Environmental Processes

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Chlorinated Byproducts of Neonicotinoids and their Metabolites: An Unrecognized Human Exposure Potential?

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ABSTRACT

We recently reported initial discovery of neonicotinoid pesticides in drinking water and potential for transformation through chlorination and alkaline hydrolysis during water treatment. The objectives of this research were to determine: (1) if neonicotinoid metabolites are relevant to drinking water exposure, and (2) the products formed from chlorination of neonicotinoids and their metabolites. Desnitro-imidacloprid and imidacloprid urea, two known metabolites of imidacloprid, are documented for the first time in drinking water. Desnitro-imidacloprid was present above the lower level of detection (0.03 ng/L) in 67% of samples (6/9) from drinking water systems but detectable in all samples (up to 0.6 ng/L). Although concentrations of desnitro-imidacloprid were lower than concentrations of parent neonicotinoids, desnitro-imidacloprid exhibits significantly more mammalian toxicity than imidacloprid. Using LC-HR-ToF-MS/MS analysis of laboratory experiments, we propose structures for novel transformation products resulting from the chlorination of clothianidin, imidacloprid, desnitro-imidacloprid, imidacloprid-urea, and hydrolysis products of thiamethoxam. Formation of chlorinated neonicotinoid byproducts occurs at timescales relevant to water treatment/distribution for the imidacloprid metabolites ($t_{1/2}$=2.4min-1.0h) and thiamethoxam hydrolysis products (4.8h). Imidacloprid metabolites in finished drinking water and potential formation of novel disinfection byproducts during treatment/distribution are relevant to evaluating the exposure and potential impacts of neonicotinoids on human health.

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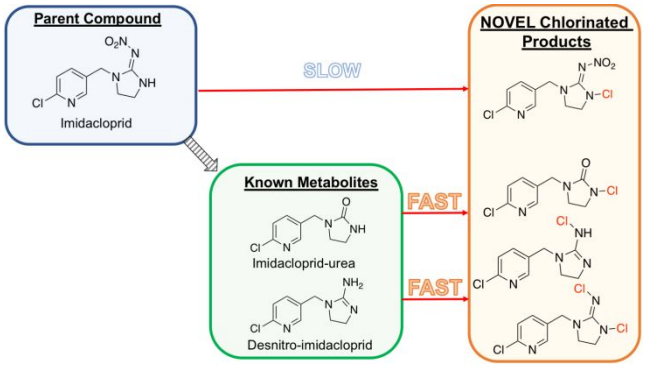
Parent Compound
Imidacloprid
SLOW
Known Metabolites
Imidacloprid-urea
Desnitro-imidacloprid
FAST
FAST
NOVEL Chlorinated Products

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INTRODUCTION

Neonicotinoids are the most widely used insecticides in the world.[1] Neonicotinoids are systemic, insect-targeting neurotoxins that have gained popularity due to their broad spectrum of control, high potency, and insect selectivity.[2–4] This insecticide class enjoys a wide range of both urban and agricultural uses, with a majority (~80% annually) of treated seeds planted in the United States coated with neonicotinoids.[1,5] Due to chemical properties (polarity, solubility) and heavy usage, neonicotinoids are commonly measured in surface waters across North America[6–10] with reported concentrations[7,11–14] up to 6900 ng/L. Neonicotinoid metabolites, such as desnitro-imidacloprid and imidacloprid-urea, are formed via microbial degradation, as well as some abiotic processes (*e.g.*, photolysis, hydrolysis).[2,3,5,15–22] As a result, these metabolites may also be present in surface waters used for drinking water.

Neonicotinoids exploit specific differences between nicotinic acetylcholine receptors (nAChR) in vertebrates and invertebrates to impart insect selectivity.[2,23] Neonicotinoids share important functional groups (nitroimines, cyanoimines, or nitromethylenes) to influence electrostatic binding potential; the negative polarity[24,25] on the neonicotinoid is rejected by the mammalian nAChR and readily accepted by the insect nAChR.[2] Although selective toxicity improves safety for non-target vertebrate organisms, the effects of chronic exposure of humans to neonicotinoids remain unknown.[26,27] Furthermore, toxicological profiles of neonicotinoid transformation products formed via degradation processes may be different from that of the parent compounds, particularly when the nitro- or cyano-groups are removed. For example, two known metabolites of imidacloprid and thiacloprid—desnitro-imidacloprid and descyano-thiacloprid—are respectively 317 and 195 times more toxic to mammals (based on IC_{50}) than their corresponding parent compounds.[3] Understanding the identity, fate, and bioactivity of transformation products

generated in natural and engineered systems is critical to understanding the full impacts of neonicotinoids on ecosystems and human health.

