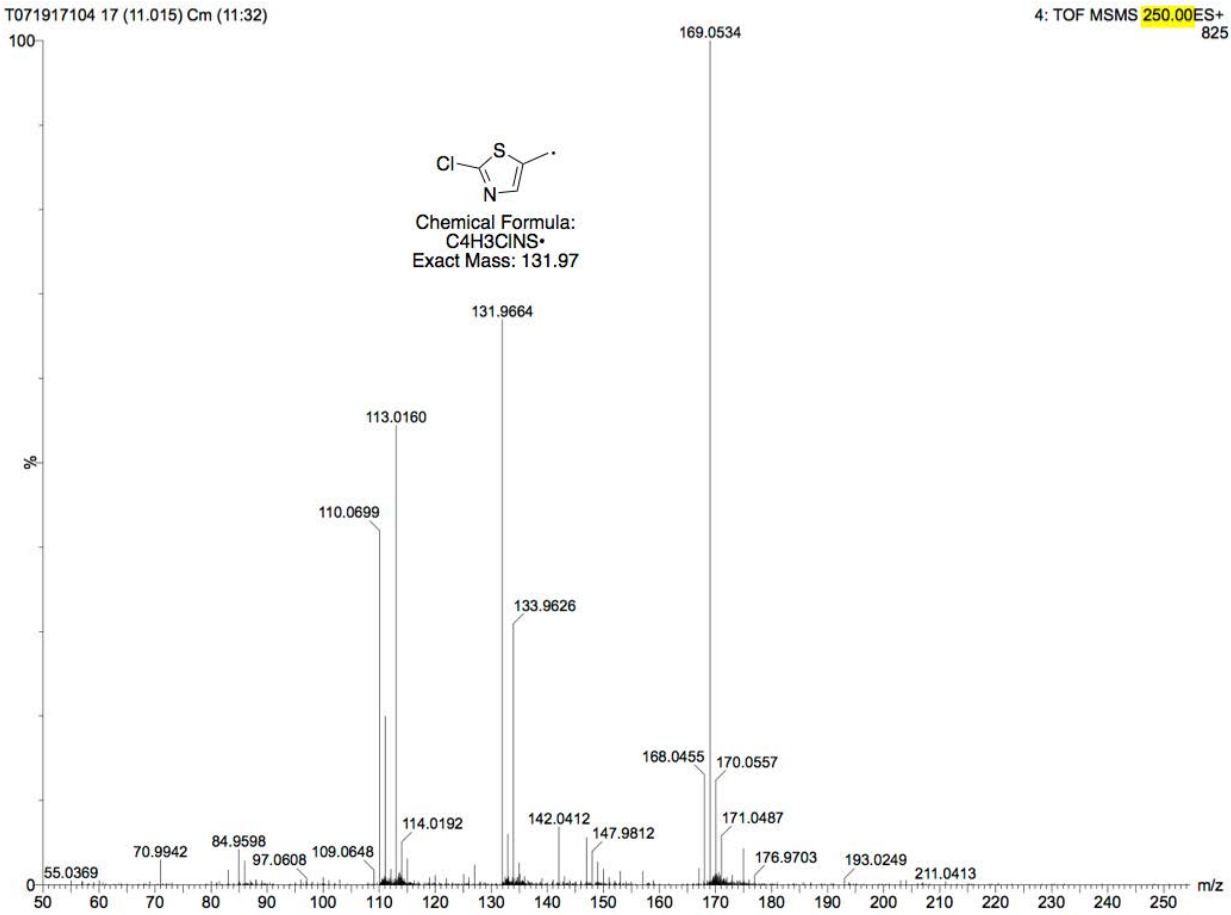


Figure A - 3: MS-MS of clothianidin with proposed fragment structures.

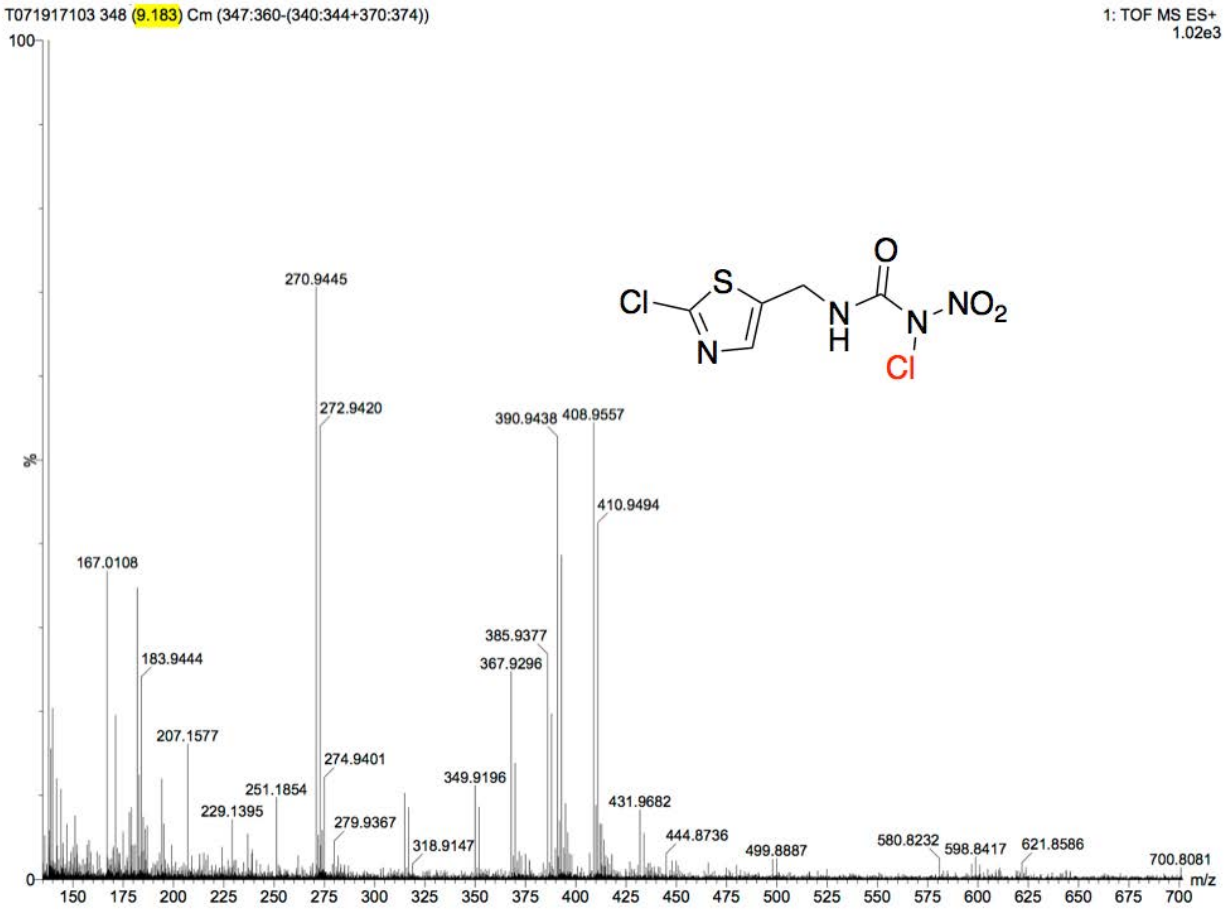


Figure A - 4: Mass spectrum and proposed structure of CLO 270.

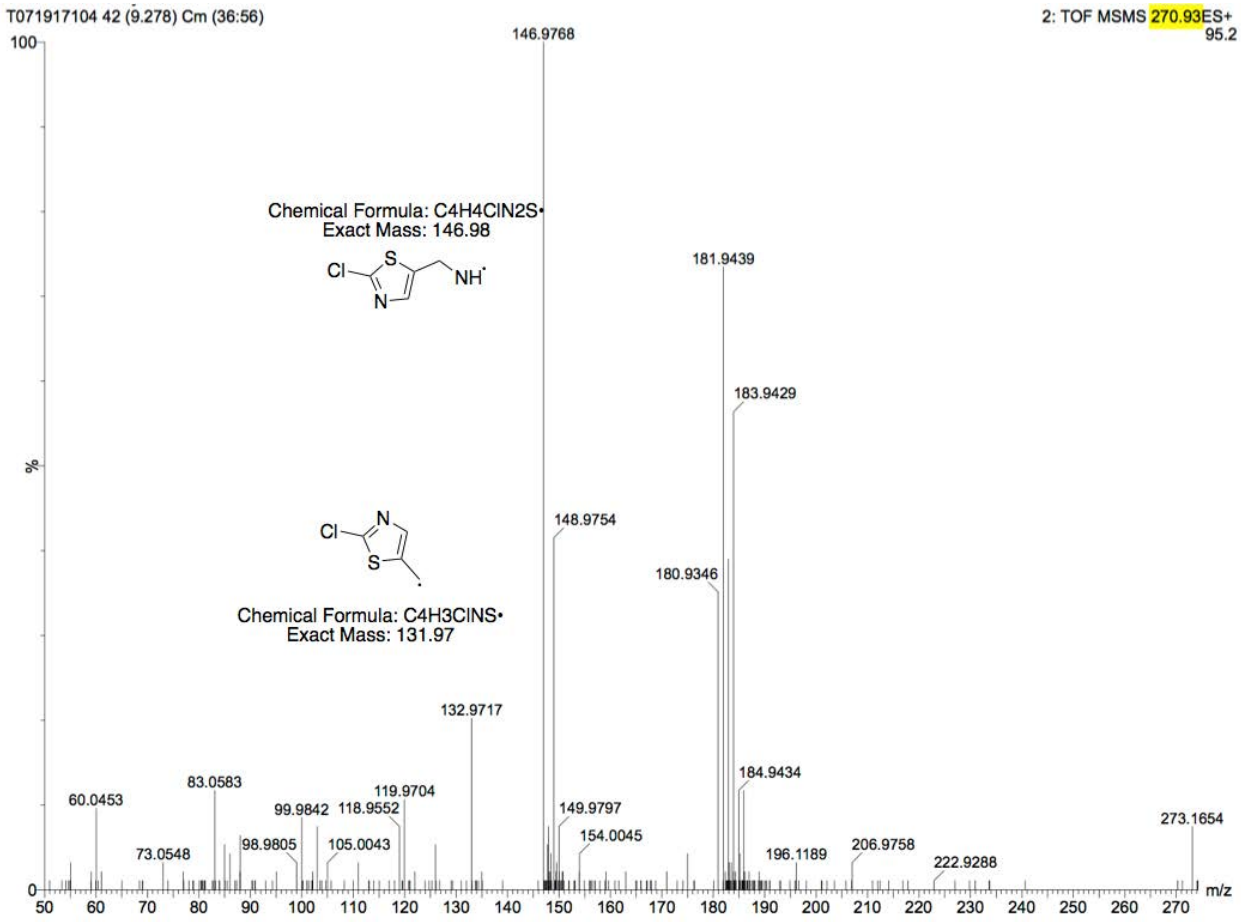


Figure A - 5: MS-MS of CLO 270 with proposed fragment structures.

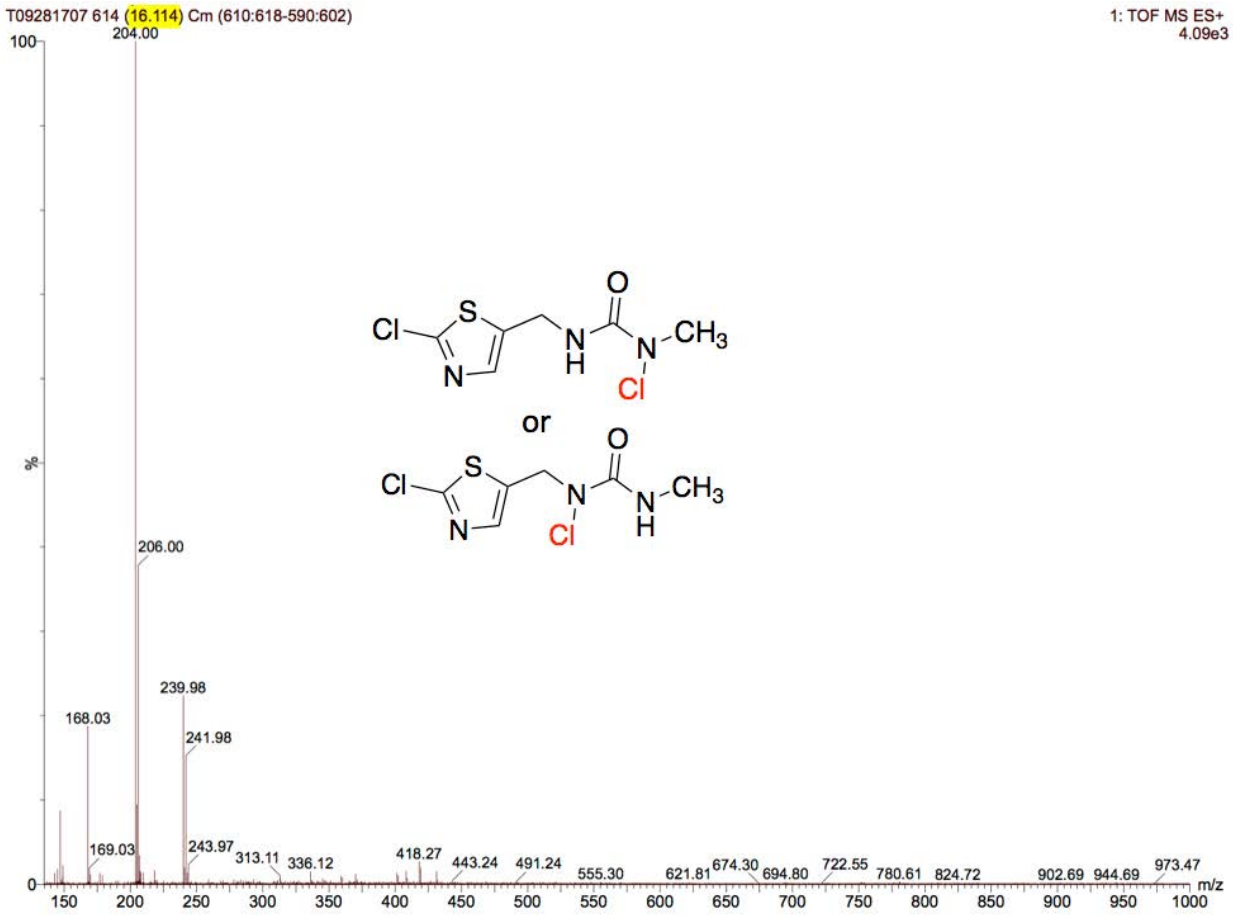


Figure A - 6: Mass spectrum and proposed structure of CLO 239a.

