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Nitenpyram degradation in finished drinking water

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RATIONALE: Neonicotinoid pesticides and their metabolites have been indicated as contributing factors in the decline of honey bee colonies. A thorough understanding of neonicotinoid toxicity requires knowledge of their metabolites and environmental breakdown products. This work investigated the rapid degradation of the neonicotinoid nitenpyram in finished drinking water.
METHODS: Nitenpyram reaction products were characterized using liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/QTOFMS). A software algorithm that compared degraded and control samples was utilized to facilitate efficient data reduction. Fragmentation pathways for six reaction products and nitenpyram were proposed using predictive software and manual product ion analysis.
RESULTS: This study showed that nitenpyram degradation in unpreserved finished drinking water was likely the result of oxidation, hydrolysis and reaction with Cl_2. Structures for six nitenpyram reaction products were proposed that suggest the C9/C11 olefin as the key reactive site.
CONCLUSIONS: Similarities between the identified nitenpyram reaction products and imidacloprid metabolites highlight the importance of this study, as the toxicity of neonicotinoids to pollinators has been linked to their metabolites. Based on the proposed reaction mechanisms, the identified nitenpyram reaction products in finished drinking water could also be present in the environment and water treatment facilities. As such, identifying these degradation products will aid in evaluating the environmental impact of neonicotinoid pesticides. Copyright © 2016 John Wiley & Sons, Ltd.

Neonicotinoids (NNIs) are neurotoxic pesticides that have seen expanding use since the introduction of the first generation NNI imidacloprid 30 years ago (Fig. 1).[1] Currently, NNIs are involved in 80% of insecticidal seed treatments and are the largest class of insecticides sold.[2] Due to their low mammalian toxicity, NNIs have been deemed relatively safe for the environment.[3] In recent years, however, the widespread use of NNIs has been associated with a decline in honeybee colonies.[2] Regulatory bodies in several countries have restricted the use of certain NNIs while their impact is assessed.[4]

Given their global use and potential impact on pollinators, there has been a plethora of research on NNIs in recent years. However, there is still a lack of monitoring data evaluating the fate and stability of NNIs in environmental waters.[5] This study started out looking at the stability of NNIs in surface, ground and finished drinking water to support an initiative by the Government of Ontario to enhance pollinator health and reduce the use of NNIs.[6,7] During stability characterization it was observed that nitenpyram degraded rapidly in unpreserved (without added sodium thiosulfate) finished drinking water while other NNIs were stable, including the structurally similar imidacloprid, (Supplementary Fig. S1, Supporting Information). This was surprising given the photolytic and pH stability (< pH 9) of nitenpyram.[5]

Studies on NNI metabolites have shown appreciable levels of toxicity.[1,5] While the majority of this data came from the study of imidacloprid metabolites in bees, prudence suggests that an understanding of potential environmental breakdown products of other NNIs is required to ensure continued pollinator health.[2,8,9] Indeed, such metabolite characterizations are becoming more important given the increasing use of reclaimed water and biosolids on food crops, which may result in the uptake of agrochemicals and their metabolites into plants.[10,11]

To understand the rapid loss of nitenpyram in unpreserved finished drinking water an investigation was carried out to identify nitenpyram degradation products using detailed fragmentation analysis. To conduct this investigation a liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/QTOFMS) method was used to acquire high-resolution, accurate mass precursor and product ion data.

EXPERIMENTAL

Reagents and sample preparation

The following chemicals were purchased from Sigma Aldrich (Ontario, Canada) and used as received: nitenpyram, imidacloprid, acetamiprid, thiacloprid, flonicamid, clothianidin,

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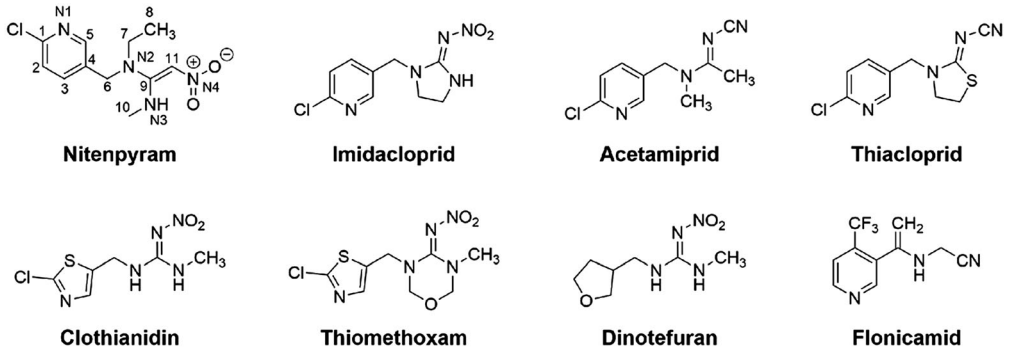

Figure 1. Structures of commonly used neonicotinoid (NNI) pesticides. The *IUPAC* numbering of nitenpyram atoms is shown to aid the reader during fragmentation analysis (*vide infra*).

thiomethoxam, dinotefuran, acetamiprid-d_3, clothianidin-d_3, imidacloprid-d_4, thiamethoxam-d_3, sodium thiosulfate (NaS_2O_3) and ACS-grade formic acid. HPLC-grade solvents were purchased from Caledon Labs (Ontario, Canada). High-purity water was produced by passing reverse osmosis water through a Barnstead NANOpure™ water purification system (Ontario, Canada). Ten milliliter solution dropper bottles for NaS_2O_3 were purchased from ACP Chemicals Inc (Quebec, Canada).