We recently reported[28] the first measurement of neonicotinoids in finished drinking water and demonstrated that select neonicotinoids can be transformed at elevated pH (thiamethoxam) or during chlorination (clothianidin, imidacloprid) over timescales relevant to water treatment and distribution. There is increasing concern about anthropogenic compounds acting as disinfection byproduct (DBP) precursors during disinfection[29] and the potential for these next-generation DBPs to exhibit retained or even enhanced bioactivity[30] (i.e., carcinogenic and/or genotoxic[31]). Objectives of this research were to determine: (1) if neonicotinoid metabolites are relevant to drinking water exposure, and (2) the products formed from chlorination of neonicotinoids and their metabolites that may be generated during drinking water treatment.

MATERIALS and METHODS

Drinking water samples. Raw and treated (entering and exiting treatment plant, respectively) drinking water samples were collected from the University of Iowa (UI) and Iowa City (IC) drinking water treatment plants (Iowa City, IA, USA). The treatment trains are detailed in the SI (Scheme S.1). The main similarities are both systems use lime softening at elevated pH (>10.3) and free chlorine disinfection, the main differences are that UI uses direct surface water and conventional coagulation/flocculation/sand-filtration with mixed powdered activated carbon during high dissolved organic matter (DOM) conditions (to control DBP formation), whereas IC uses an alluvial well-field with granulated activated carbon (GAC) filter-beds. Tap samples were collected from two buildings on the UI campus and three residences serviced by the IC plant

located throughout the city. The limited number of samples was intended to establish the presence and relevance of neonicotinoid metabolites in drinking water, but was not intended to be fully spatially / temporally representative, nor collected in a Lagrangian manner (i.e., transport time-adjusted). Samples were collected during the summer months, when neonicotinoid concentrations are highest.[6,28] UI and IC drinking water samples were analyzed for clothianidin, imidacloprid, thiamethoxam, desnitro-imidacloprid, and imidacloprid-urea. Methods for sample collection and analysis, as well as background information for both treatment and distribution systems, are described previously.[28] Analytical details, lower limits of detection (LLD), and field blank data are provided in the SI.

Hydrolysis, chlorination, and transformation product analysis. Fate during unit processes (lime softening, disinfection, and sequential lime softening and disinfection) was simulated in laboratory batch systems (described fully in the SI) using pH adjustment and free chlorine addition with neonicotinoid concentrations measured by liquid chromatography with diode array detector (LC-DAD). Experiments used free chlorine (HOCl) in a closed reactor containing 5 mM phosphate buffer (pH 7); a range of neonicotinoid (1–50 μM) and HOCl (1–50 mg/L) concentrations were tested (described in Figures S.2, S.3). Chlorination of thiamethoxam hydrolysis products occurred following initial hydrolysis at elevated pH with no chlorine (details in SI). Samples were monitored for 24-72 hours via LC-DAD, and then brought to the High-Resolution Mass Spectrometry Facility (HRMSF) at the University of Iowa for exact mass identification and MS/MS fragment analysis via LC-HR-ToF-MS/MS (Figures S6-S40). The Schymanski framework[32] was used for communicating confidence in identifying newly discovered small molecules (Table 1). Stability of chlorinated products (DN-IMI 245 chosen as representative example) was examined by adding

freshly-prepared sulfite (50 μM in the reactor) and observing back-transformation via LC-MS. Experimental details and analytical methods are provided in the SI.

RESULTS AND DISCUSSION

Occurrence of neonicotinoids and their metabolites in drinking water samples. Desnitro-imidacloprid was present above the Lower Level of Detection (LLD)[33] in 67% (6/9) of samples (raw, treated, and tap water) collected from UI (4/4) and IC (2/5) drinking water systems (Figure 1) but was detectable above the instrument signal-to-noise in all samples analyzed, representing the first known documentation of neonicotinoid metabolites in drinking water. The concentration of desnitro-imidacloprid ranged from <0.03-0.60 ng/L for all water samples. The desnitro-imidacloprid tap water concentrations ranged from 0.03-0.06 ng/L at UI and <0.03 ng/L for IC. Imidacloprid-urea was also present above the LLD in 56% (5/9) of all samples analyzed (4/4 for UI; 1/5 for IC), with measured detections ranging from 0.08-0.66 ng/L. Imidacloprid-urea was not detected in IC tap samples and ranged from 0.22-0.29 ng/L at UI taps (2/2). Clothianidin, imidacloprid, and thiamethoxam were also present in raw, treated, and tap samples with concentrations ranging from 2.34–25.34 ng/L for clothianidin, 1.02–8.79 ng/L for imidacloprid, and 0.24-5.99 ng/L for thiamethoxam. Notably, tap water concentrations for both UI and IC were similar to those we previously reported[28] (Table S.5). In contrast to our previous study, we observed removal of clothianidin and imidacloprid between the source and treated UI samples. We attribute removal to a powder activated carbon system that was added to UI for control of disinfection byproduct precursors after our initial study. This updated system is likely also removing neonicotinoid parent compounds, which we previously reported were effectively removed via activated carbon.[28]