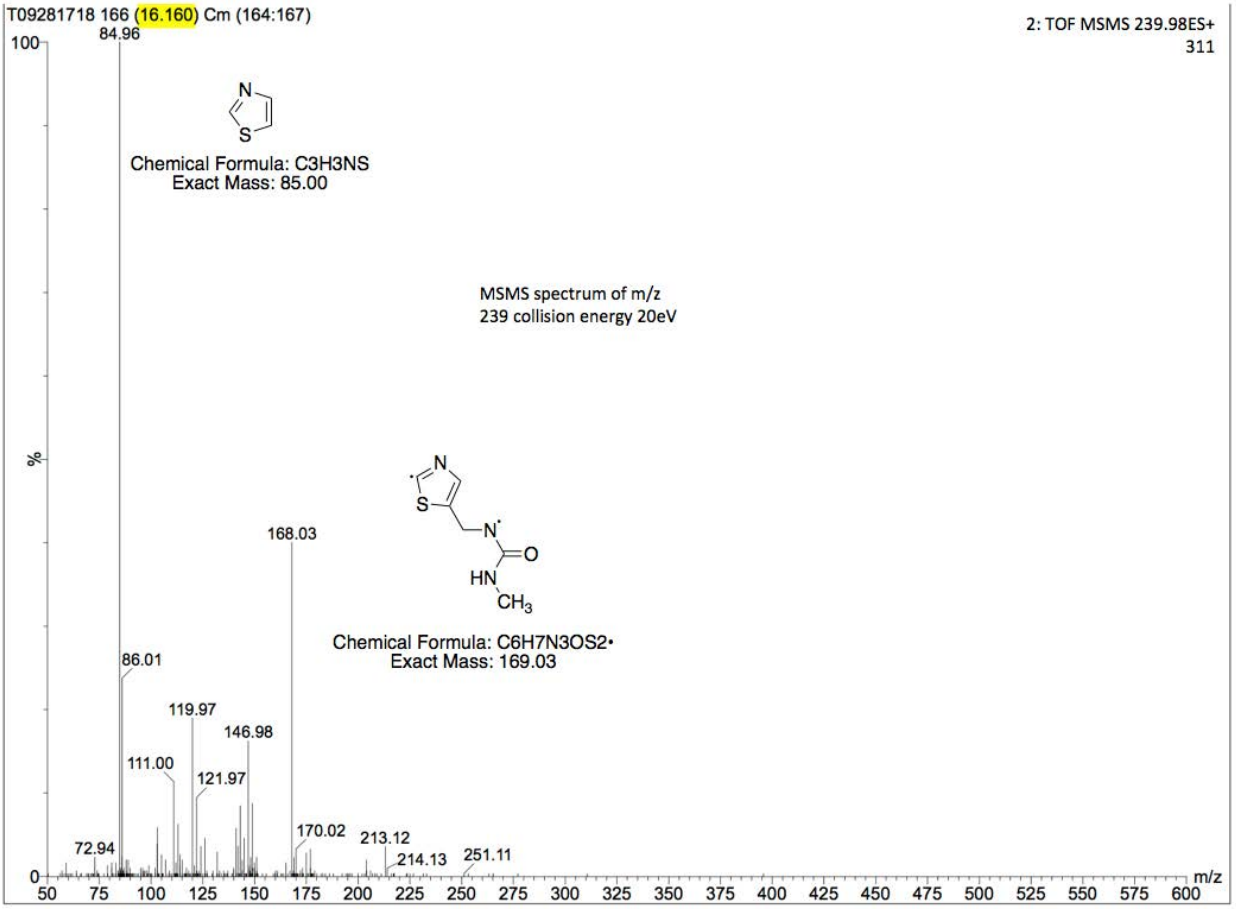


Figure A - 7: MS-MS of CLO 239a with proposed fragment structures.

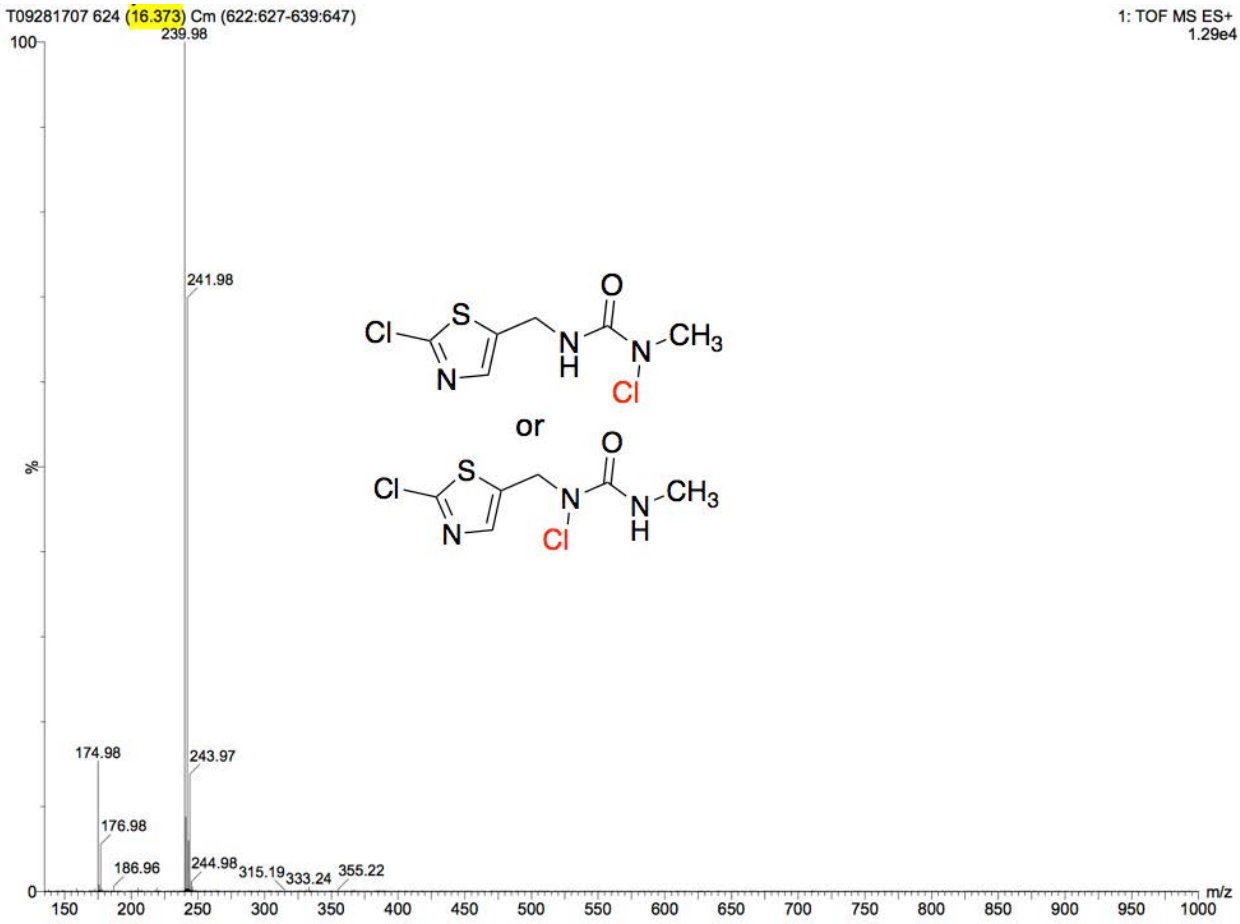


Figure A - 8: Mass spectrum and proposed structure of CLO 239b.

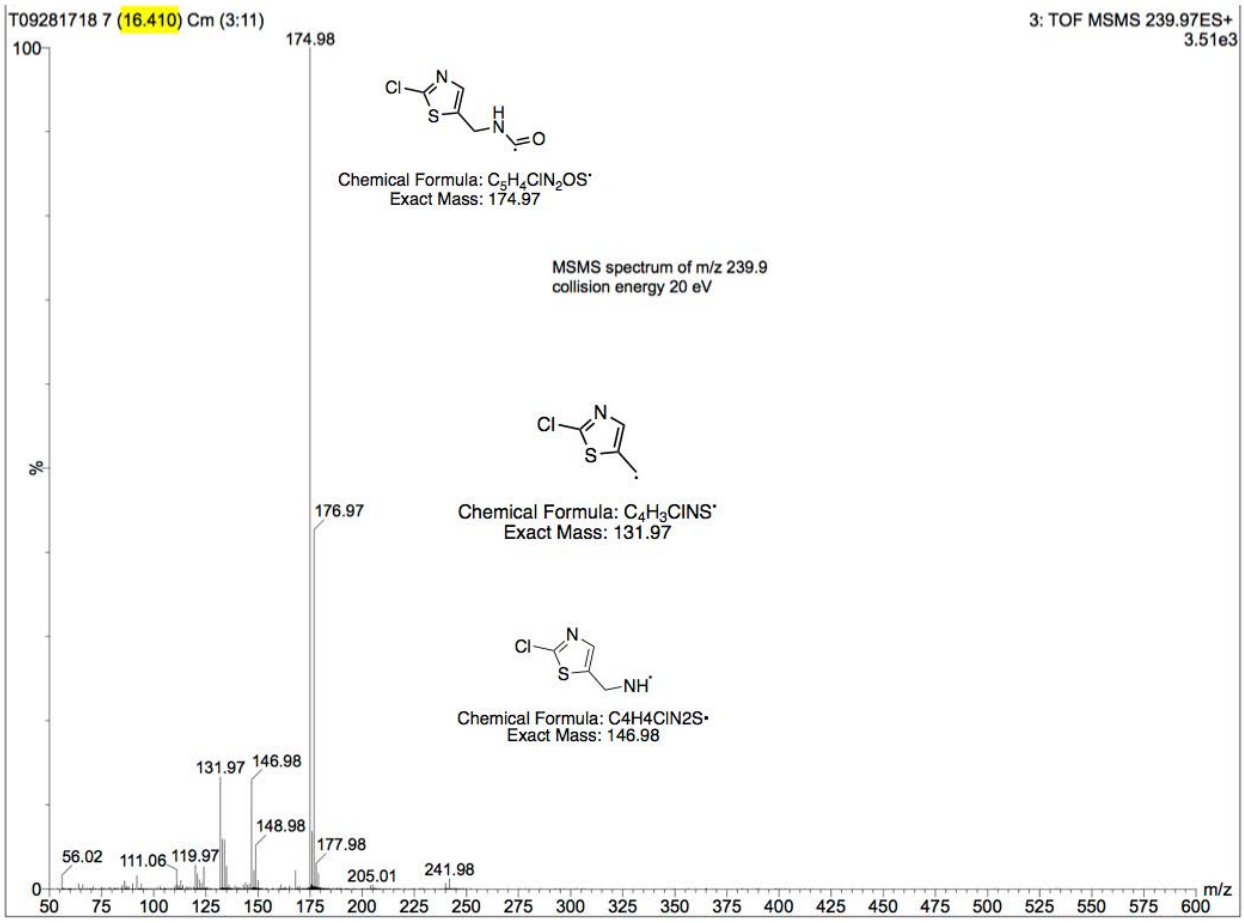


Figure A - 9: MS-MS of CLO 239b with proposed fragment structures.

Chlorination of Desnitro-imidacloprid. 10 mg/L of chlorine were added to 25 uM desnitro-imidacloprid. Samples were run on the HR-MS.

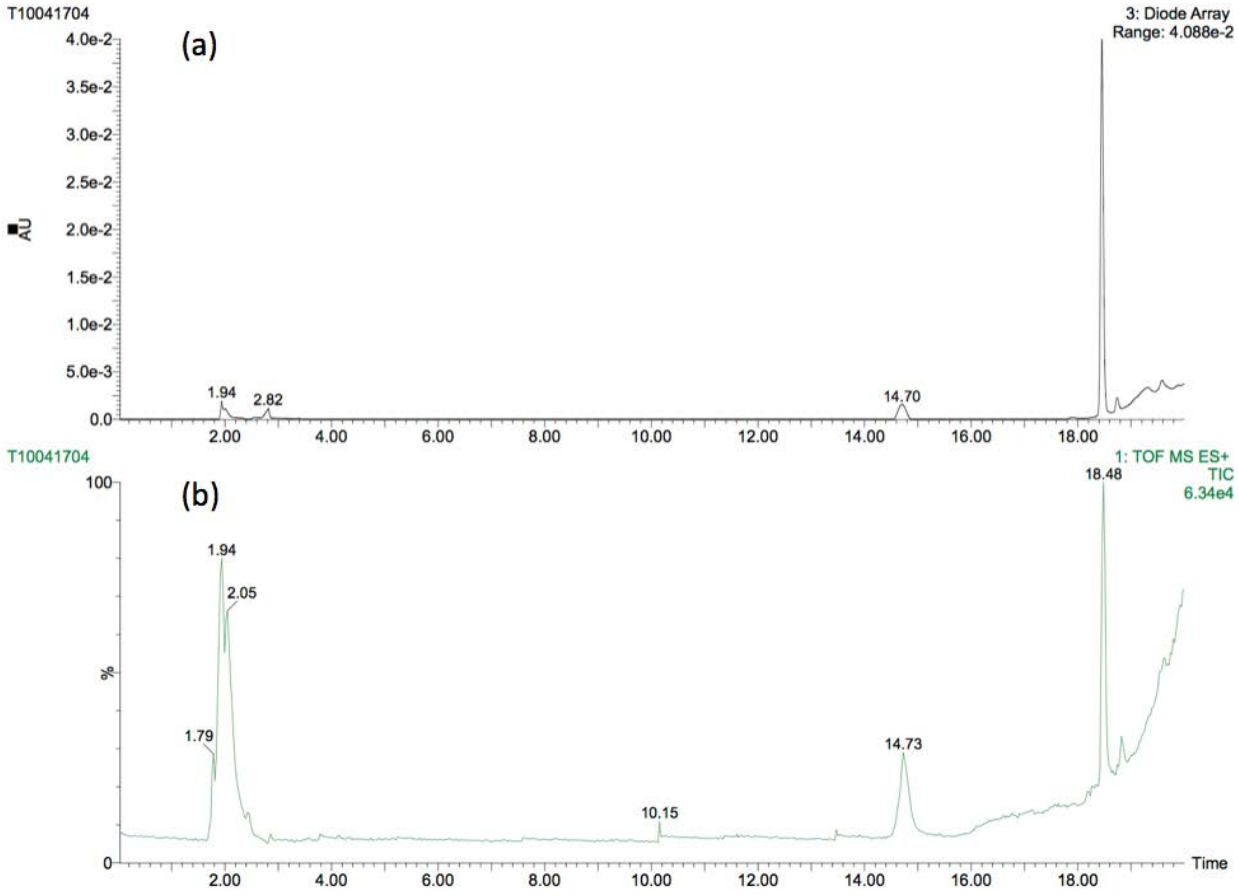


Figure A - 10: (a) UV scan and (b) TIC scan of the reaction of desnitro-imidacloprid with chlorine.

