

Supplemental Information

Quantum yields for direct photolysis of neonicotinoid insecticides in water: Implications for exposure to non-target aquatic organisms

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MATERIALS AND METHODS

Chemicals and reagents

Thiamethoxam (3-[(2-chloro-1,3-thiazol-5-yl)methyl]-5-methyl-*N*-nitro-1,3,5-oxadiazinan-4-imine), clothianidin (1-[(2-chloro-1,3-thiazol-5-yl)methyl]-2-methyl-3-nitroguanidine), imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-4,5-dihydro-1*H*-imidazol-2-amine), acetamiprid ((*1E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N'*-cyano-*N*-methylethanimidamide), and thiacloprid ({(2*Z*)-3-[(6-chloro-3-pyridinyl)methyl]-1,3-thiazolidin-2-ylidene}cyanamide) standards were purchased from Accustandard (New Haven, CT) with purity higher than or equal to 99.7%. Pyridine (PYR, $\geq 99.9\%$), *p*-nitroanisole (PNA, $\geq 97\%$) and *p*-nitroacetophenone (PNAP, $\geq 97\%$) were purchased from Sigma-Aldrich (St. Louis, MO). Solutions for direct photolysis were prepared with borate buffer (50 mM, prepared by borate acid and sodium hydroxide, pH=7.4) in nanopure water (>18 M Ω -cm, Milli-Q RG, Millipore Corp., Ann Arbor, MI). Liquid chromatographic solvents were prepared with nanopure water, HPLC grade acetonitrile or methanol (Fisher Scientific, Fair Lawn, NJ) and formic acid (95%, Sigma-Aldrich, St. Louis, MO).

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25 **Molar absorptivities of neonicotinoid insecticides**

26 The absorption spectra (200-400 nm) of neonicotinoid insecticides and actinometers are shown in
27 Figure S2A. These compounds strongly absorb UV irradiation over a wide wavelength range, from 200
28 to 360 nm. Thiamethoxam, clothianidin, and imidacloprid all have tailing absorption bands well past
29 290 nm, while acetamiprid and thiacloprid exhibit minimal absorption in the environmentally relevant
30 wavelength region (Figure S2B).

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32 **Tubes deployed in mesocosm tanks**

33 To examine contributions of non-photolytic abiotic transformation (e.g., hydrolysis), microbial
34 biotransformation and indirect photolysis on thiamethoxam degradation, four types of tubes were
35 deployed in triplicate: tube A (non-photolytic transformations only e.g., hydrolysis)-dark (wrapped in
36 aluminum foil), poisoned (10 mM HgCl₂) mesocosm water + thiamethoxam (10 µg/mL) at the water
37 surface of the mesocosm tanks; tube B (indirect photolysis and non-photolytic transformation)-poisoned
38 (10 mM HgCl₂) mesocosm water + thiamethoxam (10 µg/mL) at the water surface of the mesocosm
39 tanks; tube C-poisoned (10 mM HgCl₂) mesocosm water + thiamethoxam (10 µg/mL) at the sediment-
40 water interface (28 cm depth) and tube D (microbial transformation only)-dark (wrapped in aluminum
41 foil), mesocosm water + thiamethoxam (10 µg/mL).

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43 **Chemical analyses.**

44 UV-Vis spectra of insecticides and actinometers were recorded at 0.1 nm intervals from 200-400
45 nm using a Shimadzu UV- 2501PC spectrophotometer. Concentrations were measured with an Agilent
46 Technologies (Mississauga, ON) 1200 high performance liquid chromatograph system with a UV diode
47 array detector (HPLC-DAD). Chromatography was achieved with a Waters (Milford, MA) Symmetry

48 C₁₈, 4.6 mm×150 mm, 3.5 μm analytical column and a Phenomenex (Torrance, CA) Security Guard
49 C₁₈ Guard Cartridge (4 mm×3.0 mm ID). All neonicotinoids were resolved isocratically with a 65:35
50 (v:v) Milli-Q water:acetonitrile eluent at 1.0 mL/min at 25°C, with an injection volume of 20 μL.
51 Thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid were quantified by absorbance
52 at 256, 260, 270, 230 and 242 nm, respectively. *p*-Nitroanisole and *p*-nitroacetophenone were separated
53 with 40:60 (v:v) Milli-Q water:acetonitrile and quantified by absorbance at 320 and 260 nm,
54 respectively. All samples were kept in dark before injection.