Although the concentrations of metabolites were substantially lower than their respective parent compounds, select neonicotinoid metabolites are known to exhibit higher mammalian toxicity, based on limited available data. Desnitro-imidacloprid has a substantially lower IC_{50} value than imidacloprid for vertebrates, indicating greater binding response (8.2 vs 2600 nM [1.7 vs 550 µg/L], respectively).[3] The greater potential toxicity and frequent presence in these water samples of neonicotinoid metabolites demonstrates the need to consider their fate and persistence in drinking water treatment systems (*e.g.*, during chlorination and other treatment processes) and their potential effects on human health. Indeed, neonicotinoids have been measured year-round[10] in streams of impacted watersheds, and our results demonstrate that consumers of drinking water derived from vulnerable sources may be exposed to neonicotinoids and their metabolites.[28]

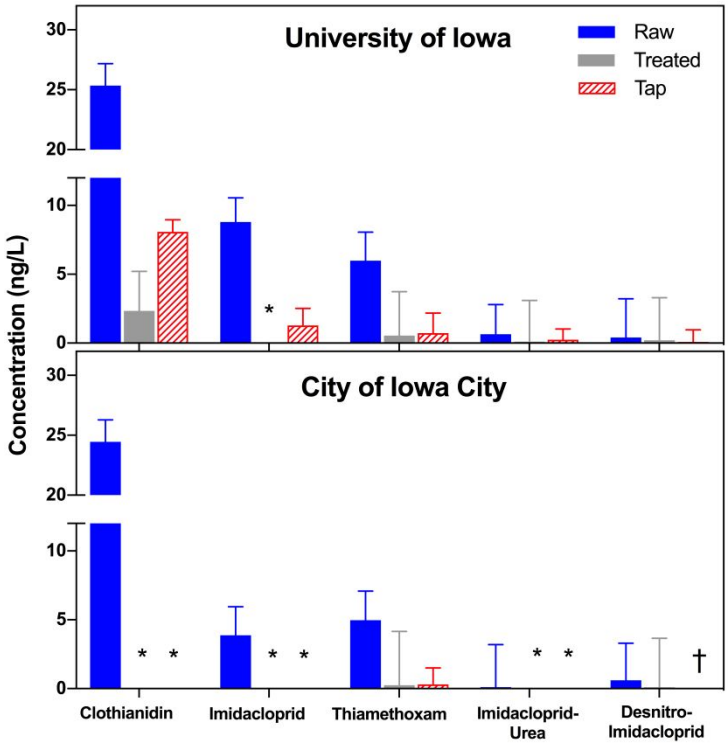

Figure 1: Clothianidin, imidacloprid, thiamethoxam, and two metabolites of imidacloprid (imidacloprid-urea and desnitro-imidacloprid) measured in raw and treated water from the University of Iowa and Iowa City water treatment plants (July 23 and 24, 2018, respectively). University of Iowa tap water was collected at two locations and Iowa City tap water was collected from three residences across Iowa City (n=2 and 3, respectively, July 17, 2018). Tap

concentrations are reported as averages (n=3, July 17, 2018), where (*) denotes non-detects while (†) denote samples present below the lower detection limit (LLD). LLD values (ng/L): clothianidin, 0.488; imidacloprid, 0.275; thiamethoxam, 0.081; desnitro-imidacloprid, 0.026; imidacloprid-urea, 0.057. Error bars represent the standard error including the variation between samples and in sample processing/analysis (associated with the composite enrichment, sample extraction, and analysis).