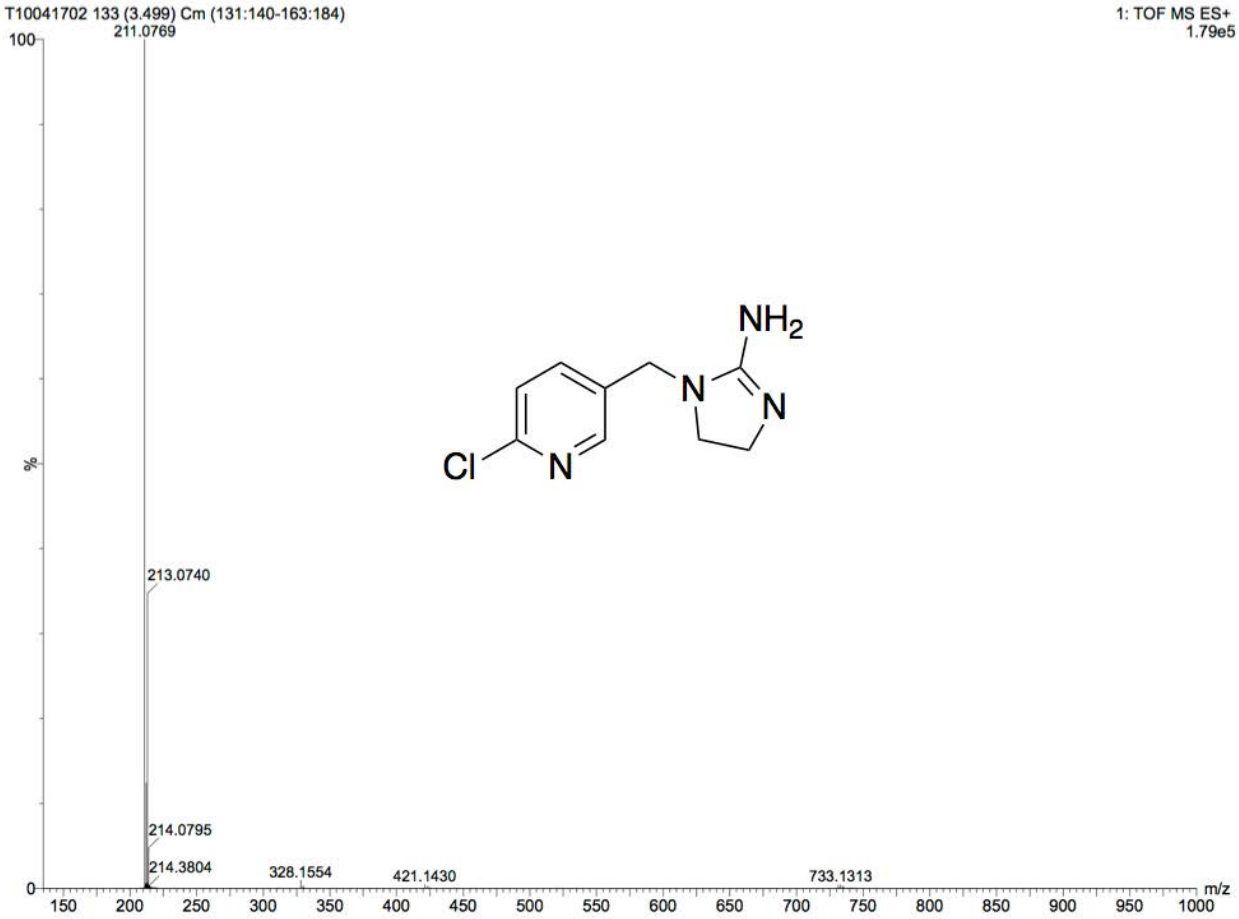


Figure A - 11: Mass spectrum and structure of desnitro-imidacloprid.

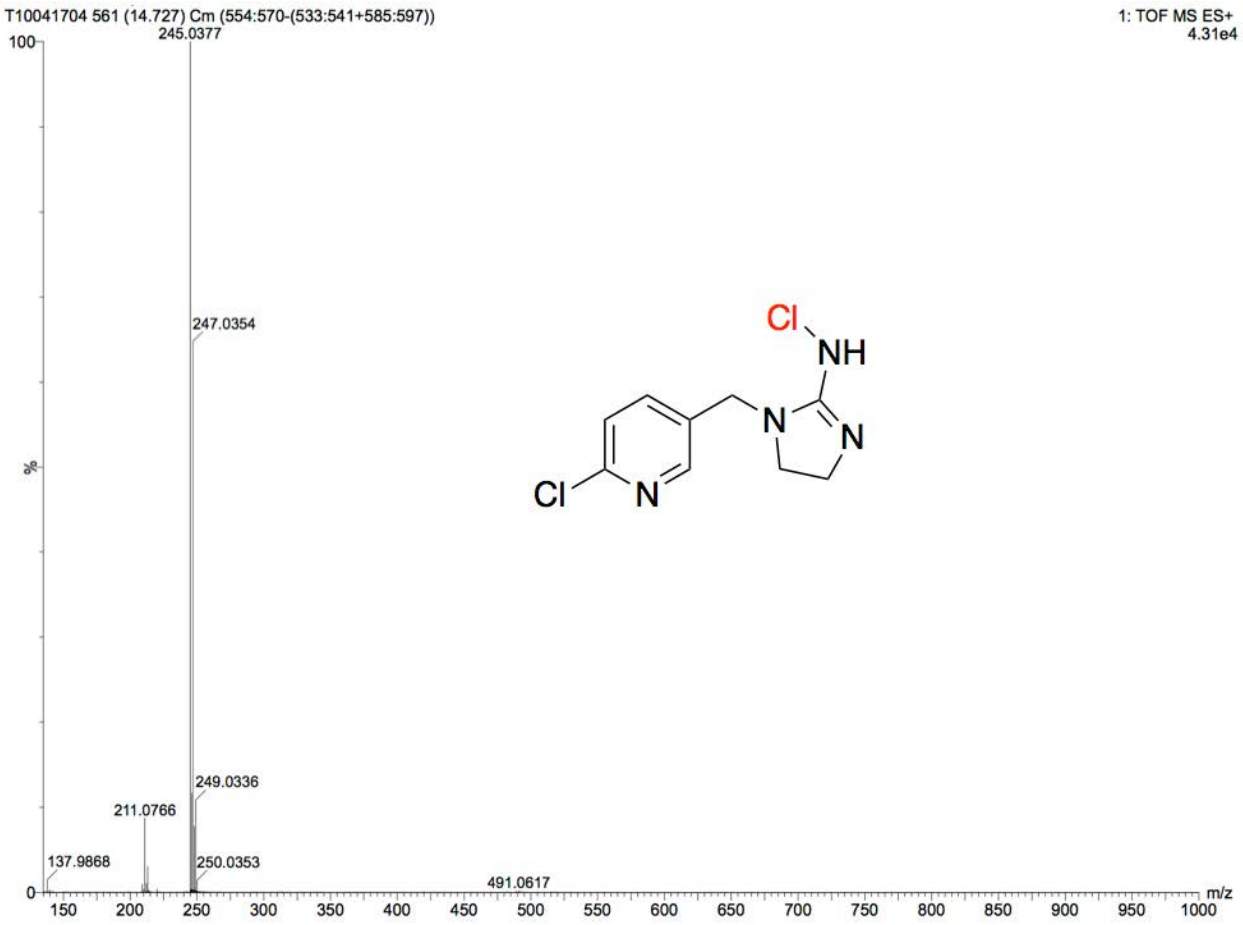


Figure A - 12: Mass spectrum and proposed structure of DN-IMI 245.

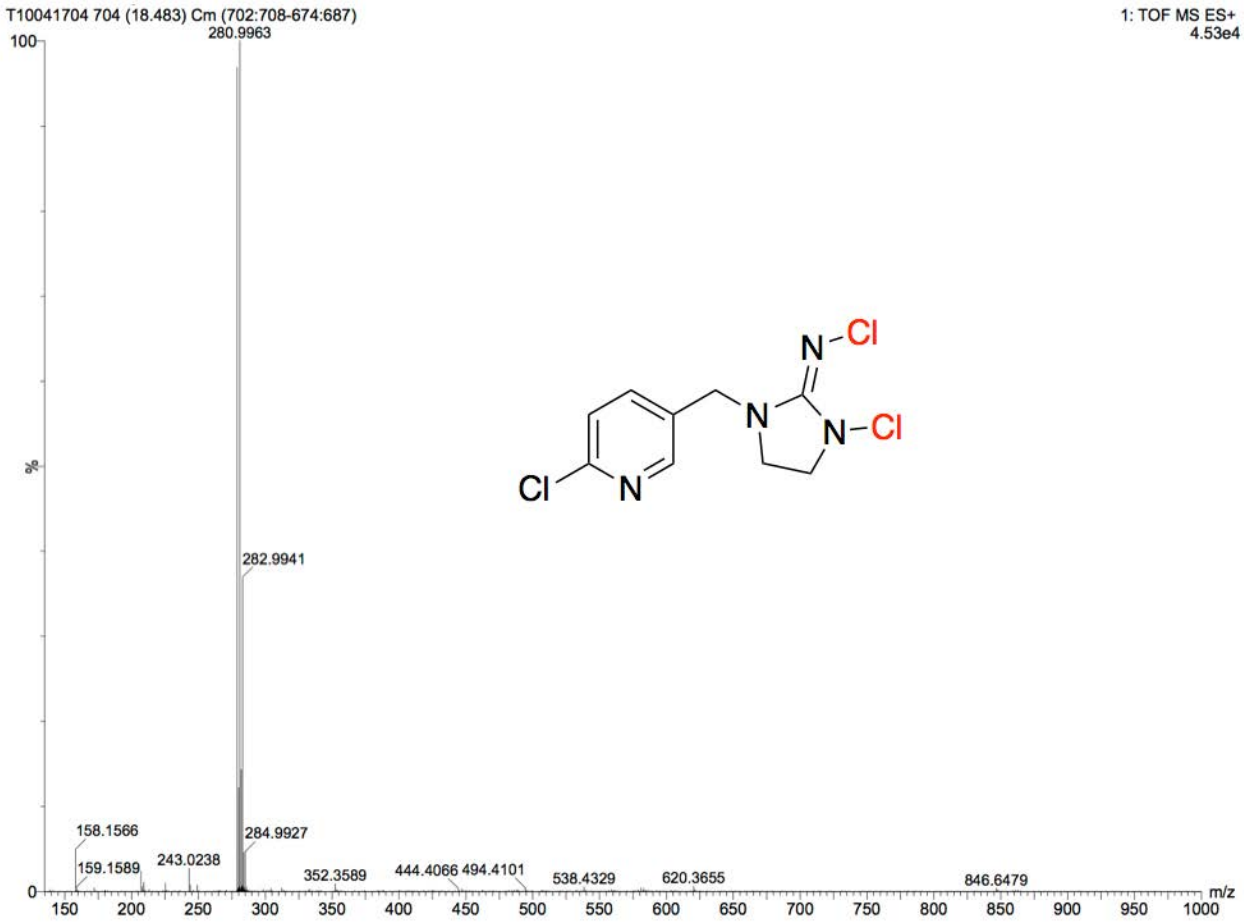


Figure A - 13: Mass spectrum and proposed structure of DN-IMI 279.

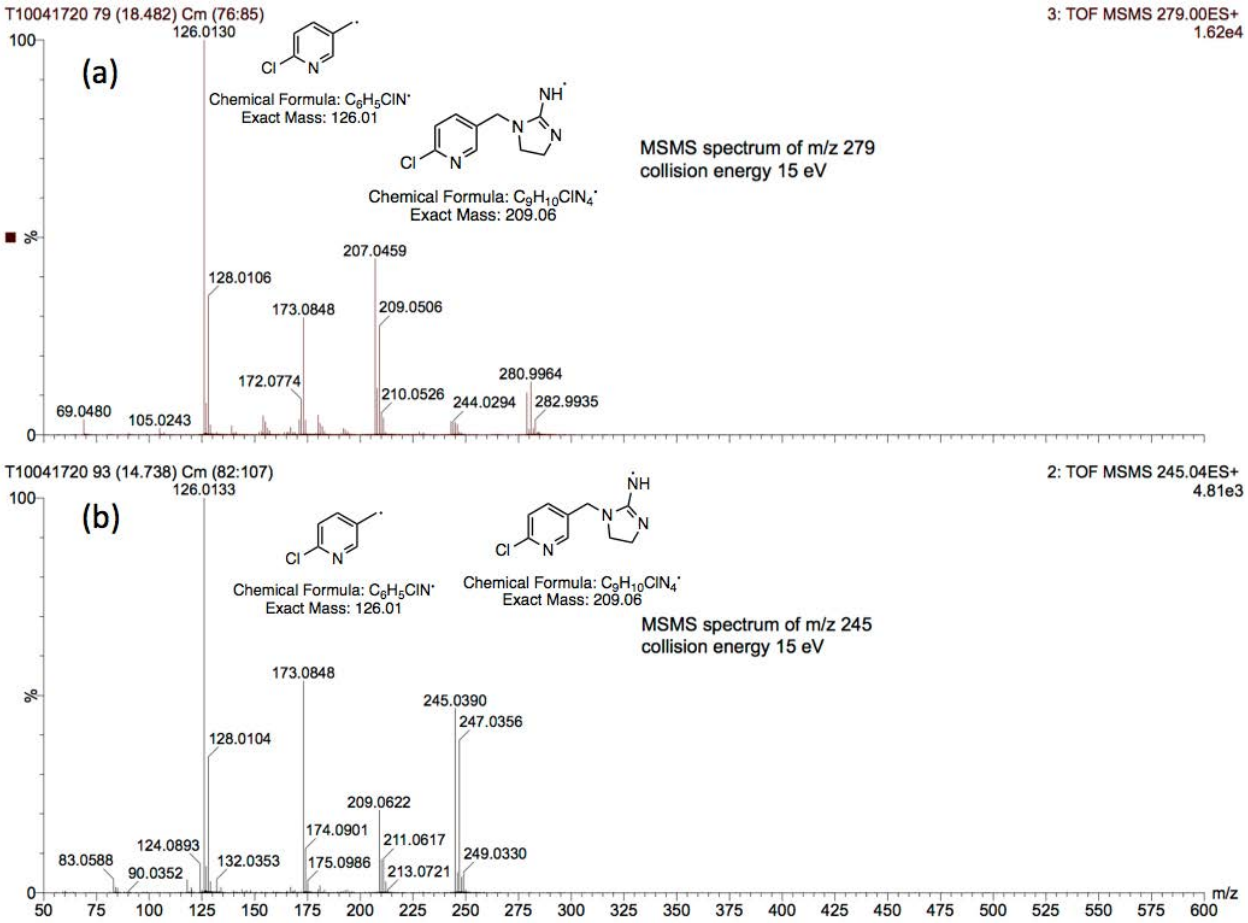


Figure A - 14: (a) MS-MS of DN-IMI 279 and (b) MS-MS of DN-IMI 245 with proposed fragment structures.

Chlorination of Imidacloprid. 50 mg/L Cl_2 was added to 25 uM imidacloprid. Samples were run on the HR-MS for product identification.