NNI and isotope-labelled (acetamiprid-d_3, clothianidin-d_3, imidacloprid-d_4 and thiamethoxam-d_3) NNI working solutions were prepared in MeOH and stored at 2–8°C for up to 6 months (Supplementary Table S1, Supporting Information). Preservative solution (25% NaS_2O_3) was prepared in dropper bottles using high-purity water.

Stability study samples were prepared by fortifying surface, ground and finished drinking water with NNI working solution and isotope-labelled NNI working solution to a final nitenpyram concentration of 50 ng/L and 100 ng/L, respectively, in 50 mL. One drop of a 25% NaS_2O_3 solution was added to a second finished drinking water sample prior to fortification. At each time point samples were prepared for analysis by adding 800 µL of fortified water sample to 100 µL of MeOH and 100 µL of internal standard stock solution (1000 ng/L). Stability samples were held at 2–8°C, with analytical samples prepared at 0, 7, 14, 21 and 28 days.

To assess nitenpyram degradation products, 200 µL of MeOH was added to 800 µL of finished drinking water. This sample was the control to aid in data processing (*vide infra*). Analytical samples were prepared by adding 200 µL of nitenpyram working solution to 800 µL of tap water. The final concentration of nitenpyram was 200 µg/mL.

Chromatography

LC/QqQ chromatography for NNI stability evaluation was performed on a Shimadzu Prominence LC20 system. LC/QTOF chromatography was performed on a SCIEX ExionLC™ AD system equipped with a binary pump, degasser, autosampler (50 µL sample loop) and column heater (SCIEX, USA). Chromatographic parameters on both LC systems were adapted from Hao *et al.*[6] The changes made include a 50 µL injection volume, a column temperature of 30°C, an aqueous mobile phase of 0.05% formic acid +10% MeOH and an organic mobile phase of 0.05% formic acid in MeOH. Chromatographic separation was achieved using a flow-gradient and non-linear mobile phase gradient (Supplementary Table S2, Supporting Information).

Mass spectrometry

LC/QqQ data were acquired with electrospray ionization (ESI) on a QTRAP® 5500 LC/MS/MS system operating in positive mode (SCIEX). The *Scheduled* MRM™ (sMRM) algorithm contained in the Analyst® 1.6.2 software was utilized for data acquisition, with a target cycle time of 1000 ms and a 60 s retention time window. The sMRM and QqQ MS source parameters are summarized in Supplementary Table S3 (Supporting Information).

LC/QTOF data were acquired on a SCIEX TripleTOF® 5600 system using the Analyst® TF 1.7 software (SCIEX). Data was collected using positive mode ESI and two acquisition workflows. The first utilized the information-dependent acquisition (IDA) algorithm of the Analyst® TF 1.7 software (Supplementary Table S4, Supporting Information). The IDA algorithm parameters were set to collect product ion spectra from four precursor ions that were identified in each full scan TOFMS spectrum. Dynamic background subtraction was used and product ion spectra were collected with a collision energy of 30 ± 15 V. Isotopes within 6 Da of a precursor ion the IDA algorithm identified were ignored. The second acquisition method used dedicated product ion scanning at collision energies of 10, 20 and 40 V. QTOFMS source parameters are summarized in Supplementary Table S4 (Supporting Information).

The QTOF mass spectrometer was mass calibrated at the start of each day and approximately every 2 h during active use and was calibrated from m/z 146.1176 to 922.0098 (eight unique ions). Product ion scans were calibrated from m/z 58.0651 to 315.1623 (eight unique ions).

Data processing

Unknown compound identification was performed using a non-targeted screening work-flow in the MasterView™ software (version 1.1; SCIEX, USA). This work-flow utilized a naïve peak-finding algorithm that permitted comparison to a control sample. To focus this study on higher responding and, assuming similar response factors, higher abundance degradation products, only candidate precursor ions present at >5× the response in a control sample were evaluated. This threshold represents an empirical assessment of mass spectral

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responses for the control and degradation samples. For candidate ions that passed this filter, extracted ion chromatograms (EICs) were generated using an extraction window of 5 mDa and a retention time tolerance of 30 s. An EIC intensity threshold of >100 counts and a signal-to-noise ratio > 5 were applied as data quality filters. The remaining candidate ions were processed using a formula finding algorithm, with $C_{50}H_{200}N_{10}O_{10}S_5Cl_3$ as the maximum permitted elemental compositions. If no suitable formula could be generated from within those elemental limitations the candidate ion was discarded. Proposed empirical formulae were assessed for precursor (<5 ppm) and production ion (<10 ppm) mass accuracy. As well, the isotope ratio difference was calculated by comparing the isotopic ratio of the proposed empirical precursor formulae to the experimental data. A final data review was accomplished by manually comparing the generated empirical formulae and product ion spectra to the nitenpyram parent formula and fragmentation pattern.