Desnitro-imidacloprid and Imidacloprid-urea Reactivity with Chlorine. Desnitro-imidacloprid and imidacloprid-urea react relatively rapidly during chlorination (Figure 2). Second-order rate coefficients (±SE) for imidacloprid-urea (2.7±0.2 $M^{-1}s^{-1}$) and desnitro-imidacloprid (72±5 $M^{-1}s^{-1}$) chlorination were calculated from measured pseudo-first-order rate constants (Figure S1-S2) assuming a constant HOCl concentration during reaction ($k_2=k_{obs}/[HOCl]$). At a typical chlorine concentration 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 h and ~2.4 min, respectively. As such, the metabolites of imidacloprid could be expected to degrade readily in a chlorine contactor and during distribution.

Notably, the half-life of desnitro-imidacloprid is much shorter than those we previously reported for clothianidin, imidacloprid, or thiamethoxam[28]—on the order of minutes compared to hours or days for other neonicotinoids. We hypothesize that tautomerization[34] within the guanidine functionality of desnitro-imidacloprid (Figure 2, Scheme S2) contributes to its greater reactivity, resulting in an amino tautomer that would be expected to rapidly chloraminate based on the high reactivity of primary amines toward free chlorine.[35] It remains unclear why imidacloprid-urea is faster reacting than clothianidin and imidacloprid. Secondary and tertiary amides, such as those in imidacloprid-urea, are known to be several orders of magnitude less reactive toward hypochlorous acid than imine and guanidine analogs.[36] We therefore attribute the lower reactivity of clothianidin

and imidacloprid relative to imidacloprid-urea to the well-established electron-withdrawing nature of the nitro group.[37]

Using HR-MS/MS fragment analysis, we propose structures for byproducts observed during chlorination of desnitro-imidacloprid and imidacloprid urea. Chlorination of desnitro-imidacloprid results in the formation of two major identifiable products (hereafter desnitro-IMI 245 and desnitro-IMI 279), corresponding to the addition of either one or two chlorines (*i.e.*, the formation of one dichloro- and one trichloro-transformation product, respectively). Analysis of HR-MS/MS fragmentation patterns indicates chlorine addition occurring in the guanidine-containing portion of the molecule rather than the chloro-pyridine moiety (Fig S19) most likely via N-Cl bond formation; however, the exact site cannot be determined and thus desnitro-IMI 245 is reported at a Level 3 confidence.[32] Consistent with the formation of reactive N-Cl compounds, addition of excess sulfite to product mixtures after desnitro-imidacloprid chlorination resulted in the loss of detectable products and a corresponding increase in desnitro-imidacloprid (Figure S3). Such byproduct reversibility in the presence of a reducing agent is indicative of chloramine formation, as has been previously reported during chlorination of amine-containing pharmaceuticals.[38] Notably, this instability of desnitro-imidacloprid chlorination products may help to explain our detection of desnitro-imidacloprid in finished tap water (Figure 1) despite its very high reactivity toward free chlorine; decomposition of reactive byproducts could result in its regeneration during dechlorination with a reductant or via incidental reactions that occur within the distribution system.

We propose that desnitro-IMI 245 forms via chloramination of the amino tautomer of desnitro-imidacloprid (Figure 2, Scheme S2), which we expect to preferentially chlorinate prior to the corresponding imino tautomer based on established trends in the chlorination of structurally analogous N-containing compounds.[36,39] At higher chlorine concentrations or contact times, we

174 further hypothesize that sequential chlorination of desnitro-IMI-245 occurs through a chlorimino
175 derivative, where the added chlorine stabilizes the imino tautomer akin to the electron-withdrawing
176 nitro-group in imidacloprid. Although speculative, the secondary amine moiety in the chlorimino
177 tautomer would again be expected to exhibit greater reactivity toward chlorine than the
178 corresponding imine moiety in the chloramino tautomer.

179 Chlorination of imidacloprid-urea yielded one major identifiable product (hereafter IMI-urea
180 246). This corresponds to the addition of chlorine to the imidacloprid-urea structure. Once again,
181 HR-MS/MS fragment analysis is most consistent with chlorination occurring at the secondary
182 amide (Figure 2; Figures S39-S40).