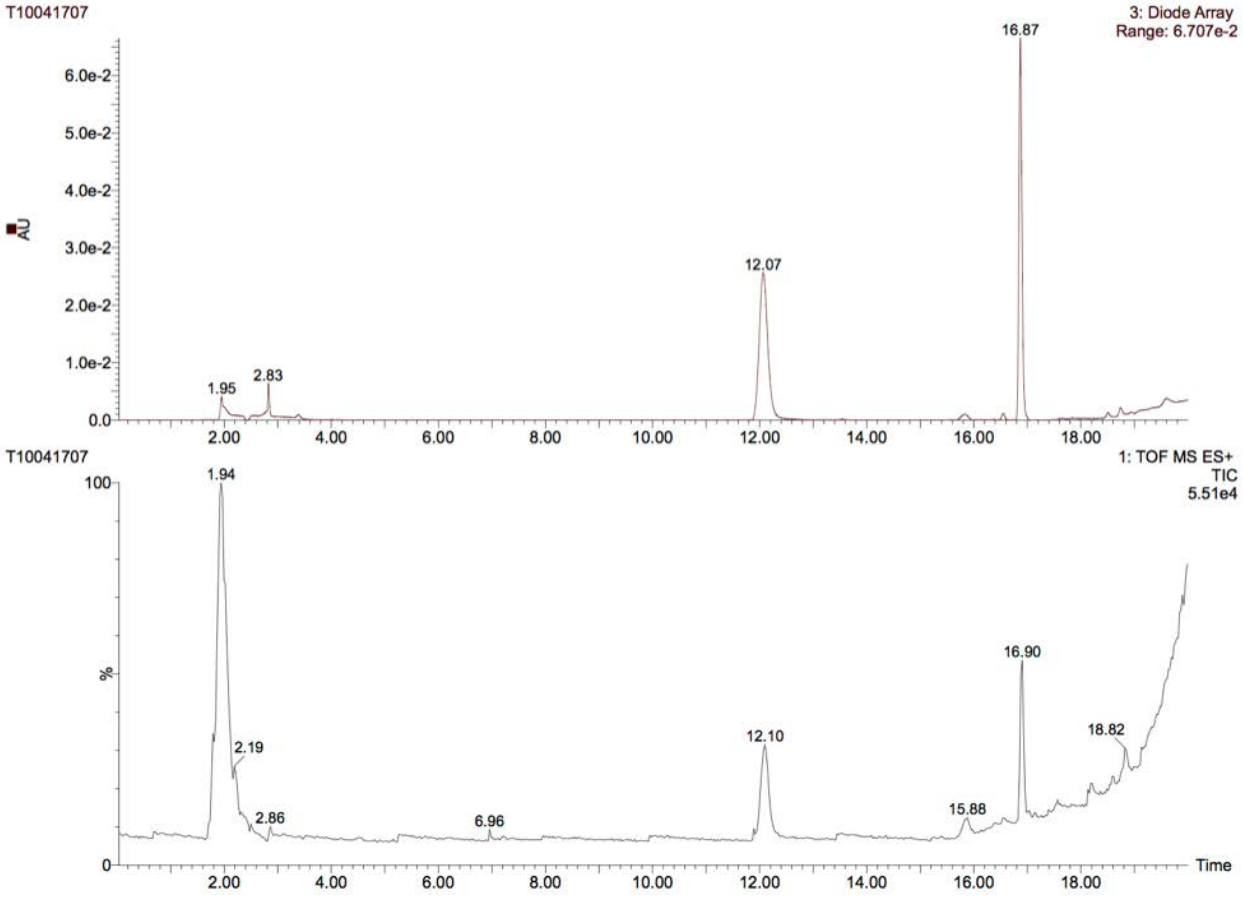


Figure A - 15: (a) UV scan (b) TIC scan of imidacloprid reaction with chlorine.

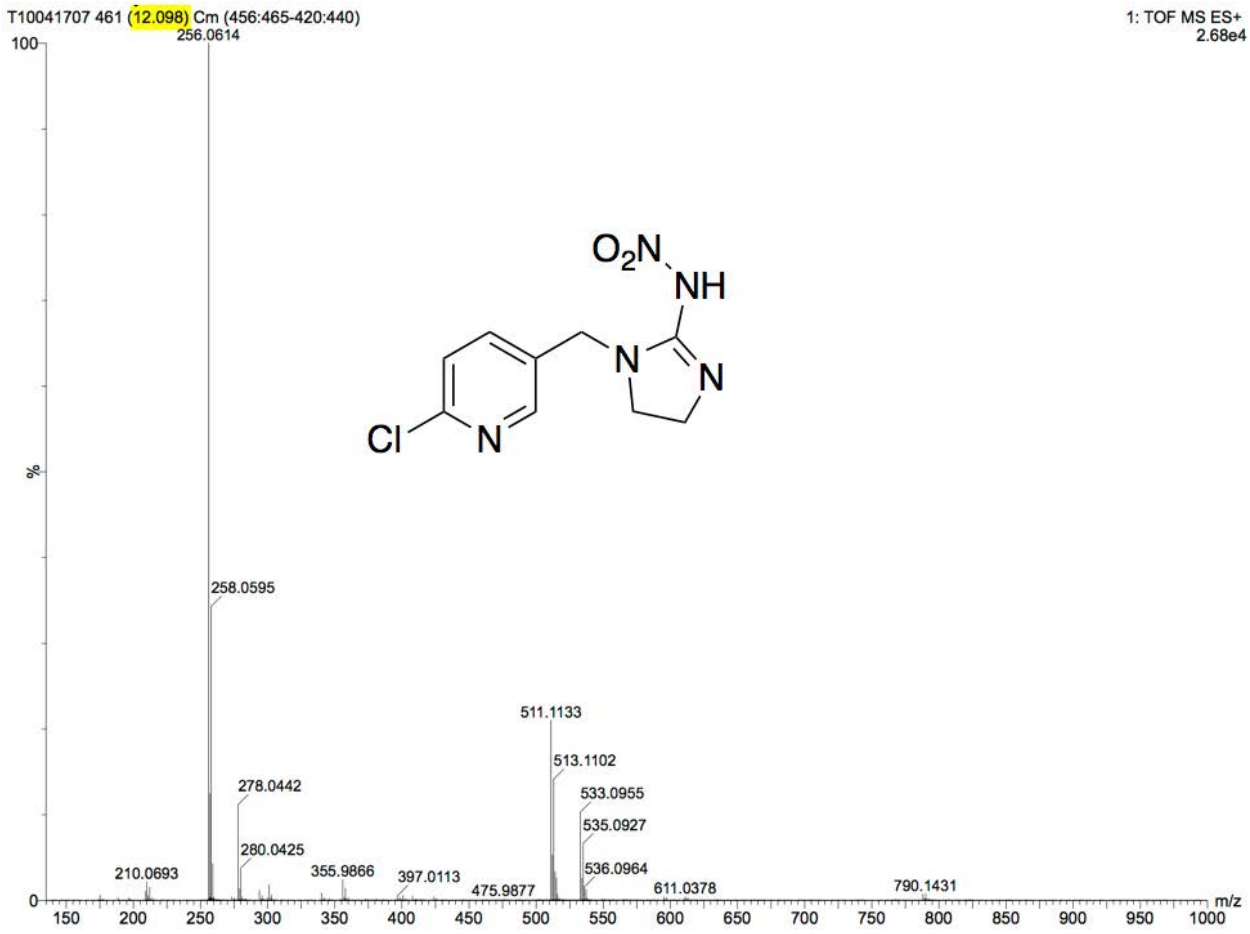


Figure A - 16: Mass spectrum and structure of imidacloprid.

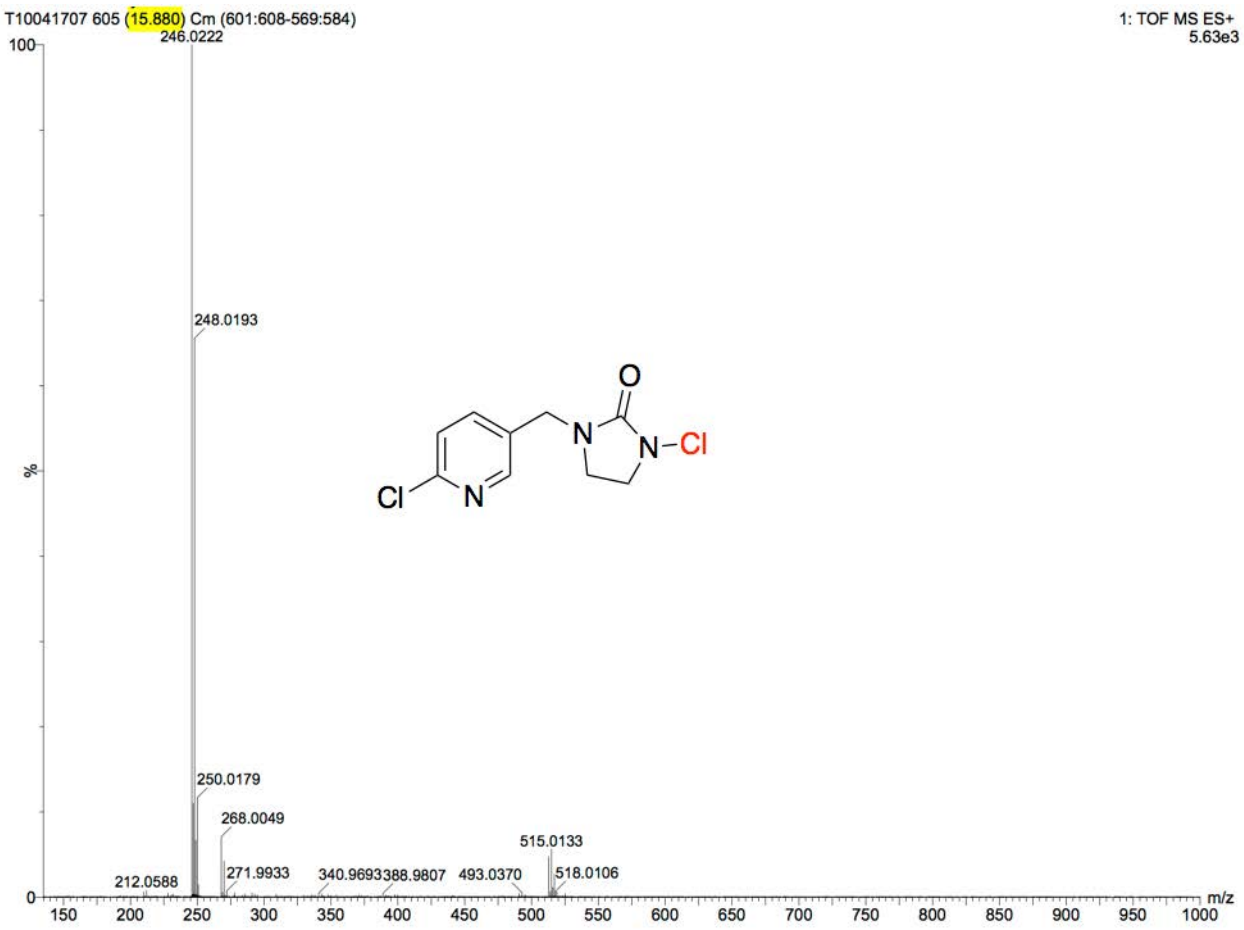


Figure A - 17: Mass spectrum and proposed structure of IMI 246.

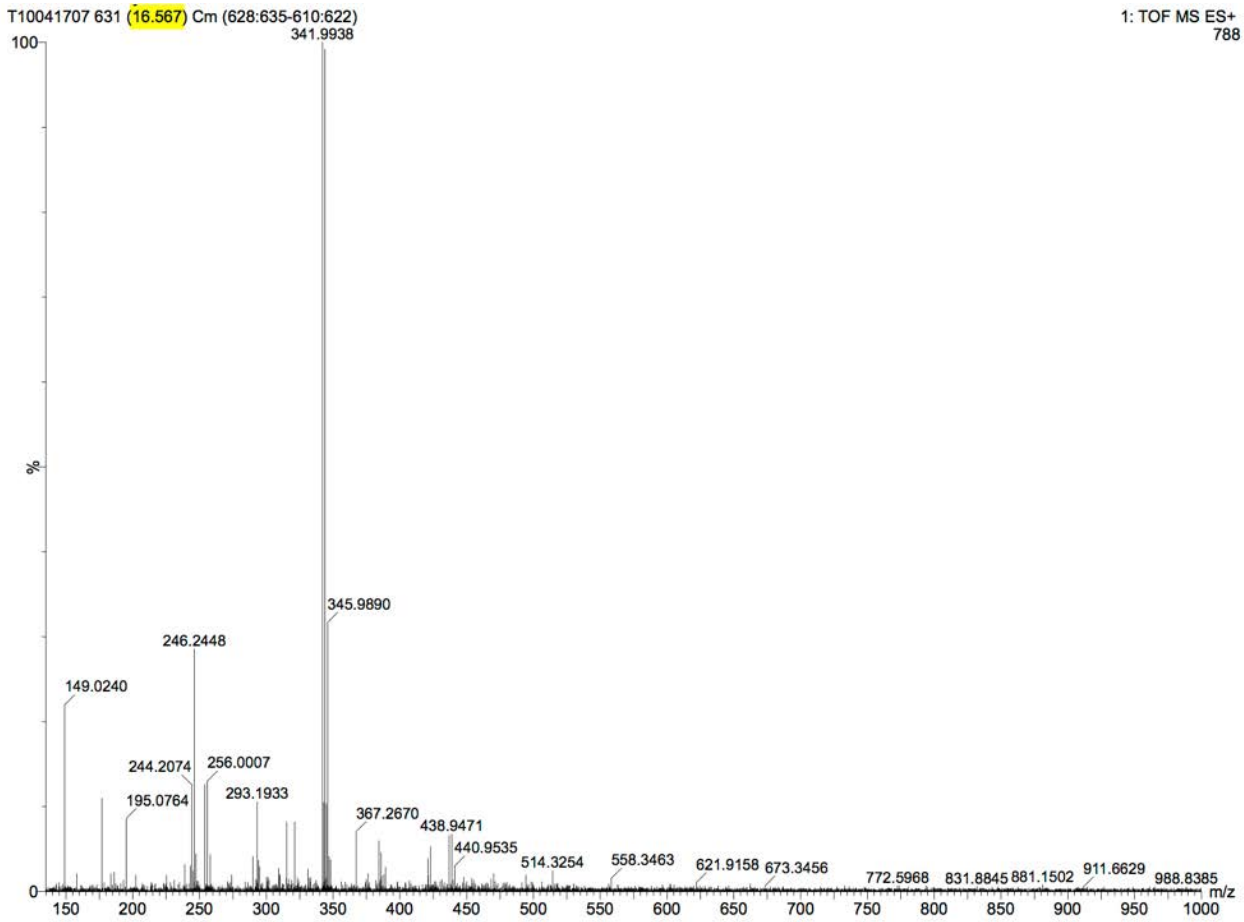


Figure A - 18: Mass spectrum of IMI 341.