55 Photoproduct identification was done initially via Agilent 1200 ultra-high performance liquid
56 chromatography–tandem mass spectrometry coupled to a 6410 triple quadrupole tandem mass
57 spectrometry (UPLC-MS-MS) (Agilent Technologies, Mississauga, ON). Separation was achieved as
58 above using isocratic elution at 1.0 mL/min commencing with 50:50 Milli-Q H₂O: methanol (0.05%
59 formic acid) at 40 °C. Injection volume was 1 μL. Analytes were ionized using an electrospray
60 interface operating in positive mode with the following conditions: capillary voltage, 4000V; nebulizer
61 pressure, 15-55 psi; drying gas flow, 10-11 L/min; drying gas temperature, 300 °C. The source
62 fragmentor voltage was +135 V. Nitrogen was the collision gas, with 0-12V collision energies. The cell
63 accelerator voltage was maintained at 7 V for all analyses. MS2 scan mode (mass range 100-400) and
64 the precursor ion scan mode were used for photoproduct identification. MS spectra of the non-
65 irradiated and matrix matched blank samples were compared to the irradiated samples to identify
66 potential photoproducts.

67 The photoproducts were further identified by high resolution mass spectrometry (HRMS) using a
68 Time-of-Flight (TOF) AB SCIEX TripleTOF® 5600 mass spectrometer equipped with a DuoSpray ion
69 source coupled to a micro-LC 200 AB SCIEX Eksigent pump (Concord, ON). HRMS data was
70 analyzed using PeakView® 2.0 Software. Sample introduction was achieved using the same solvent

71 conditions as for the LC-MS/MS experiments above, with a flow rate of 20 $\mu\text{L}/\text{min}$, injection volume
72 of 2 μL and a HALO C₁₈, 2.7 μm , 90A, 0.5 \times 50 mm column (Eksigent). All QTOF experiments were
73 conducted in positive ionization mode. IDA (information dependent acquisition) experiments were
74 conducted on the blank and irradiated samples to identify potential photoproducts and elucidate
75 structural information. IDA experiments involved a combination of TOF-MS scans with a specified
76 number of TOF-MS/MS scans being triggered to obtain exact mass products of certain precursors
77 identified in the initial TOF-MS scan.

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79 Sunlight photolysis estimations

80 The photolysis rate constants (k_{dcE}) (days^{-1}) and half-lives ($t_{(1/2)E}$) (days) estimations for
81 neonicotinoid insecticides in surface water at 50° N latitude for spring, summer, autumn and winter by
82 natural sunlight were calculated by the equations:¹

$$83 \quad k_{dcE} = \phi_{dc} \sum_{\lambda} \varepsilon_{\lambda c} L_{\lambda}$$

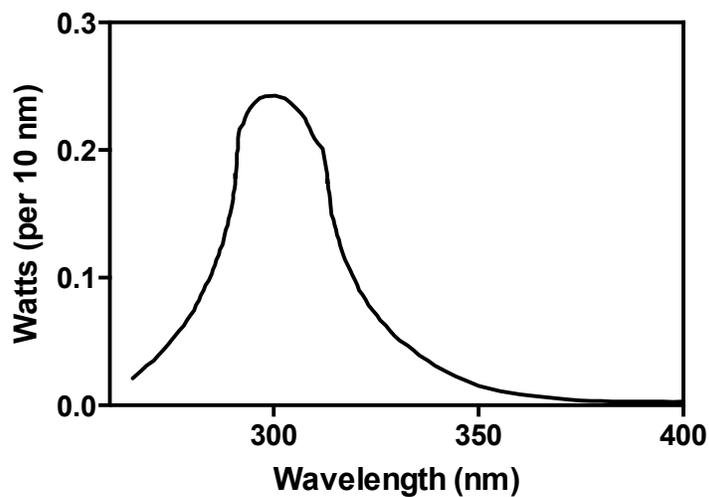
$$84 \quad t_{(1/2)E} = 0.693 / k_{dcE}$$

85 where k_{dcE} and $t_{(1/2)E}$ represent the natural sunlight rate constant and half-life respectively. The L_{λ} value
86 is the solar irradiance parameter, which was obtained from the OECD.² The wavelength region
87 considered was 297.5 to 360 nm.

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90 DATA AND RESULTS



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93 **Figure S1:** Photoreactor light output. Plot represents re-plotted data provided by the manufacturer (The