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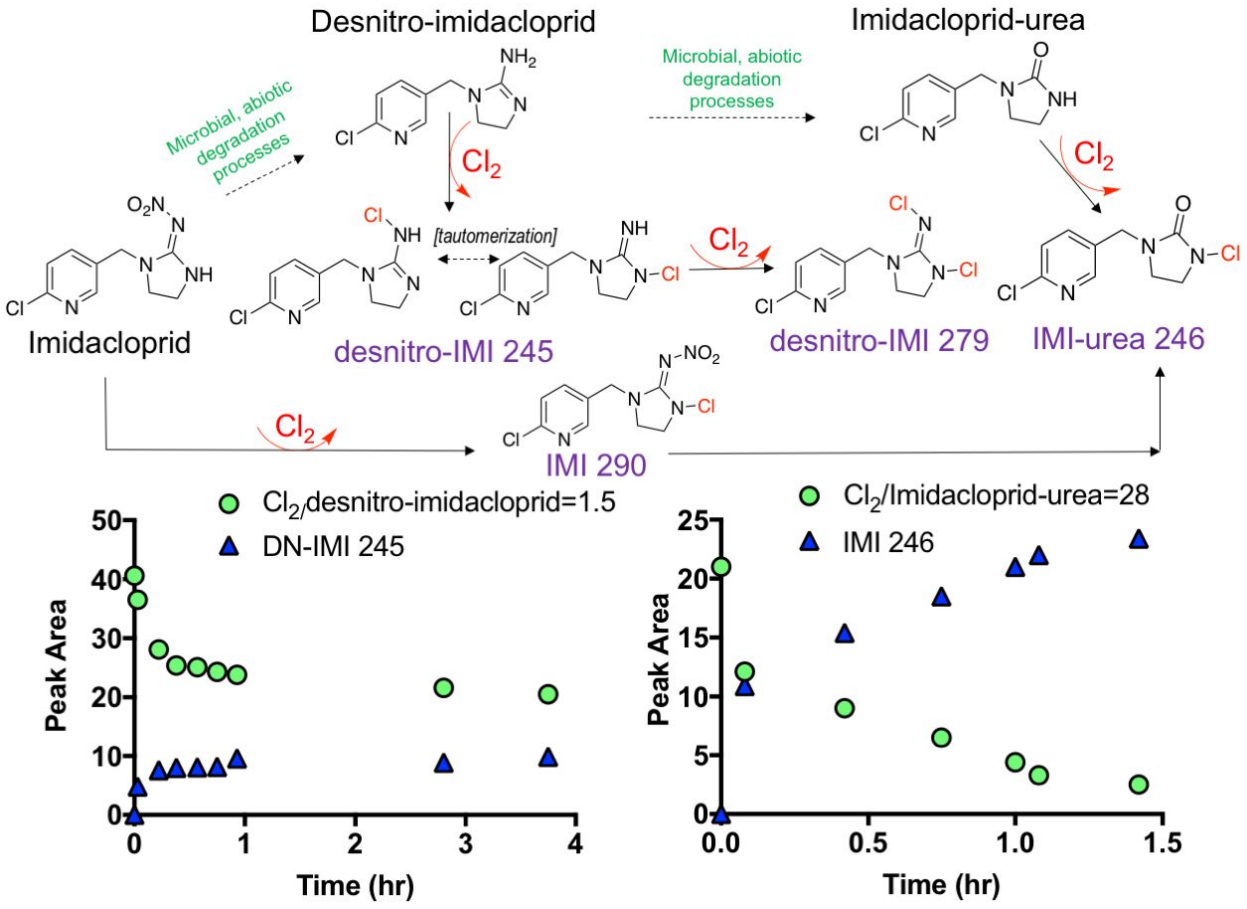

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Figure 2: Chlorination of (left) desnitro-imidacloprid and (right) imidacloprid-urea to form chlorinated products desnitro-IMI 245, desnitro-IMI 279 and IMI-urea 246. Chlorination kinetics of desnitro-imidacloprid to desnitro-IMI 245 and imidacloprid urea to IMI-urea 246 are shown. Peak area shown is the HPLC-DAD response λ= 260 nm for imidacloprid-urea; 273 nm for desnitro-IMI; relative values are shown because no authentic standards of chlorinated products are available. Initial concentration conditions (molar ratios shown in figures): desnitro-imidacloprid= 10μM, 1 mg/L HOCl as Cl_2; imidacloprid urea= 5μM, 1 mg/L HOCl as Cl_2. Full kinetics data, conditions in Figures S1, S2.

Hydrolysis Products of Thiamethoxam and Reactivity with Chlorine. The alkaline hydrolysis of thiamethoxam (at pH 10; relevant to lime softening) results in two products (hereafter THX-H 248 and THX-H 237), both of which have been previously identified with proposed pathways.[5,15,20,40] Imines are known to easily hydrolyze in water to yield ketones,[41,42] and the electron-withdrawing -NO_2 substituent makes the carbon in the guanidine portion of thiamethoxam more electrophilic, thus inviting hydroxide attack under alkaline conditions.[20] THX-H 248 is formed through the simple hydrolysis of the nitro-imine group into a ketone.[20] THX-H 237 was reported by Maienfisch[5] and corresponds to a ring opening with hydroxide attack at the imine carbon.