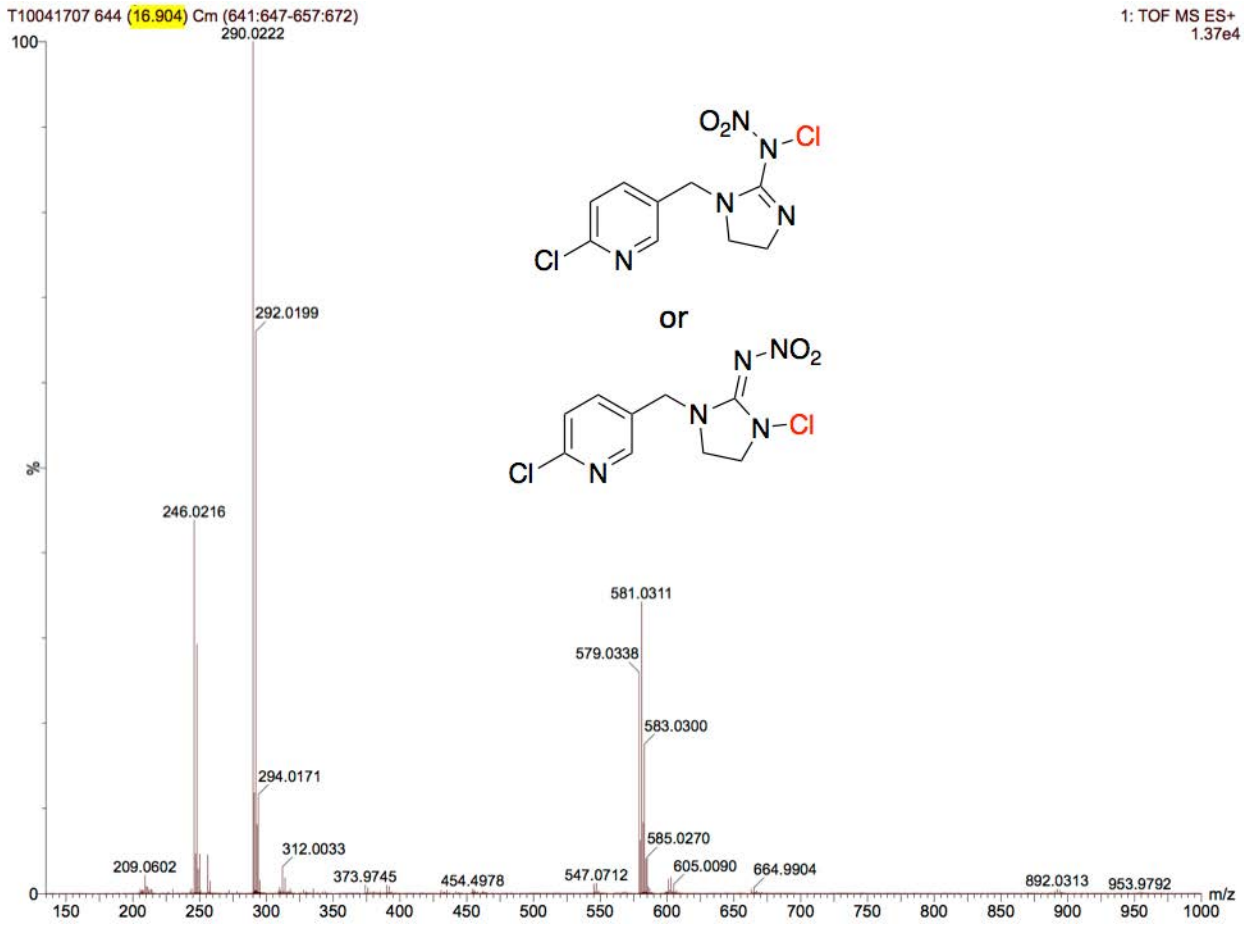


Figure A - 19: Mass spectrum and proposed structure of IMI 290.

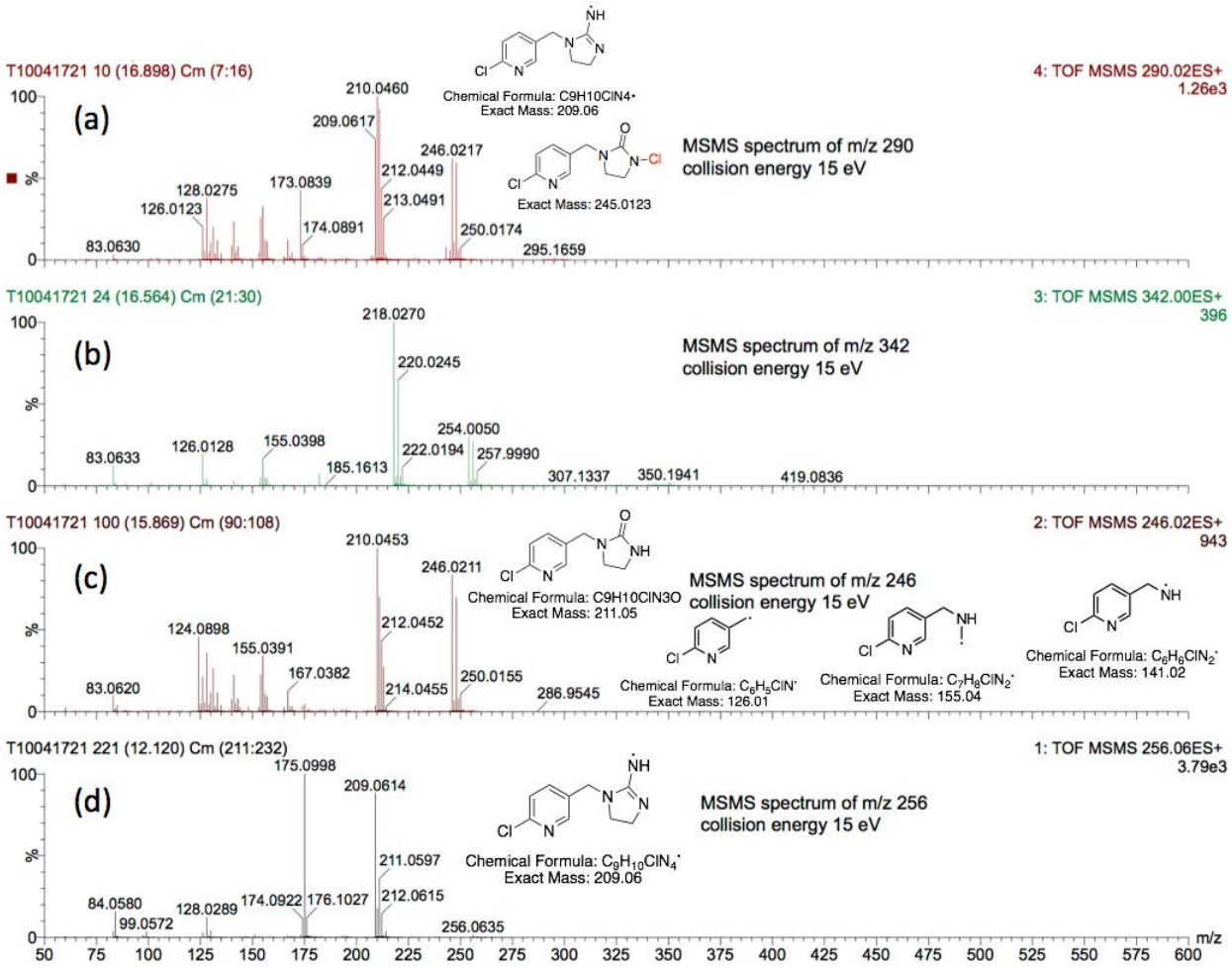


Figure A - 20: (a) MS-MS of IMI 290, (b) MS-MS of IMI 341, (c) MS-MS of IMI 290 and (d) MS-MS of imidacloprid with proposed fragment structures.

Sequential Hydrolysis and Chlorination of Thiamethoxam. Thiamethoxam (25 uM) was added to pH 10 phosphate buffer and allowed to react for at least 48 hours. Samples were run on the HR-MS to identify hydrolysis products. Then 50 mg/L of chlorine was added to the mixture. Samples were run on the HR-MS to identify chlorination products.

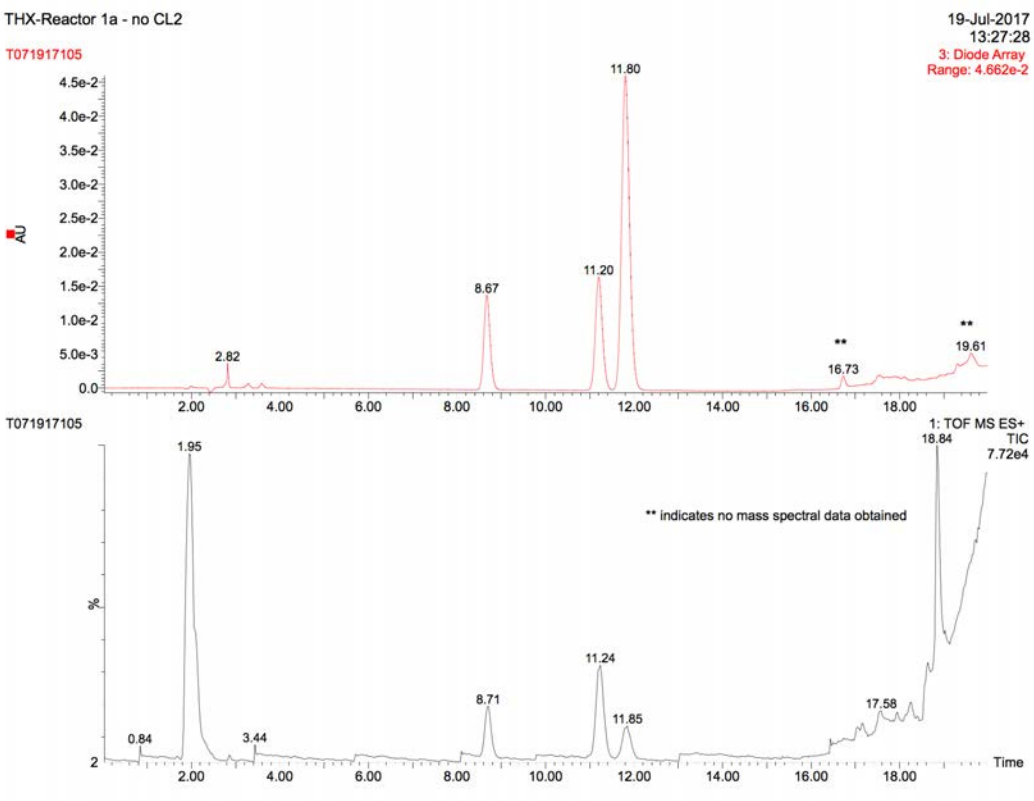


Figure A - 21: (a) UV scan and (b) TIC scan of thiamethoxam hydrolysis (no chlorine).

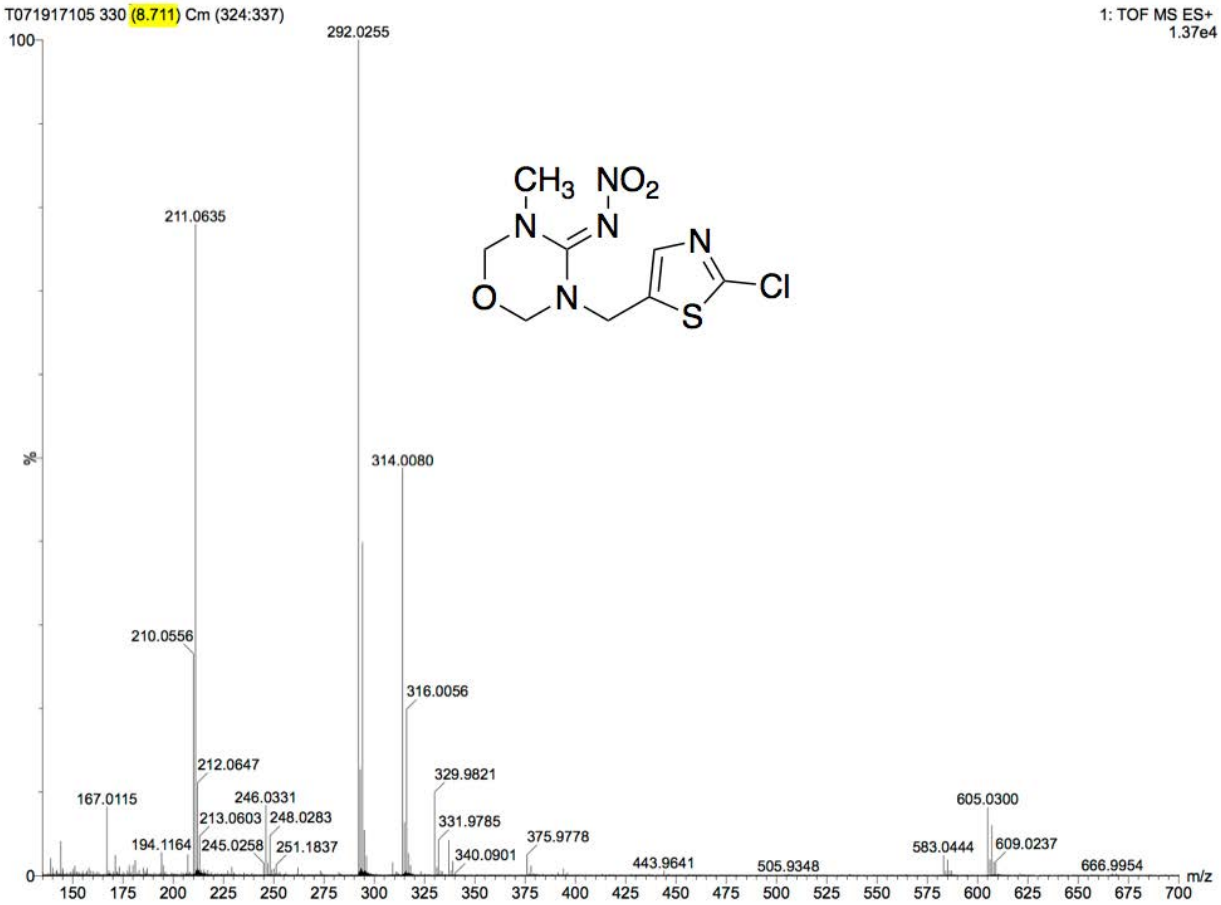


Figure A - 22: Mass spectrum and structure of thiamethoxam.

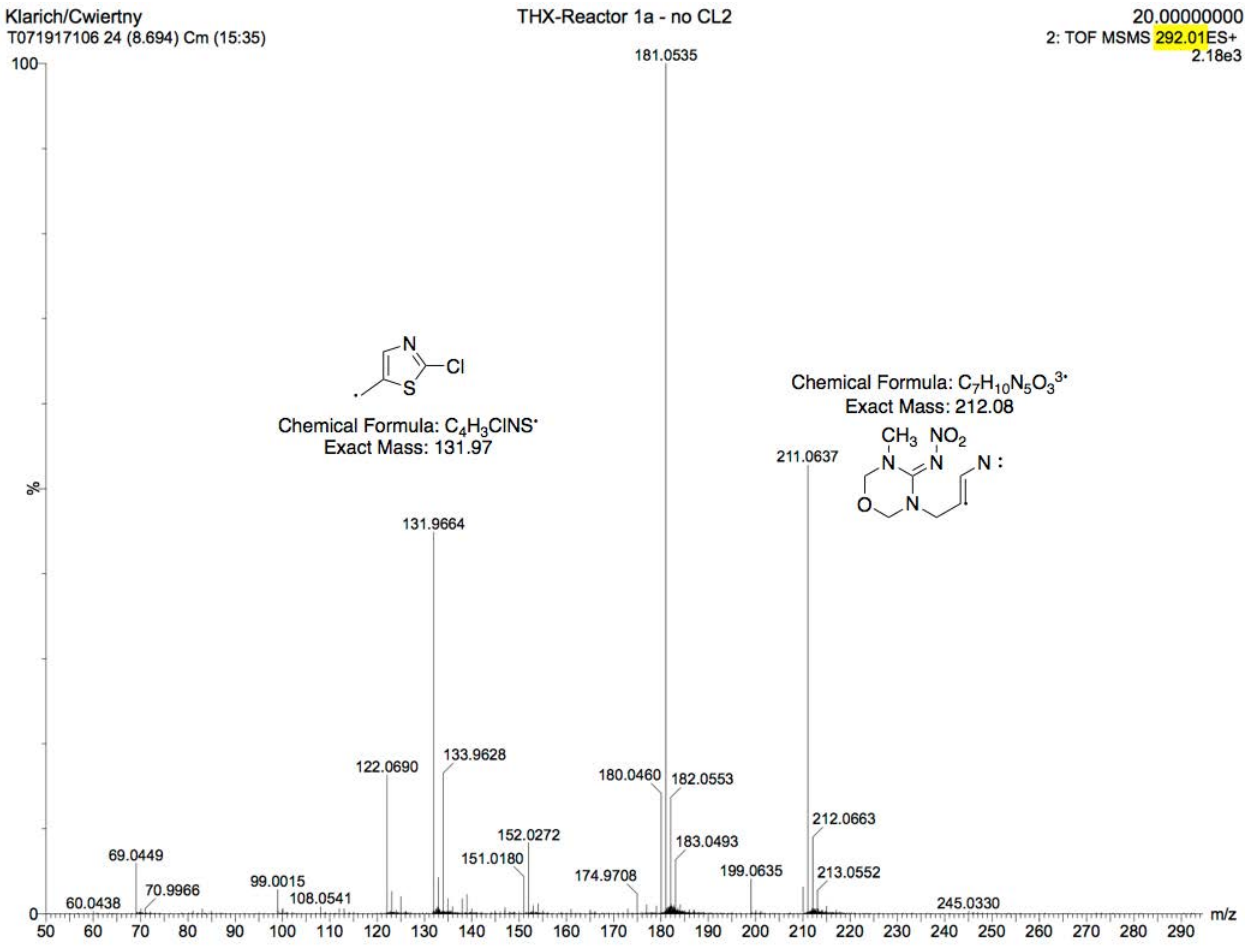


Figure A - 23: MS-MS of thiamethoxam with proposed fragment structures.