Fragmentation analysis was performed manually and with the aid of a fragmentation prediction tool contained in the PeakView™ version 2.2 software (SCIEX). Proposed primary fragmentation pathways for nitenpyram were labelled P1 → P9 and for reaction products were labelled according to their assigned numbers (e.g., Deg_01 = D1–1, etc.). Proposed higher order fragments were assigned a lower-case letter designation (e.g., D1–1a).

RESULTS AND DISCUSSION

NNI stability in finished drinking water

To support a government action plan in Ontario, Canada, aimed at enhancing pollinator health and reducing the use of NNIs, an LC/MS/MS method was developed to assess environmental waters and finished drinking water. This analysis included eight NNIs: acetamiprid, imidacloprid, nitenpyram, thiacloprid, clothianidin, thiamethoxam, dinotefuran and flonicamid (Fig. 1). During development of this method it was observed that nitenpyram was stable in surface and ground waters, but completely disappeared shortly after fortifying unpreserved finished drinking water (Fig. 2). This was quite surprising as none of the structurally related NNIs (e.g., imidacloprid) displayed similar behavior (Supplementary Fig. S1, Supporting Information). Stabilization of nitenpyram in finished drinking water was achieved by adding NaS_2O_3 as a dechlorinating agent, which extended nitenpyram stability through 4 weeks (Fig. 2).

Identification of nitenpyram degradation products

To investigate the rapid loss of nitenpyram, a study was conducted to identify degradation products in unpreserved finished drinking water. Such work is important not only

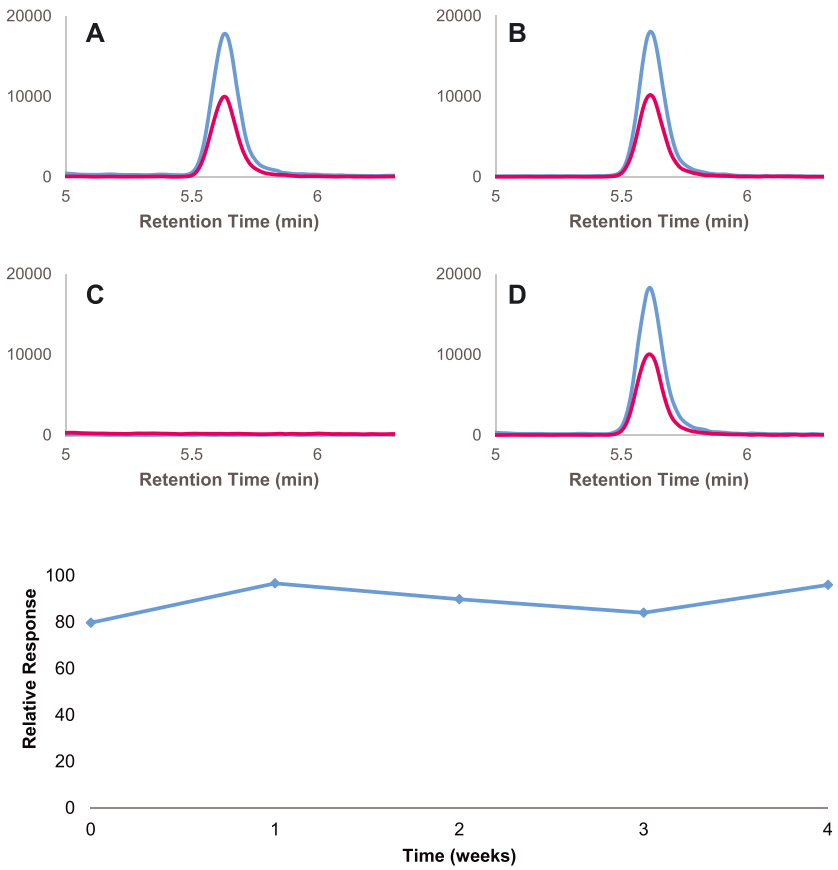

Figure 2. Nitenpyram is stable in surface (A) and ground waters (B). Degradation of nitenpyram in unpreserved finished drinking water (C). The nitenpyram response remains after fortification in finished drinking water with the addition of a NaS_2O_3 (D). Chromatograms are shown with quantifier (blue) and qualifier (pink) MRM transitions overlaid. Nitenpyram is stable for 4 weeks in NaS_2O_3-preserved finished drinking water (bottom).

for understanding how nitenpyram degrades in finished drinking water, but may also provide insight into the mechanism of NNI degradation in the environment and water treatment facilities. As a starting point, nitenpyram was fortified into finished drinking water and analyzed by LC/QTOFMS 6 h after fortification. To aid in the identification of reaction products an information-dependent acquisition algorithm was utilized in tandem with a software package that permits comparative analysis to a control sample. For this study the control sample was finished drinking water fortified with MeOH. This approach maximized the information obtained from a single injection and ensured that the percentage of relevant compounds identified was high.