Upon addition of chlorine, THX-H 237 is reactive, while THX-H 248 is recalcitrant over the timescales / conditions investigated (Figure 3). We attribute the greater reactivity of THX-H 237 toward chlorine to the presence of its two secondary amides. The second-order rate coefficient (±SE) for the reaction of free chlorine with THX-H 237 (0.67±0.02 $M^{-1}s^{-1}$) was calculated from the measured pseudo-first-order rate constant (Figure S4). Assuming a constant chlorine residual (5 mg/L Cl_2), the half-life of THX-H 237 would be 4.8 h.

THX-H 237 reacts with chlorine to produce a single species hereafter referred to as CLO-THX-H 270 (see also Table 1). We propose that chlorine addition occurs at the secondary amide group

without the electron-withdrawing nitro substituent (Figure 3). Our MS/MS fragmentation results reveal a corresponding chlorinated fragment to support this proposed structure (Figure S9-S10). We anticipate that THX-H 237 will react to generate CLO-THX-H 270 at time-scales relevant to disinfection and distribution in systems that also employ chemical (e.g., lime-soda) softening earlier in the treatment process train.

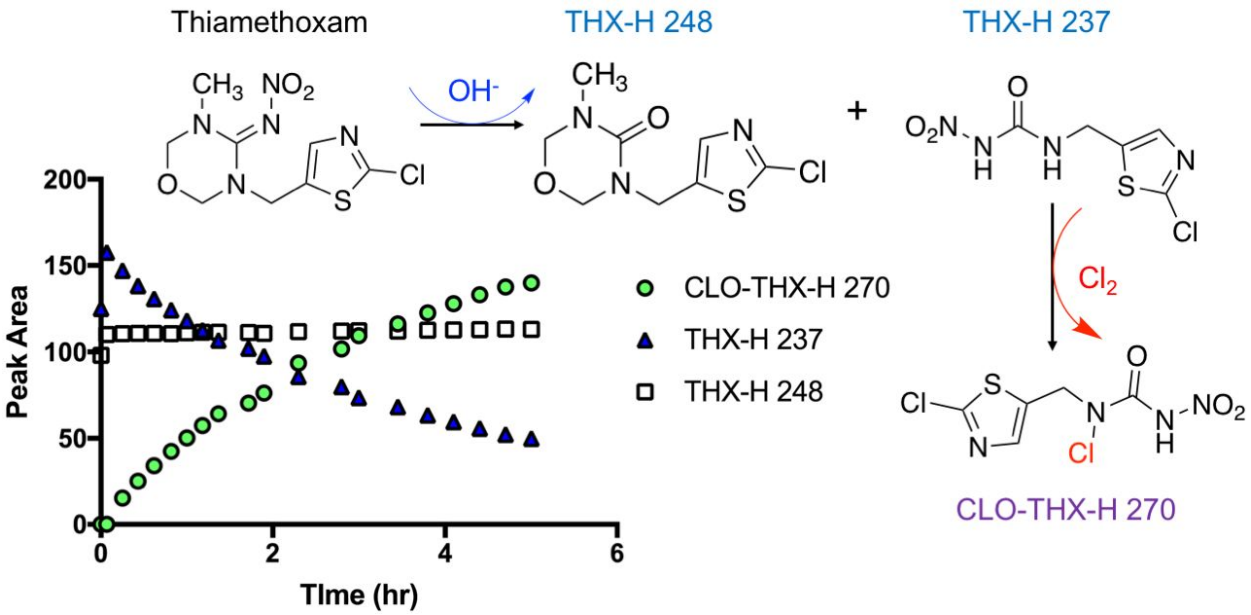


Figure 3: Chlorination of the hydrolysis products of thiamethoxam (THX-H 237 and THX-H 248) to form novel chlorinated product CLO-THX-H 270. Chlorination kinetics (represented by HPLC-DAD peak area λ = 260 nm; authentic standards unavailable; 50 mg/L Cl_2) of thiamethoxam hydrolysis product THX-H 237 to CLO-THX 270 is shown (THX-H 248 was unreactive) at pH 10. The structure of CLO-THX 270 (the same as generated through chlorination of clothianidin) is presented as shown to be consistent with Table 1; chlorination occurs at either amine farther from the nitro-group as determined by HR-MS/MS fragmentation (Figures S9, S10, S34, S35).