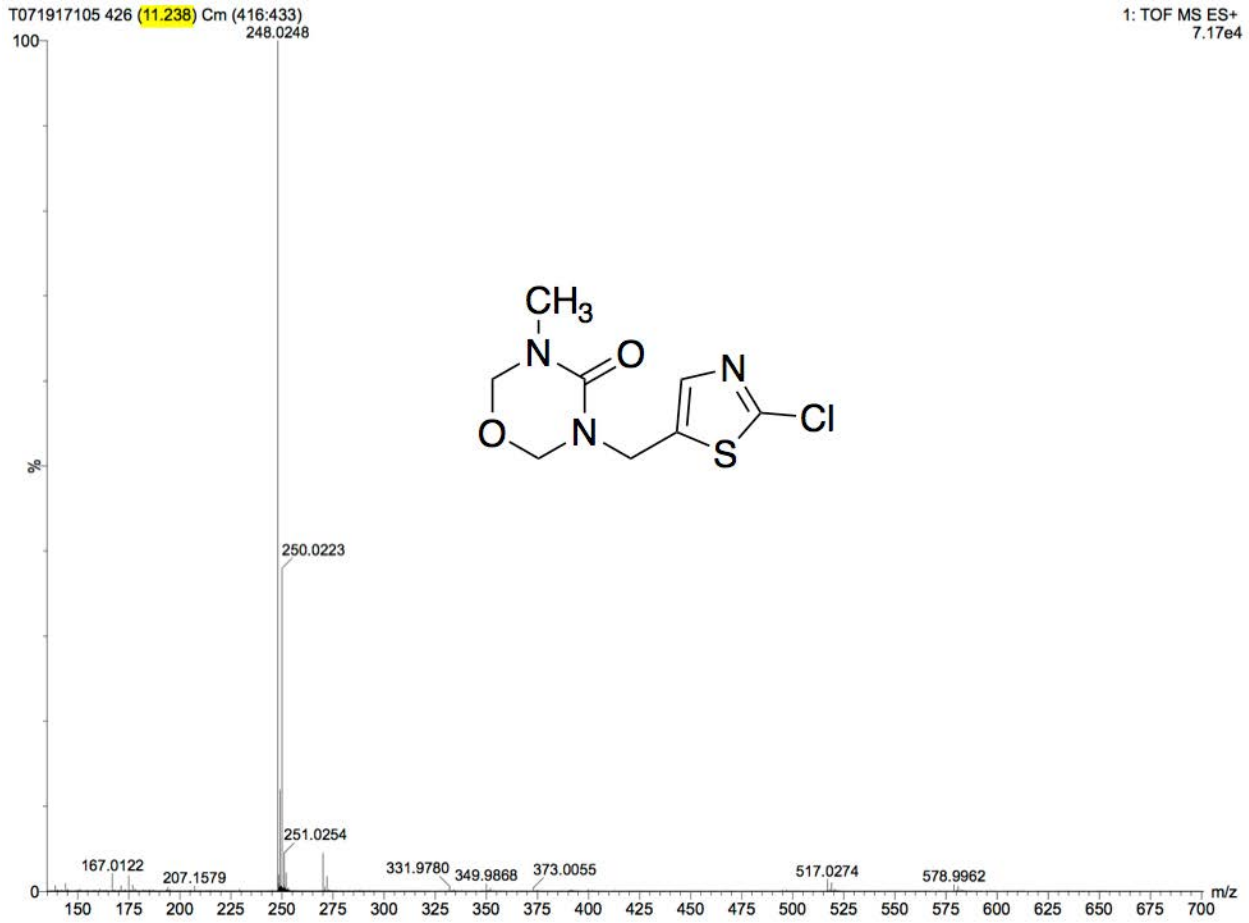


Figure A - 24: Mass spectrum and structure of THX 248.

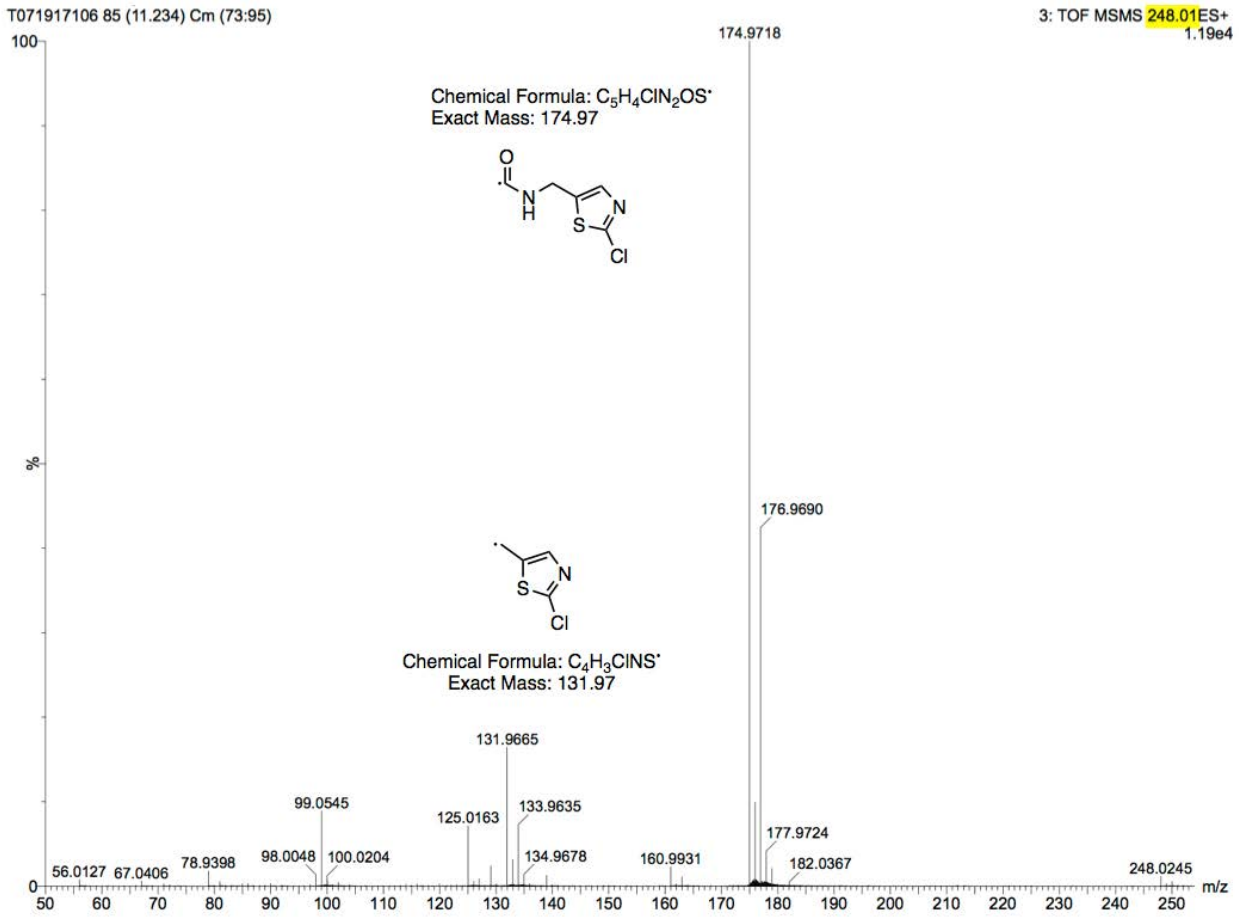


Figure A - 25: MS-MS of THX 248 with proposed fragment structures.

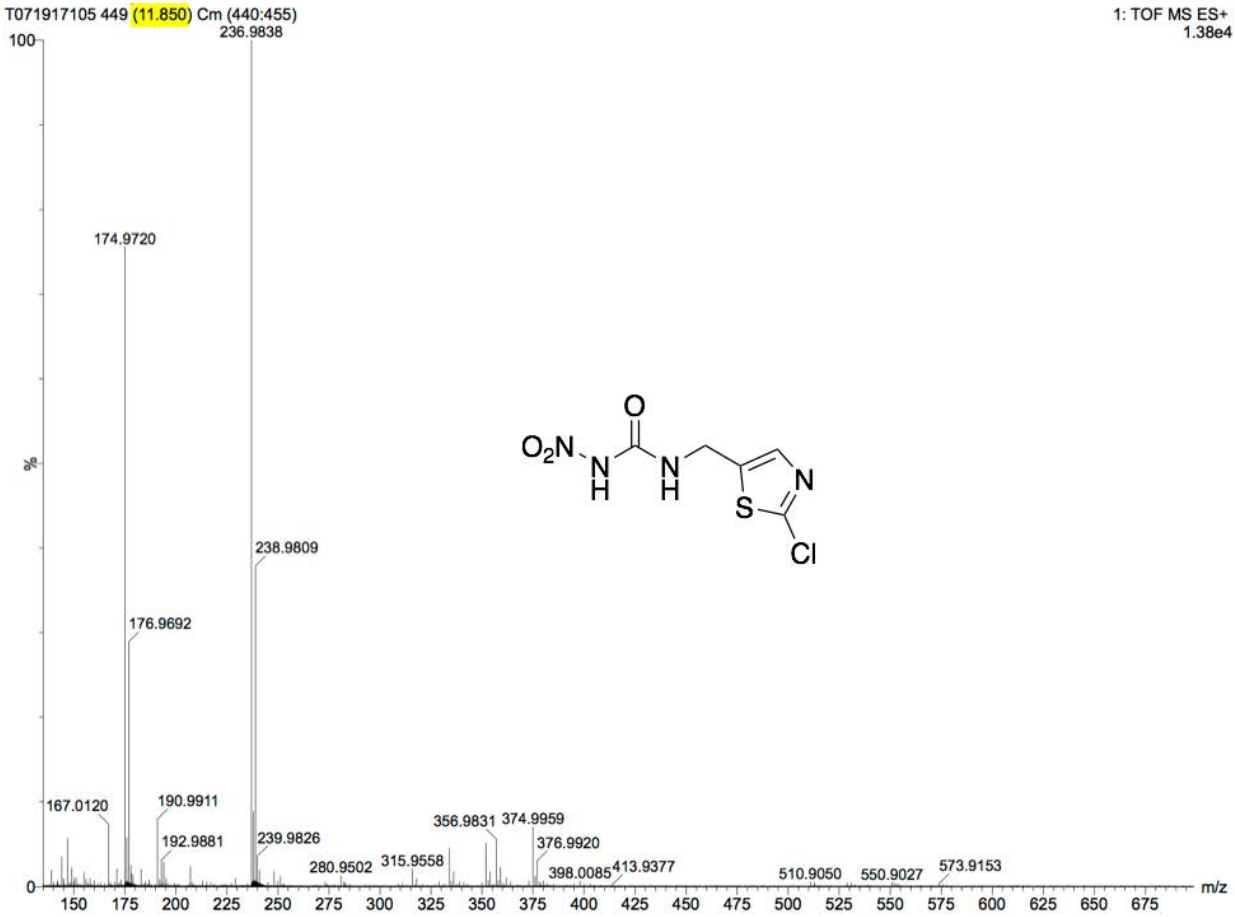


Figure A - 26: Mass spectrum and proposed structure of TXH 237.

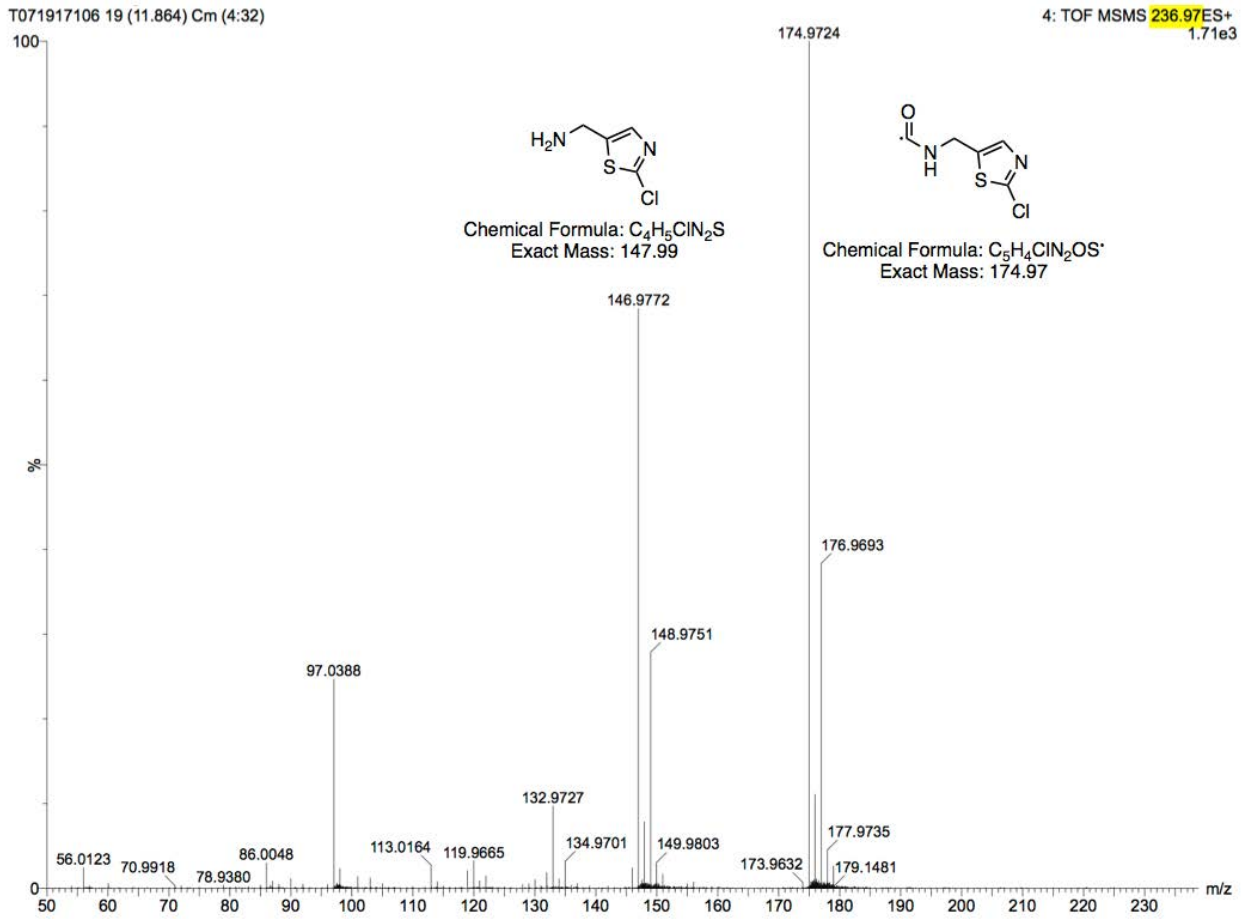


Figure A - 27: MS-MS of THX 237 with proposed fragment structures.