Using this work-flow 20 potential reaction products were identified in nitenpyram-fortified finished drinking water (Table 1). Since the comparative analysis used by the MasterView™ software was used, it was known that all 20 compounds were present in the fortified finished drinking water at >5× the response observed in the control sample. This provided strong evidence that the compounds identified were all related to nitenpyram. However, to increase confidence in the identifications and aid in the assignment of empirical formulae mass accuracy (TOFMS and MS/MS), theoretical isotopic distribution and MS/MS fragmentation analysis were also evaluated. Such data quality objectives are consistent with common publishing requirements for unknown identification using LC/MS/MS.[12,13]

The predicted isotope ratio was very useful for this study given the presence of chlorine in the parent and proposed reaction products (*vide infra*). The isotope ratio difference provided a metric for evaluating how the theoretical isotope ratio of a proposed formula matched with the experimental data, with values <10 indicating good agreement. All of the proposed empirical formulae show excellent agreement with the theoretical isotope ratios (Table 1).

For TOFMS data the accurate mass was calculated based on the proposed empirical formulae, with the identified reaction product list showing < ±1 ppm mass accuracy (Table 1). For product ion mass accuracy, the MasterView™ software used for data processing contained an embedded feature that used the assigned precursor formula to interrogate the acquired product ion spectra and suggest plausible formulae. The software then averaged the suggested product ion formulae mass accuracy. While the proposed product ion formulae cannot be used to blindly deduce reaction product structures, the average mass accuracy does provide an additional level of confidence in the proposed empirical precursor formulae. The average product ion mass error calculated using this approach was < ±7 ppm for the identified reaction products (Table 1).

Nitenpyram fragmentation analysis

With high confidence in the empirical precursor ion formulae, the product ion spectra were interrogated to assign plausible reaction product structures based on predicted ionization and fragmentation characteristics. Rather than view each reaction product separately, a thorough understanding of nitenpyram fragmentation was pursued first (Fig. 3). This step was critical since diagnostic fragment ions from nitenpyram would guide the proposal of structures for the identified reaction products.

The product ion spectrum of nitenpyram was feature rich, with ten major components (>30% of the base peak) and a plethora of minor product ions. However, since the information dependent acquisition algorithm used a collision energy of

Table 1 Proposed empirical formulae, retention times (RT) and area responses for nitenpyram reaction products identified in unpreserved finished drinking water

Name	RT (min)	Empirical formula	Exact mass (Da)	Adduct	TOFMS error (ppm)	MS/MS error (ppm)	Isotope ratio difference	Area
Nitenpyram	5.08	$C_{11}H_{15}ClN_4O_2$	270.0884	+H	0.7	6.8	9.6	1,504,245
Deg_01	3.31	$C_8H_{11}ClN_2$	170.0611	+H	0.3	3.4	1.0	107,935
Deg_02	9.44	$C_8H_{10}ClN_3O$	199.0512	+H	0.2	6.5	1.1	2,451
Deg_03	3.75	$C_{10}H_{14}ClN_3$	211.0876	+H	0.6	2.4	1.2	70,936
Deg_04	7.58	$C_{10}H_{14}ClN_3O$	227.0825	+H	0.9	2.8	1.3	12,232
Deg_05	8.64	$C_{10}H_{12}ClN_3O_3$	257.0567	+H	0.8	3.6	1.7	161,312
Deg_06	7.32	$C_{12}H_{15}ClN_4O_2$	282.0884	+H	−0.9	3.0	0.9	4,181
Deg_07	5.61	$C_{12}H_{15}ClN_4O_2$	282.0884	+H	0.9	3.9	2.0	157,568
Deg_08	4.76	$C_{12}H_{17}ClN_4O_2$	284.1040	+H	0.5	5.5	2.5	2,539
Deg_09	4.58	$C_{12}H_{17}ClN_4O_2$	284.1040	+H	0.4	6.2	2.0	2,751
Deg_10	8.79	$C_{12}H_{17}ClN_4O_2$	284.1040	+H	0.8	3.7	4.1	39,021
Deg_11	6.04	$C_{12}H_{17}ClN_4O_2$	284.1040	+H	0.8	3.1	0.7	31,224
Deg_12	8.42	$C_{14}H_{15}Cl_2N_3$	295.0643	+H	0.9	2.8	0.2	11,375
Deg_13	10.72	$C_{12}H_{16}ClN_3O_4$	301.0829	+H	0.8	3.7	2.0	41,687
Deg_14	7.41	$C_{11}H_{14}Cl_2N_4O_2$	304.0494	+H	0.9	1.7	1.3	53,831
Deg_15	8.83	$C_{16}H_{17}Cl_2N_5O_2$	381.0759	+H	−0.6	1.8	0.7	7,716
Deg_16	8.58	$C_{15}H_{20}ClN_5O_6$	401.1102	+H	1.0	3.7	4.4	27,919
Deg_17	11.85	$C_{18}H_{21}Cl_2N_5O_2$	409.1072	+H	0.8	1.5	2.6	39,050
Deg_18	10.23	$C_{23}H_{28}Cl_2N_6O_3$	506.1600	+H	0.5	3.1	5.3	1,988
Deg_19	11.30	$C_{22}H_{27}Cl_2N_7O_5$	539.1451	+H	−0.1	1.0	5.0	9,882

Mass error and Isotope ratio difference data are reported as determined by the MasterView™ software.