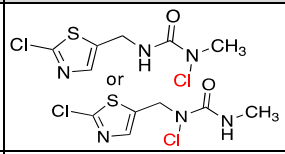
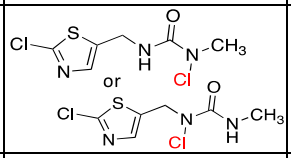
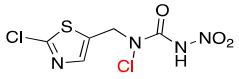
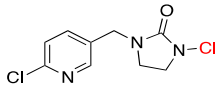
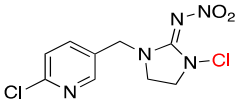
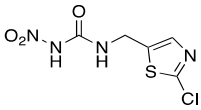
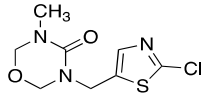
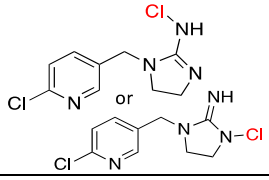
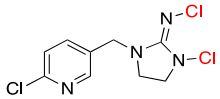
Products of Imidacloprid and Clothianidin Chlorination. We previously reported timescales for the reaction of imidacloprid and clothianidin with chlorine.[28] Herein, we propose structures using the Schymanski framework[32] to communicate confidence of novel products discovery for

229 the products of these reactions (Table 1) based on HR-MS/MS fragment analysis of these product
230 mixtures (Table S.7 describes compounds prior to chlorination).

231 Chlorination of clothianidin results in three major products. Two products have with the same
232 mass (hereafter CLO-239a and CLO-239b) but different retention times, while the third has an
233 exact mass $[M+H]^+$ of 270.9442. The latter product appears identical to the product formed during
234 chlorination of thiamethoxam hydrolysis products, and is thus also referred to as CLO-THX-H
235 270. Clothianidin is a known product of thiamethoxam degradation through multiple reported
236 biologically-mediated pathways[43,44] (e.g., in insects, mammals, plants, and soil) where the two
237 compounds share common metabolites;[25,45] however, abiotic and biological pathways may
238 generate different products. CLO 239a and CLO 239b correlate to loss of the nitro group,
239 formation of the ketone (C=O), and chlorination of a remaining secondary amide. We suspect these
240 reactions occur in a step-wise fashion and involve both oxidation with chlorine and hydrolysis
241 (*e.g.,* imine hydrolysis to a ketone) reactions, potentially involving intermediates we were unable
242 to identify. The exact location of the chlorine on two of the clothianidin products (CLO 239a, CLO
243 239b) could not be confirmed with certainty because MS/MS fragmentation did not yield the
244 chlorinated component (Figure S11-S14; Level 3 confidence). Nevertheless, chlorination is most
245 likely to occur at either of the secondary amides because HR-MS/MS fragment analysis indicated
246 that the chlorothiazole component was not further chlorinated (Figure S8-S14). Fragmentation
247 analysis of CLO-THX-H 270 generated either with clothianidin or thiamethoxan as the parent
248 compounds suggests that chlorination occurs at the nitrogen farther from the nitro group because
249 a chlorinated fragment consistent with this structure was present (Figures S9, S10; S34, S35; Level
250 2b confidence).

Chlorination of imidacloprid forms three major transformation products (hereafter: IMI-urea 246, IMI 290, and IMI 341). Product IMI-urea-246 is chlorinated imidacloprid-urea, which we previously identified in our independent analysis of products generated from the chlorination of an imidacloprid-urea standard (described above). IMI 290 is chlorinated imidacloprid (without loss of the nitro group), with chlorination most likely occurring at the secondary nitrogen in its guanidine moiety. One product, IMI 341, could only be confirmed to level 5 confidence,[32] thus no structure is proposed.

Table 1: Transformation products of clothianidin, imidacloprid, desnitro-imidacloprid, imidacloprid-urea, and thiamethoxam.