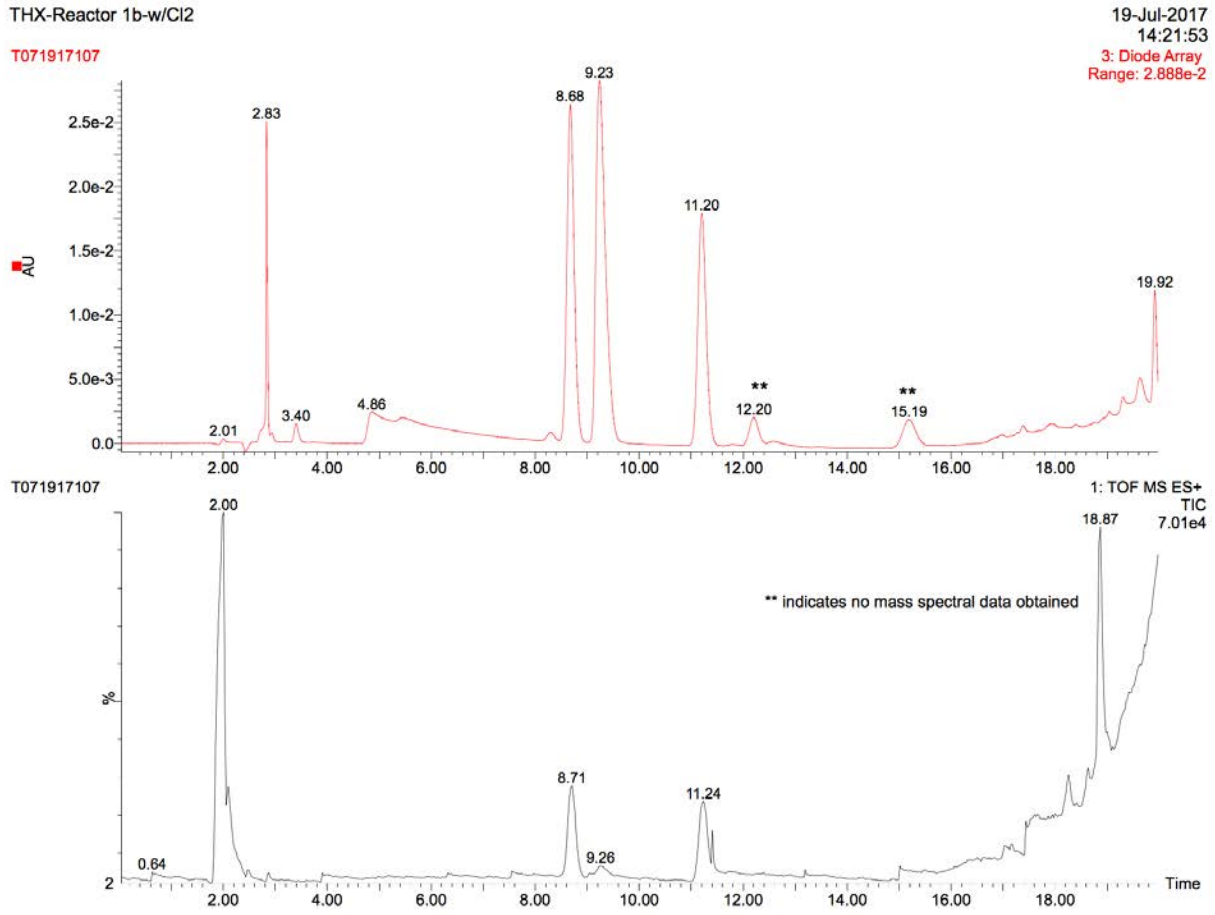


Figure A - 28: (a) UV scan (b) TIC scan of chlorination of thiamethoxam hydrolysis plus chlorination.

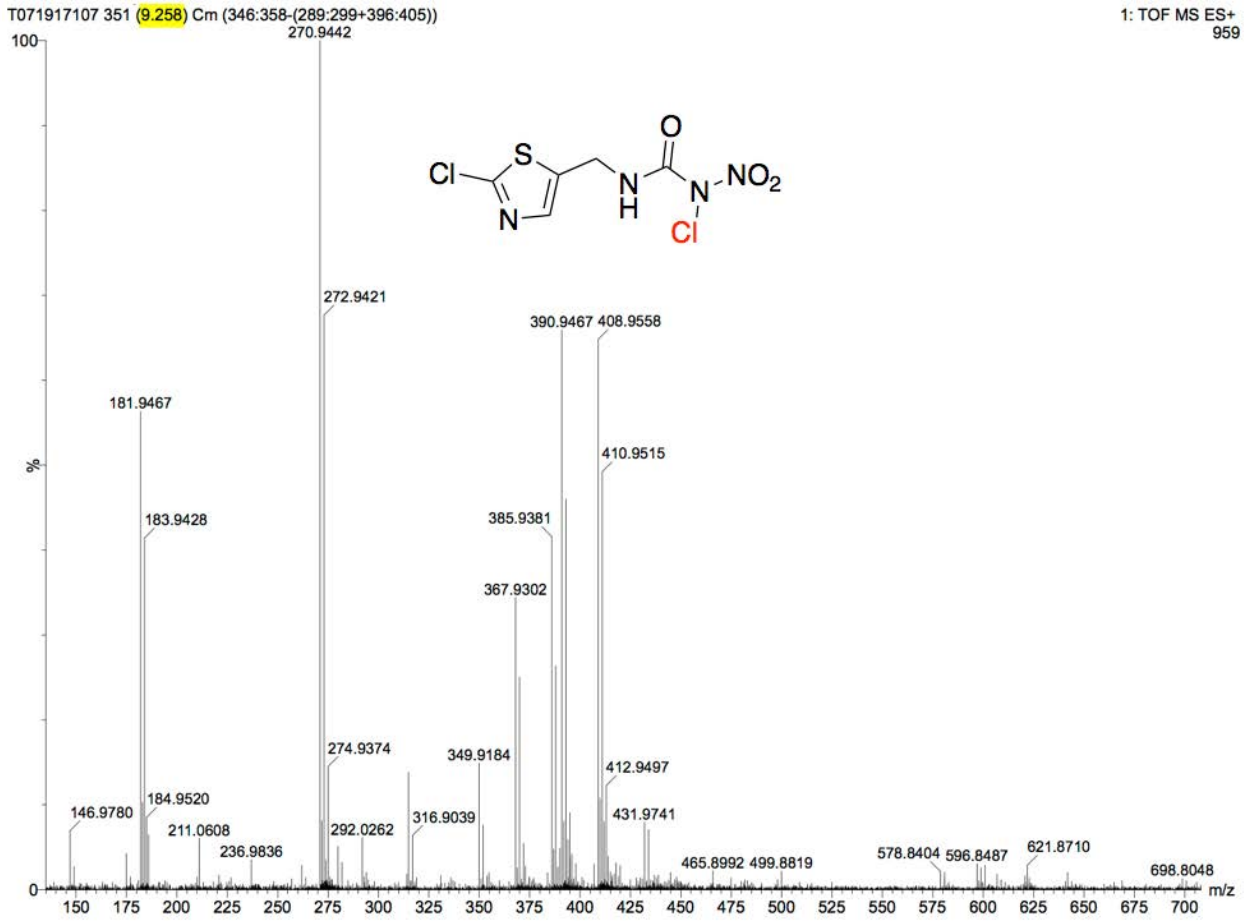


Figure A - 29: Mass spectrum and proposed structure of THX 270.

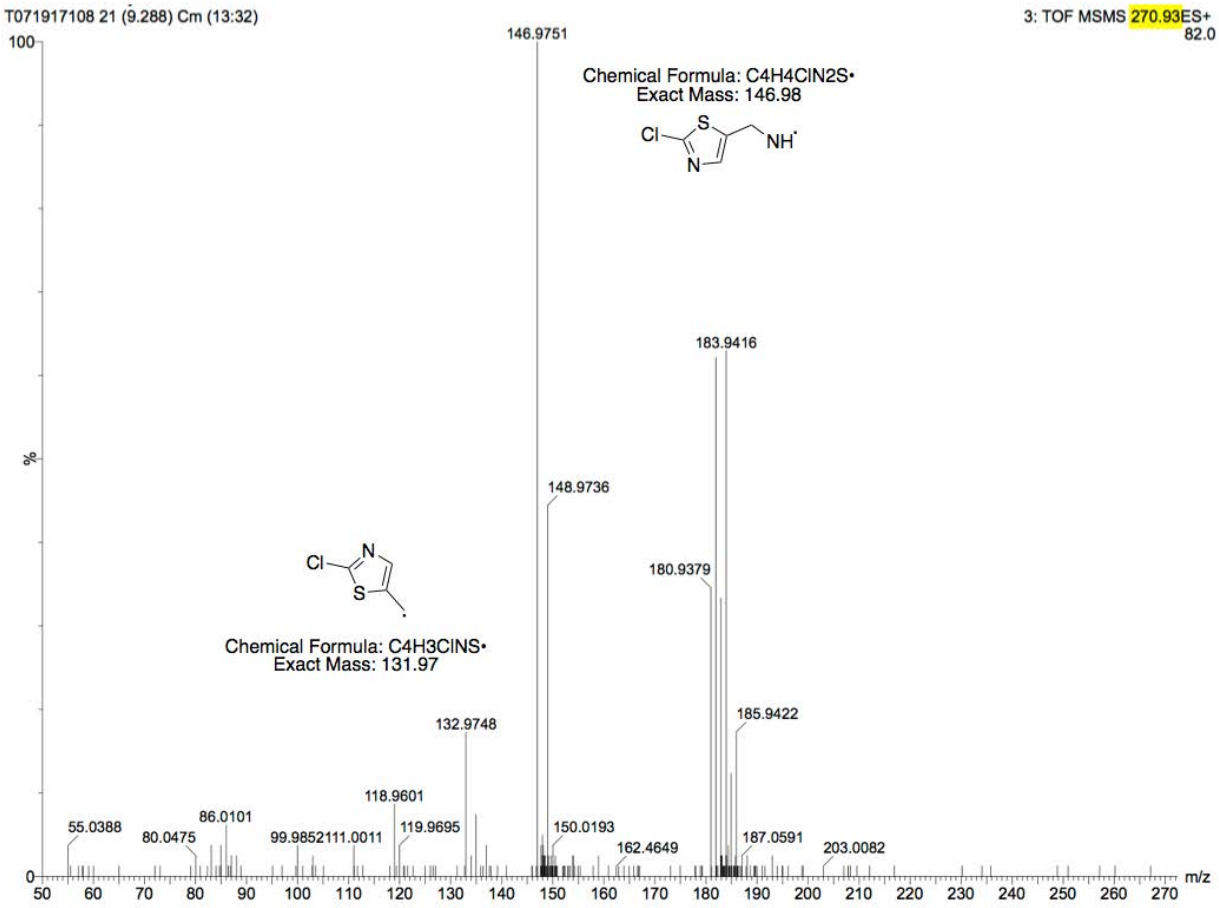


Figure A - 30: MS-MS of THX 270 with proposed fragment structures.

Chlorination of Imidacloprid-urea. 5 mg/L of chlorine were added to 10 uM imidacloprid-urea. Samples were run on the HR-MS.

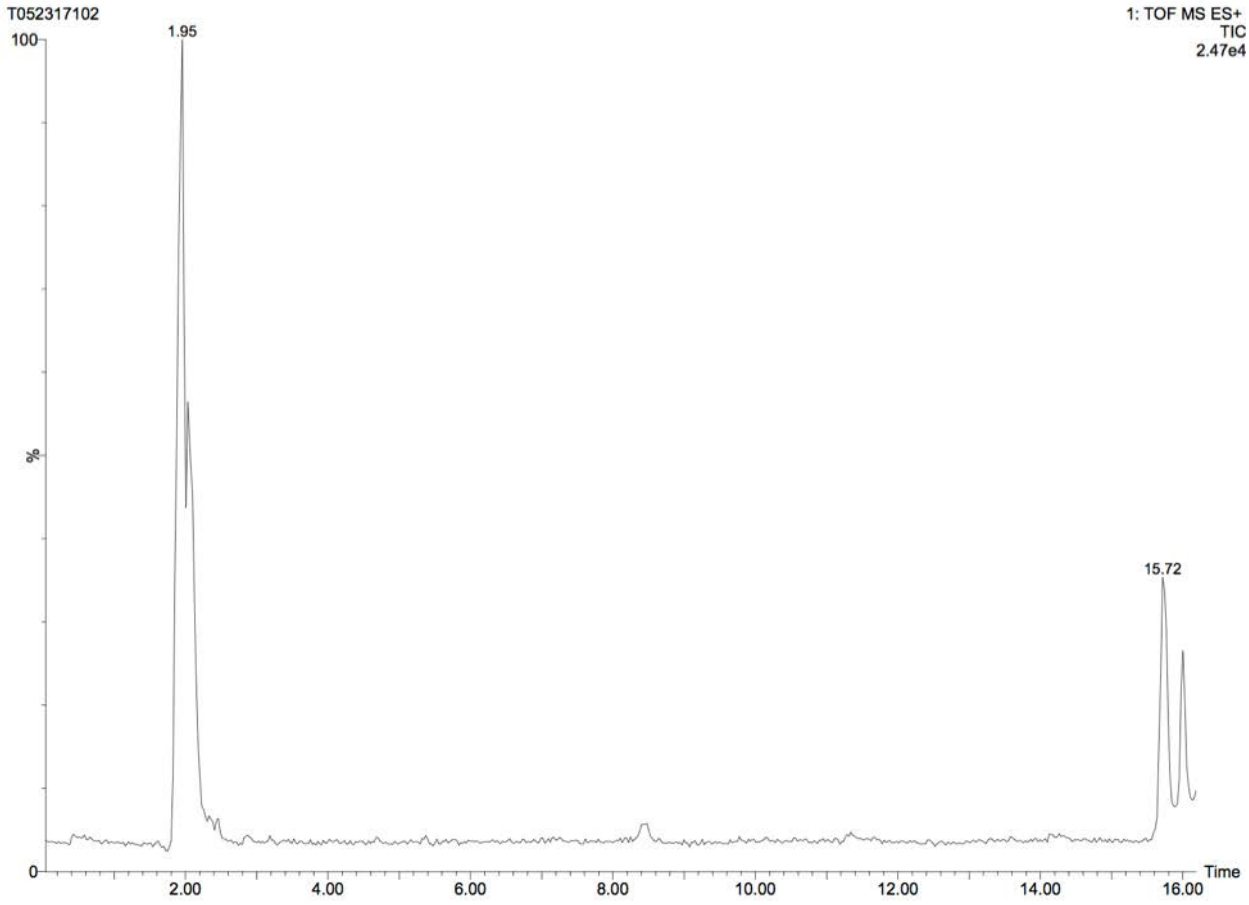


Figure A - 31: TIC scan of imidacloprid-urea reaction with chlorine.