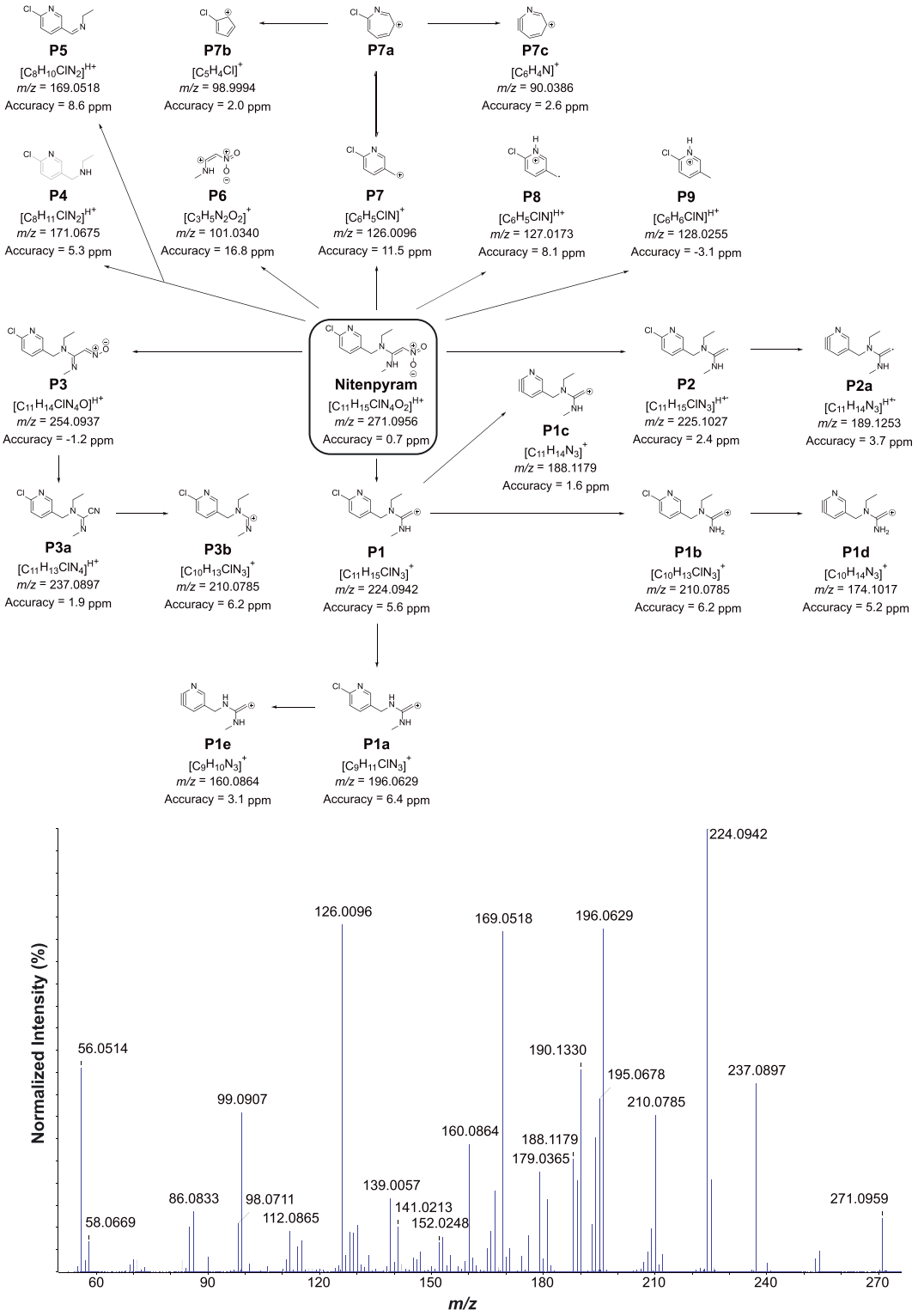

Figure 3. Nitenpyram fragmentation analysis (top) and the nitenpyram product ion spectrum (bottom) that was acquired with a collision energy (CE) of 35 ± 15 V. Proposed primary fragments are denoted P1 → P9. Higher order fragments are denoted by a lower case letter (e.g., P3a).

30 ± 15 V the spectrum was a complex mixture of primary and higher-order product ions that was difficult to interpret (Fig. 3). To facilitate thorough characterization dedicated product ion scans were acquired at discrete collision energies (10, 20, 30, 40 V; Supplementary Fig. S2, Supporting Information).

The first product ions identified were a series initiating from loss and decomposition of the nitro group. P1 and P2 represent the neutral and radical loss of NO_2, respectively, with the neutral loss to the carbocation dominating at higher collision energies. It was proposed that P1a forms from P1 via the

neutral loss of ethylene, also at higher collision energies. P1 and P2 showed secondary and tertiary fragmentation products that terminated with the neutral loss of HCl.

The P3 fragmentation series started from the breakdown of the nitro group via sequential loss of hydroxide from the protonated nitenpyram.[14,15] It is proposed that P3a loses neutral HCN to give the carbocation P3b. Note that P1b and P3b are structural isomers and it is not clear if one or both of these fragmentation products are formed.