	Neonicotinoid Chlorination and Hydrolysis Transformation Products						Fragment Ions	
Parent Compound	Product Name	Proposed Structure	Proposed Formula	Schymanski‡ Confidence Level	RT (min)	Accurate Mass $[M+H]^+$	Accurate mass (m/z)	Proposed Molecular Formula
Clothianidin	CLO 239 a		$C_6H_7Cl_2N_3OS$	Level 3	16.1	239.9792	168.0261	C_6H6N_3OS
							174.9774	$C_5H_4ClN_2OS$
							204.0124	$C_6H_7ClN_3OS$
							119.9693	C_3H_2ClNS
							86.0095	C_3H_3NS
Clothianidin	CLO 239 b		$C_6H_7Cl_2N_3OS$	Level 3	16.4	239.9798	174.9771	$C_5H_4ClN_2OS$
							146.982	$C_4H_4ClN_2S$
							131.9711	C_4H_4ClNS
							168.0261	$C_6H_6N_3OS$
							119.9788	C_3H_2ClNS
Clothianidin, THX-H 237	CLO-THX-H 270		$C_5H_4Cl_2N_4O_3S$	Level 2b	9.2	270.9442	181.9439	$C_4H_3Cl_2N_2S$
							146.9768	$C_4H_5ClN_2S$
							132.9717	C_4H_4ClNS
							118.9552	C_3HClNS
Imidacloprid, Imidacloprid-urea	IMI-urea 246		$C_9H_9Cl_2N_3O$	Level 2b	15.9	246.0222	211.0487	$C_9H_{10}ClN_3O$
							155.0348	$C_7H_8ClN_2$
							141.0206	$C_6H_6ClN_2$
							126.0097	C_6H_5ClN
Imidacloprid	IMI 341	Unknown	Ambiguous	Level 5	16.6	341.9938	218.0239	Unknown
							155.0367	Unknown
							126.0104	Unknown
Imidacloprid	IMI 290		$C_9H_9Cl_2N_5O_2$	Level 2b	16.9	290.0222	246.0217	$C_9H_9Cl_2N_3O$
							209.0617	$C_9H_{10}ClN_4$
							173.0839	$C_9H_{10}N_4$
							126.0123	C_6H_5ClN
Thiamethoxam	THX-H 237		$C_5H_5ClN_4O_3S$	Level 2b	11.8	236.9838	174.9724	$C_5H_4ClN_2OS$
							147.9772	$C_4H_5ClN_2S$
							97.0388	C_4H3NS
Thiamethoxam	THX-H 248		$C_8H_{10}ClN_3O_2S$	Level 2a	11.2	248.0248	174.9718	$C_5H_4ClN_2OS$
							98.0048	C_4H_4NS
							131.9665	C_4H_3ClNS
Desnitro-imidacloprid	desnitro-IMI 245		$C_9H_{10}Cl_2N_4$	Level 3	14.7	245.0377	209.0622	$C_9H_{10}ClN_4$
							173.0848	$C_9H_{10}N_4$
							211.0766	$C_9H_{11}ClN_4$
							83.0588	$C_3H_5N_3$
							132.0353	$C_4H_7ClN_3$
							126.0133	C_6H_5ClN
Desnitro-imidacloprid	desnitro-IMI 279		$C_9H_9Cl_3N_4$	Level 2b	18.5	279.0004	209.0506	$C_9H_{10}ClN_4$
							173.0848	$C_9H_{10}N_4$
							126.0130	C_6H_5ClN

‡The confidence level and structure of each product is characterized according to the Schymanski et al. 2014 framework for identifying small molecules via high resolution mass spectrometry.[32] All samples were analyzed in in ESI positive mode (i.e., ion $[M+H]^+$ =compound exact mass+H). High-resolution fragmentation patterns are presented in Figures S6-S40.

Environmental Implications. This is the first known study to report neonicotinoid metabolites in drinking water, and builds upon our prior research[28] and a subsequent publication from Canada[46] demonstrating neonicotinoids in drinking water. We also show that neonicotinoids and their known metabolites can form transformation products during disinfection and/or lime softening (hydrolysis at elevated pH) at timescales relevant to water treatment / distribution. The mammalian toxicity of transformation products formed during water treatment processes remains unknown. It is possible that chlorination of neonicotinoids and their metabolites will impact receptor binding interactions and alter their bioactivity relative to that of the parent neonicotinoids or known metabolites, a scenario that requires further investigation. Several transformation products identified (CLO 239a, CLO 239b, CLO-THX-H 270, IMI 246, THX-H 248, DN-IMI 245 and DN-IMI 279) appear to lose the nitro-group through chlorination or hydrolysis, and/or gain one or more chlorines—both characteristics that may increase mammalian toxicity.[3,4,23,29,31,47] Additional studies are needed to better assess temporal and spatial trends in metabolite occurrence / toxicity of chlorinated DBPs formed during drinking water treatment (including synthesized standards), especially in waters impacted by parent neonicotinoid insecticides.

SUPPORTING INFORMATION. Additional method details, statistical analysis, quality assurance / control, additional detailed data / results / analysis in figures and tables.

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NOTES. The authors declare no competing financial interest.

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