94 Southern New England Ultraviolet Company)¹

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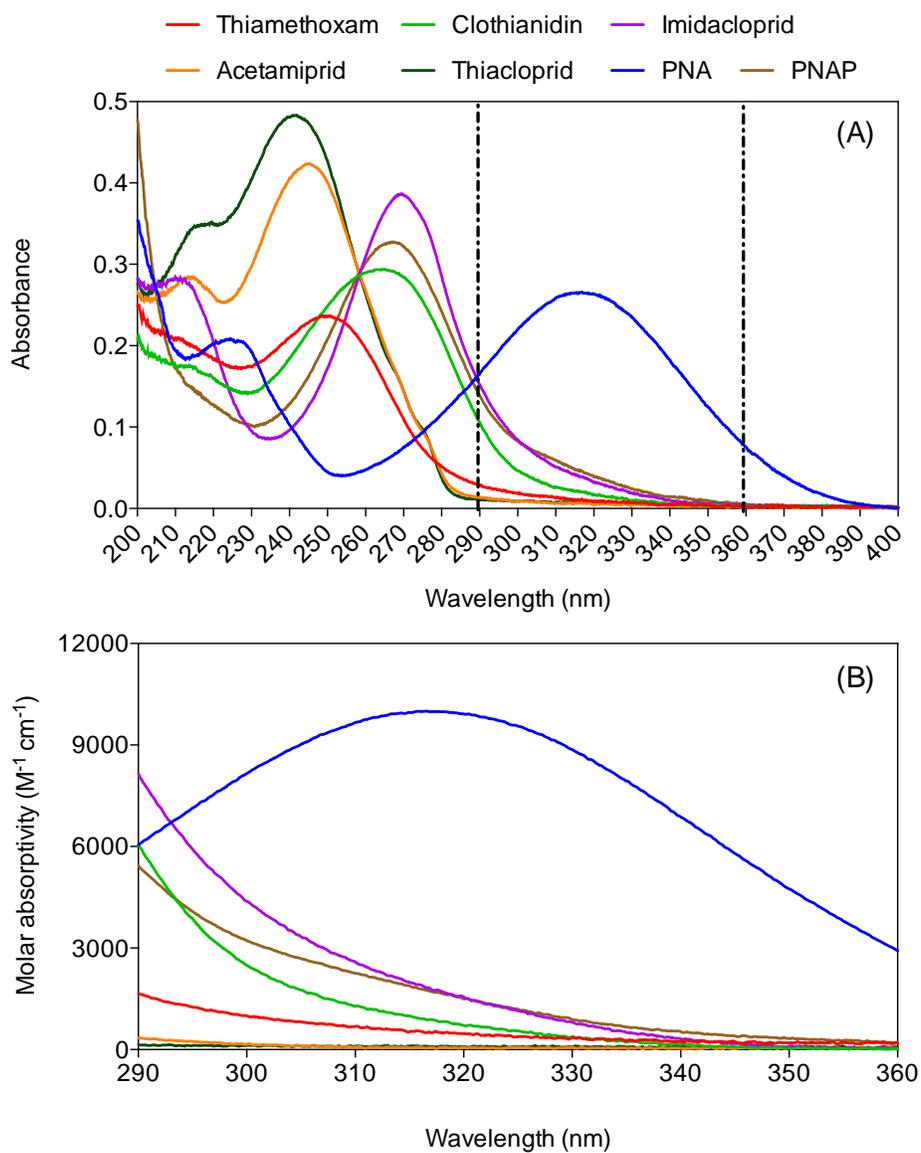
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109 **Light absorption and photolysis kinetics**