The product ions at m/z 171.0675 (P4) and 169.0518 (P5) likely originate from a pyridine-protonated nitenpyram (see Fig. 1 for numbering of nitenpyram atoms). The more abundant P5 could form via a six-membered transition state that leads to imine formation, with the loss of NO_2 and acetylenamine providing an entropic driving force. Alternatively, a five-membered transition state with proton transfer between N2 and N3 could yield P4. The high mass accuracy for these (and other) product ion assignments was attributed to their low relative response (<2% of the base peak). It should be noted that the above proposals do not preclude alternate structures/mechanisms. Regardless, the conservation of these product ions (and analogues) throughout the observed nitenpyram reaction products underscores their diagnostic potential.

Assessing the smaller m/z product ions (P7–P9) started with m/z 126.0096, which was proposed as a homolytic fragmentation from N2-protonated nitenpyram (P7). The same product ion was observed for the fragmentation of the structurally related NNI imidacloprid and its metabolites.[16]

An understanding of the other small m/z nitenpyram product ions was obtained via a thorough characterization of Deg_01 (Fig. 4). The empirical formula for Deg_01 suggested it was formed by cleavage between N2 and C9 to give the free secondary amine (P4 in Fig. 3). The product ion spectrum for Deg_01 was information-rich under m/z 130, with the majority

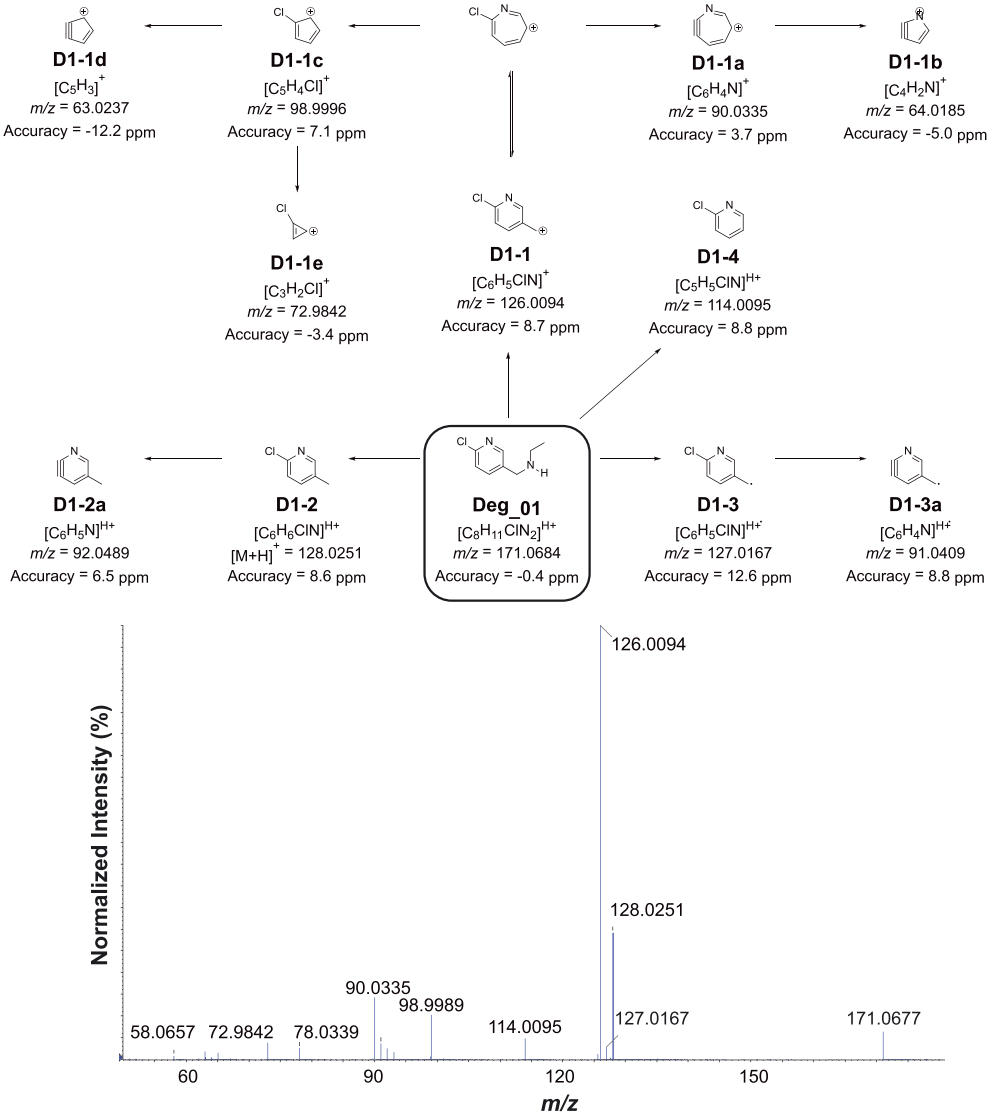

Figure 4. Deg_01 fragmentation analysis (top) and the Deg_01 product ion spectrum (bottom) that was acquired with a CE of 35 ± 15 V. Detailed analyses of the fragmentation pathways of Deg_01 were used in the proposal of nitenpyram reaction products. Proposed primary fragments are denoted D1–1 → D1–4. Higher order fragments are denoted by a lower case letter (e.g., D1–3a).

of the observed product ions shared with nitenpyram. However, the lower overall complexity of the Deg_01 spectrum (relative to nitenpyram) permitted the assignment of detailed fragmentation patterns with increased confidence. It was postulated that an understanding of these small m/z product ions would prove useful based on potentially diagnostic shared product ions observed for nitenpyram and its reaction products (*vide infra*).