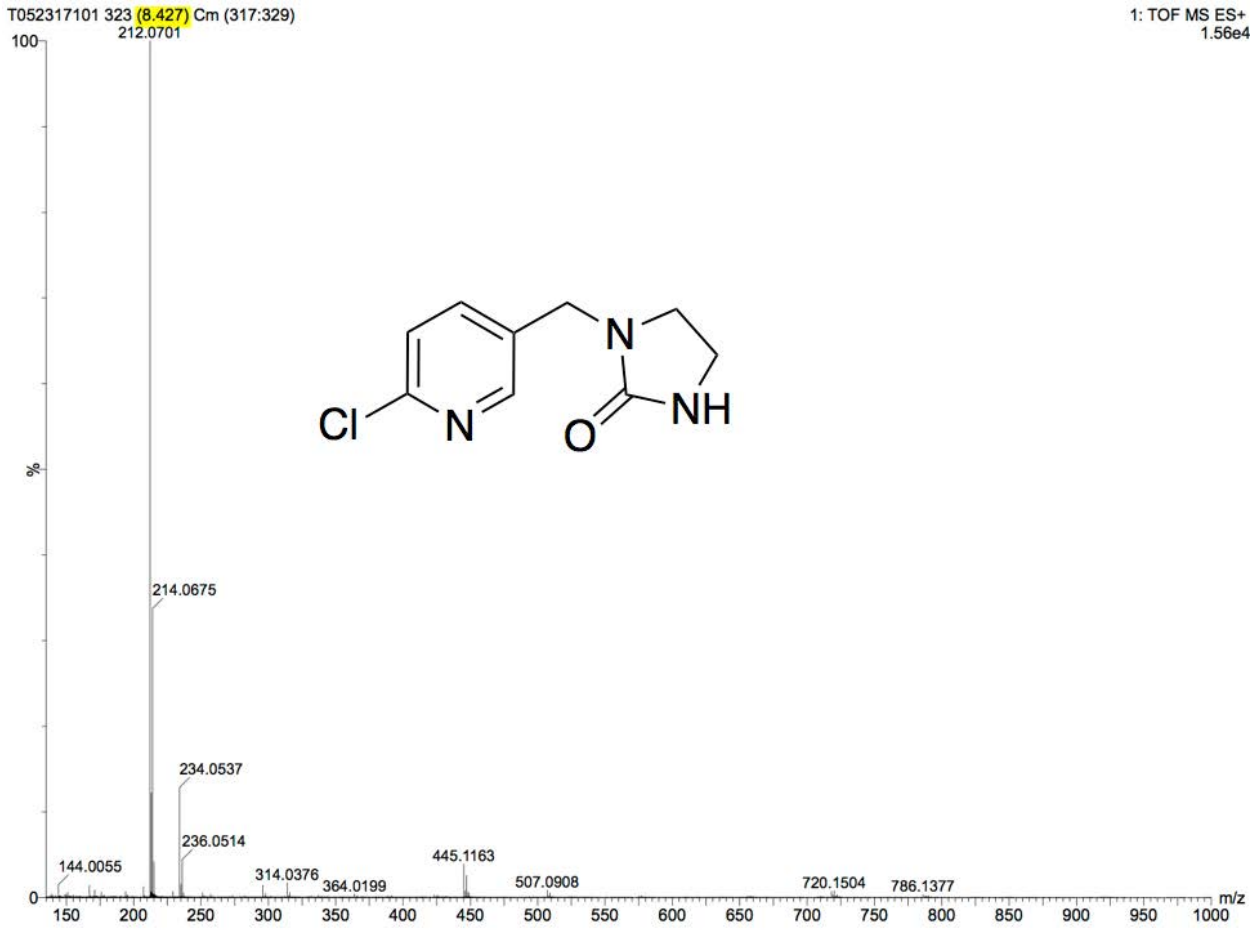


Figure A - 32: Mass spectrum and structure of imidacloprid-urea.

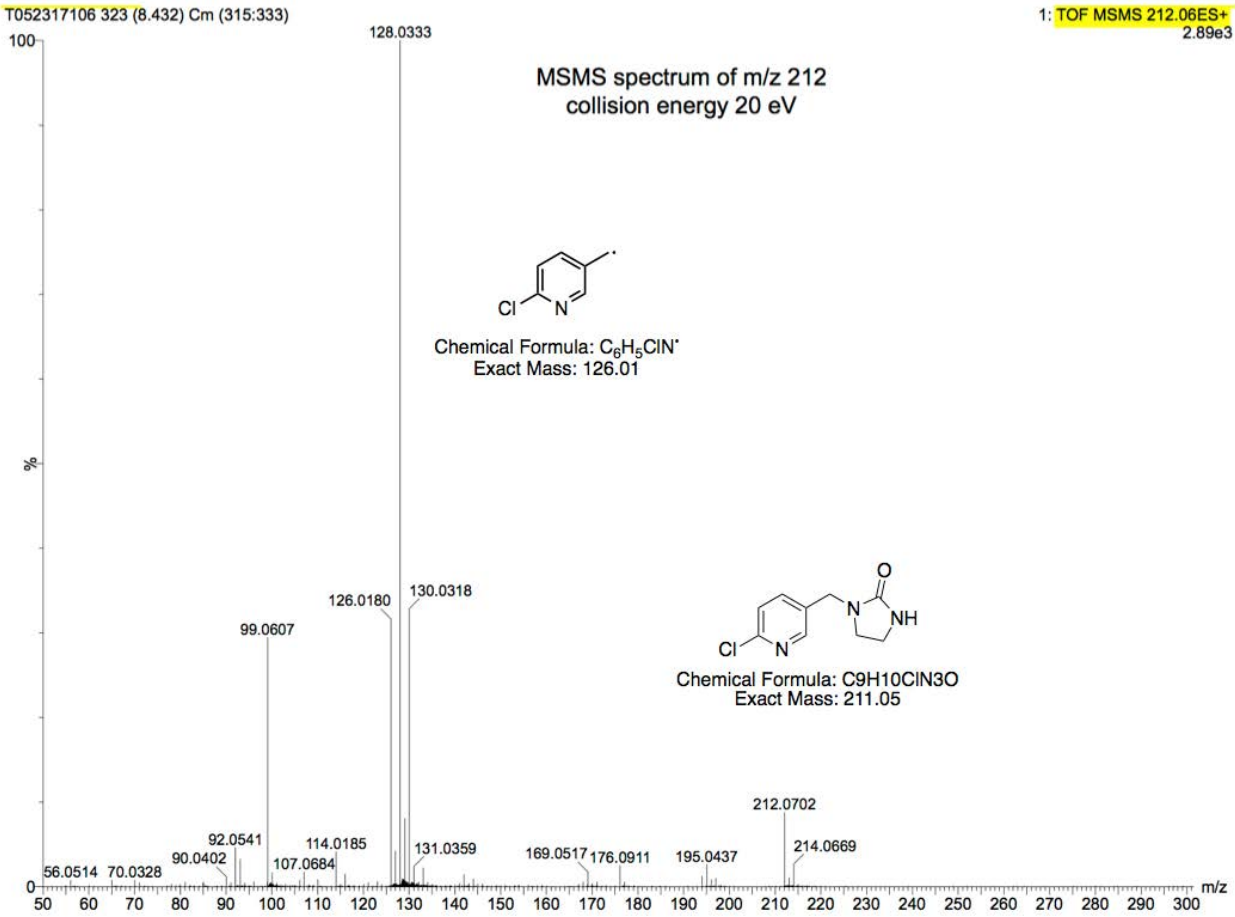


Figure A - 33: MS-MS of imidacloprid-urea with proposed fragment structures.

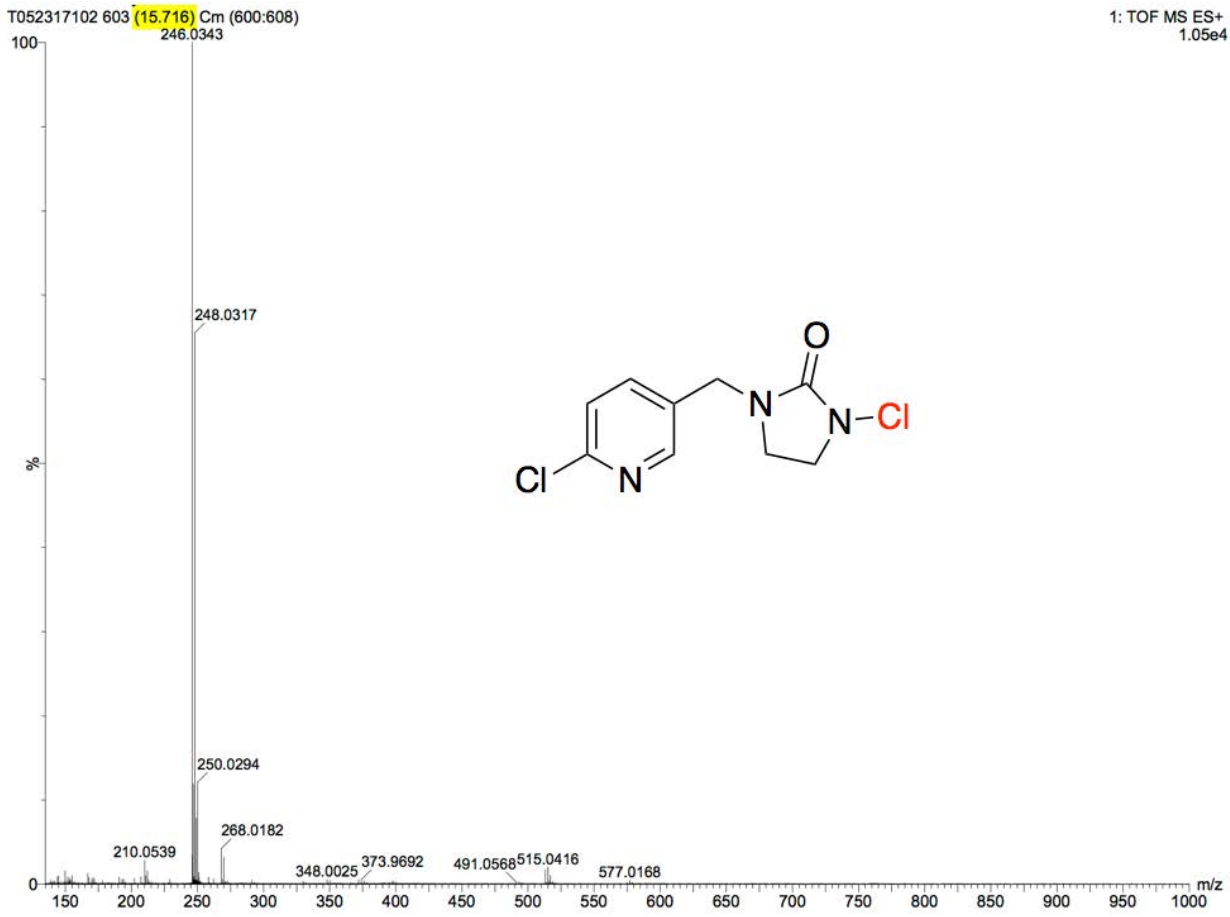


Figure A - 34: Mass spectrum and proposed structure of IMI 246.

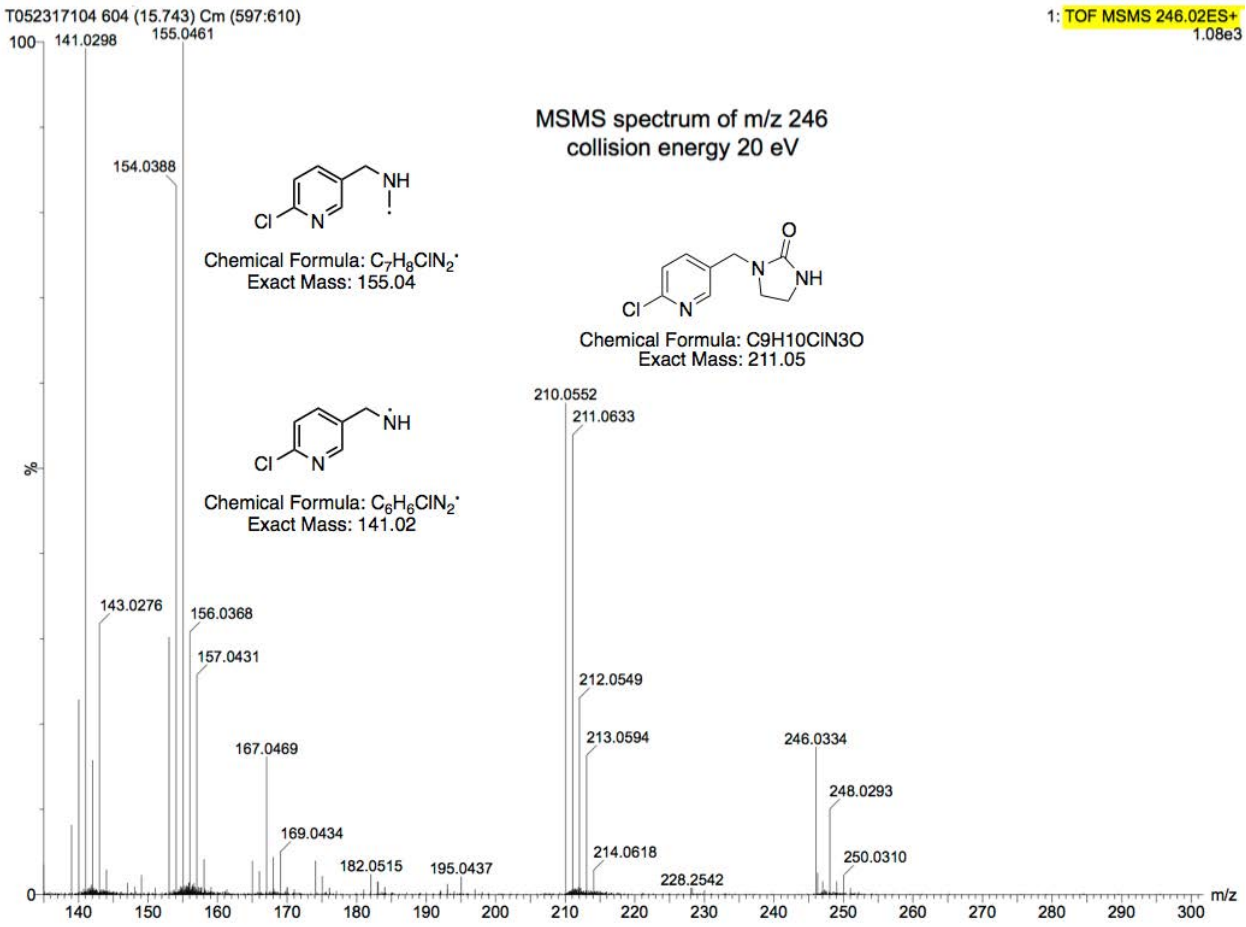


Figure A - 35: MS-MS of IMI 246 with proposed fragment structures.

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