The key fragments for Deg_01 were D1–1, D1–2 and D1–3, which differ nominally by 1 Da. Similar to P7 of nitenpyram (Fig. 3), these three product ions were observed for the imidacloprid and its metabolites.[16] These product ions resulted from different fragmentation mechanisms between C6 and N2 of Deg_01. The D1–1 carbocation (m/z 126.0105) forms through neutral loss of ethylamine and permits isomerization to a tropylium-like intermediate (Fig. 4). This is a well-established mechanism for the fragmentation of benzyl cations and their derivatives.[15,17] Following isomerization two different product ion pathways were proposed. The first involved the sequential neutral loss of HCl (D1–1a) and ethylene (D1–1b), with the latter characteristic of tropylium fragmentation. The second pathway involved loss of neutral HCN (D1–1c), likely in a mechanism analogous to the loss of neutral ethylene. D1–1c then appeared to yield product ions corresponding to the neutral loss of HCl (D1–1d) and ethylene (D1–1e).

D1–2 and D1–3 resulted from fragmentation of the pyridine-protonated Deg_01. Moving beyond the site of protonation, D1–2 and D1–3 are the homo- and heterolytic cleavage products, respectively, between C6 and N2. D1–2 and D1–3 fragment further via neutral loss of HCl to give D1–2a and D1–3a. Neutral loss of the alkylamine side chain was proposed as the origin of product ion D1–4. Following from these proposed fragmentation pathways for Deg_01, fragmentation pathways P7–P9 were assigned for nitenpyram (Fig. 3).

Proposed structures of nitenpyram degradation products

Using the detailed product ion analysis of nitenpyram and Deg_01, a review of the reaction products in Table 1 was conducted and structures were proposed for a total of six reaction products that had strong MS/MS spectral support (Fig. 5).

The empirical formula for Deg_14 suggested the substitution of chlorine for hydrogen (Table 1). This formula was supported by the MS/MS spectrum for Deg_14, which displayed a nominal shift of m/z 34 for all of the proposed nitenpyram product ions in Fig. 3 (Supplementary Fig. S3, Supporting Information). Proposed structures D14–7–D14–9 provided strong evidence that the second chlorine was added to the pyridine ring and D14–6 confirmed that the modification was not on the C9/C11 olefin. While no product ions were observed that definitively localized the position of the second chlorine on the pyridine ring, D14–7b suggested that it was on C2 or C3. This assignment was based on the proposed neutral loss of HCN that mechanistically involved C5 and the fact that C1 and C4 did not have hydrogens to lose.

Deg_16 corresponded to the net addition C_5H_2ClN to nitenpyram, which represented the addition of chloropyridine. As with Deg_14, Deg_16 possessed an MS/MS spectrum with similar features to nitenpyram, although this time a nominal shift of m/z 111 was observed for all of the proposed nitenpyram product ions in Fig. 3 (Supplementary Fig. S4, Supporting Information). To aid in the identification of diagnostic product ions for Deg_16, the monoisotopic mass and the strong A + 2 isotope resulting from the presence of two chlorines were subjected to MS/MS scanning. Analyzing the A + 2 isotope clearly showed when fragmentation involved the loss of one chlorine, as the resulting m/z was a doublet due to the loss of either ^{35}Cl or ^{37}Cl during fragmentation. Product ions D16–5 and D16–7–D16–9 demonstrated that the aliphatic tertiary amine and chloropyridine were, respectively, unchanged from nitenpyram. This meant the modification was present on the olefinic side of N2. All of the analogous product ions associated with fragmentation of the nitro group of nitenpyram were observed for Deg_16 (D16–1–D16–3). As well, D16–3c and D16–3d were proposed as product ions that were not observed for nitenpyram. It was postulated that these product ions were formed as a result of the addition of chloropyridine at C10. Further supporting this were the nitenpyram product ions analogous to D16–1b/3b. For nitenpyram they were proposed as structural isomers, but for Deg_16 they were clearly differentiated due to the proposed substitution of chloropyridine at C10.

Closely related to Deg_16 was Deg_18, which shared a number of product ions and displayed several that were nominally shifted by m/z 28 from those observed for Deg_16 (Supplementary Fig. S5, Supporting Information). The empirical formula and observed product ions suggested the

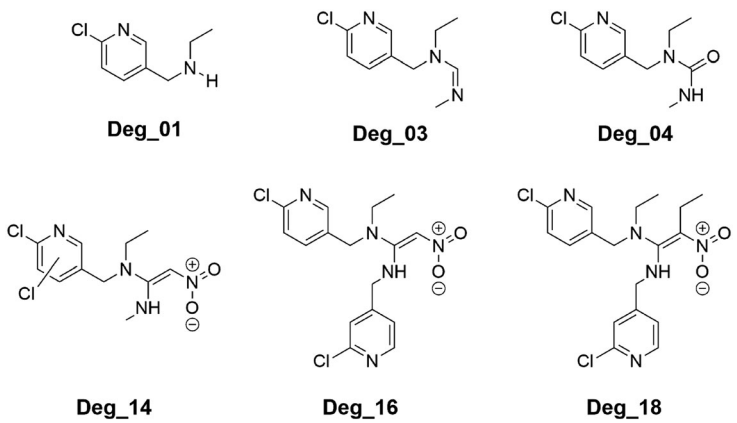

Figure 5. Structures of the nitenpyram reaction products identified in unpreserved finished drinking water. Only those reaction products that had strong MS/MS spectral support were discussed herein.

net addition of C_2H_4 to Deg_16, with the modification proposed at C11 (Fig. 5). The presence of D18–4, D18–5 and D18–7–D18–9 again provided evidence that the modification was on the olefinic side of N2. In tandem with all of the analogous product ions shared with Deg_16, strong support for modification at C10 was obtained by comparing D16–3a/b to the predicted structures for Deg_18. If C10 was modified as proposed, then the formation of analogous product ions would be expected to differ from Deg_16, which was the observed behavior of Deg_18. There was an m/z value that suggested a product ion similar to D16–3a, but the experimental m/z was off by one hydrogen. If, as proposed, C10 of Deg_16 was modified by the addition of ethylene, then D18–3f was the plausible product ion that would follow a nitro fragmentation similar to D18–3. The absence of product ions analogous to D16–3b provided strong support for the modification of C10. Starting from the proposed D18–3f it would not be favorable to form a product ion analogous to D16–3b, as there was not a strong leaving group present to facilitate this mechanism.

Deg_03 and Deg_04 are related structures that followed from oxidation of the C9/C11 olefin (Fig. 5). The product ion spectra and empirical formula of Deg_04 supported the presence of an unmodified benzyl chloropyridine (D4–1–D4–4; Supplementary Fig. S6, Supporting Information). After evaluating the proposed empirical formula and the plausible reactivity of nitenpyram it was deduced that C9 of the nitenpyram olefin was fully oxidized to the urea derivative Deg_04 (Fig. 5). This assignment was strongly supported by D4–7/8. Also supporting the proposed Deg_04 structure was the ratio of D4–6:D4–9. For nitenpyram this ratio favoured D4–9 (P5, Fig. 3), while, for Deg_04, it was reversed with a preference for D4–6 due to the loss of the more favourable leaving group (methyl isocyanate). It was also viewed as diagnostic that the ratio of D4–1:D4–2:D4–3 favoured D4–3, whereas D4–1 (P7, Fig. 3) was favoured for nitenpyram. This likely occurs due to the formation of the urea functional group and the loss of the strongly electron-withdrawing nitro group. The resulting decreased basicity of N2/N3 would make the pyridine nitrogen (relatively) more basic and. Thus, more likely to be the site of proton-adduct formation.

It is postulated that Deg_03 formed in parallel with Deg_04 via dehydration of a shared secondary alcohol intermediate. The diagnostic product ions for Deg_03 started with the absence of a product ion analogous to D4–9. For Deg_03 this product ion was not formed due to the lower stability of the required leaving group when compared to Deg_04 or nitenpyram (Supplementary Fig. S7, Supporting Information). D3–7 also supported the imine functional group that was proposed for Deg_03. There is also literature precedent suggesting Deg_03 as a nitenpyram metabolite in plants.[18]

The remaining degradation products identified by retention time and empirical formula did not contain sufficient information in the acquired product ion spectra to propose reaction product structures with a high degree of empirical support. Indeed, it is worth noting that LC/QTOFMS alone is not capable of identifying all of the nitenpyram reaction products, as some could be short-lived or not readily ionize in positive electrospray. Orthogonal analytical techniques (e.g. NMR) would be needed to obtain a more complete understanding of all of the nitenpyram reaction products formed in finished drinking water. However, from the reaction product structures that were proposed herein, it appears that a combination of oxidation/hydrolysis and reaction with Cl_2 lead to the observed rapid loss of nitenpyram in unpreserved finished drinking water. For instance, Deg_14 clearly suggests a direct role for Cl_2, while Deg_03/04 strongly suggests oxidative/hydrolytic mechanisms.

CONCLUSIONS

While not exhaustive in the characterization of the observed degradation products, the results presented herein show that nitenpyram degradation in unpreserved finished drinking water is likely mediated by a combination of oxidation/hydrolysis and reaction with Cl_2. Structures for a variety of reaction products were proposed that all point to the C9/C11 olefin as the key labile site. The proposed reaction products are consistent with reported metabolites of the structurally related NNI, imidacloprid.[2,19] Such similarities highlight the importance of identifying these reaction products given that the toxicity of NNIs to pollinators has been linked to NNI metabolites.[5] Based on the proposed degradation mechanisms, the identified nitenpyram reaction products in finished drinking water could also be present in aquatic environments and water treatment facilities. Thus, identifying these degradation products will aid in evaluating the overall risks/impact of NNIs to pollinators.

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