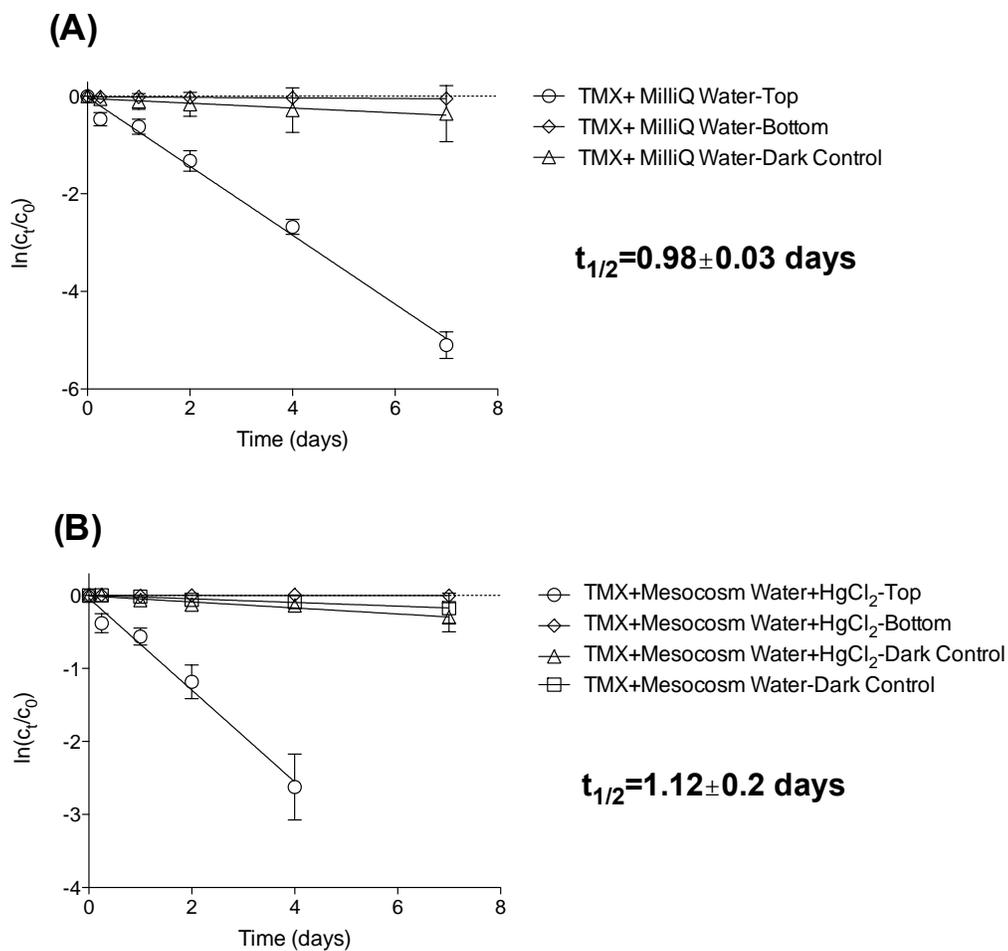


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111 **Figure S2:** Absorption spectra of (A) neonicotinoid insecticides investigated in this study and (B)

112 actinometers used. PNA: *p*-nitroanisole; PNAP: *p*-nitroacetophenone.

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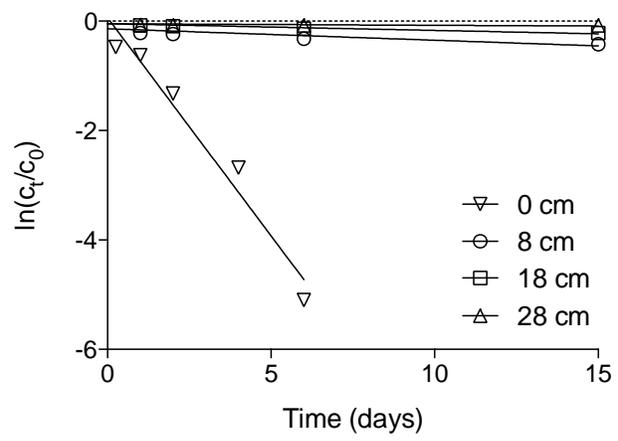
115 **Figure S3:** Photodegradation kinetics of (A) thiamethoxam (TMX) in MilliQ water and (B) TMX in
 116 mesocosm water. Data are shown as mean \pm SD. Top: 0 cm; Bottom: 28 cm.

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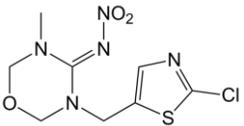
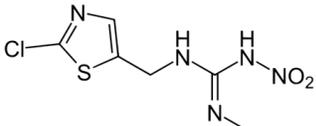
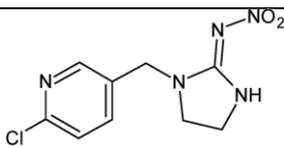
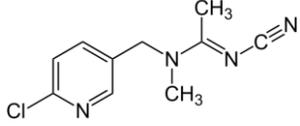
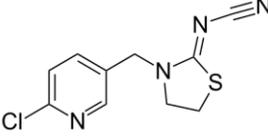


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122 **Figure S4:** Photodegradation kinetics of thiamethoxam in MilliQ water at different depths in
123 mesocosm tanks.

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125 **Table S1** Physical-chemical parameters of neonicotinoid insecticides.³

Compound	Structure	Mass (g/mol)	pKa	log <i>K</i> _{ow}
Thiamethoxam		291.7	No dissociation	-0.13
Clothianidin		249.7	11.1	0.9
Imidacloprid		255.7	No dissociation	0.6
Acetamiprid		222.7	0.7	0.8
Thiacloprid		252.7	No dissociation	1.3

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128 **Table S2** Light intensity distribution of the photoreactor used in quantum yield determination.

Photoreactor light ^a		Sunlight ^b	
λ distribution		λ distribution	
Section (nm)	% of total light ^c	Section (nm)	% of total light ^c
290 - 300	0.238	300 - 305	0.015
300 - 310	0.245	305 - 310	0.029
310 - 320	0.158	310 - 315	0.048
320 - 330	0.076	315 - 320	0.063
330 - 340	0.043	320 - 325	0.079
340 - 350	0.023	325 - 330	0.095
350 - 360	0.012	330 - 335	0.103
		335 - 340	0.105
		340 - 345	0.109
		345 - 350	0.115
		350 - 355	0.119
		355 - 360	0.121

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130 ^a Data was provided by manufacturer (Rayonet Southern New England Ultraviolet Company)

131 ^b Dulin and Mill (1982)⁴

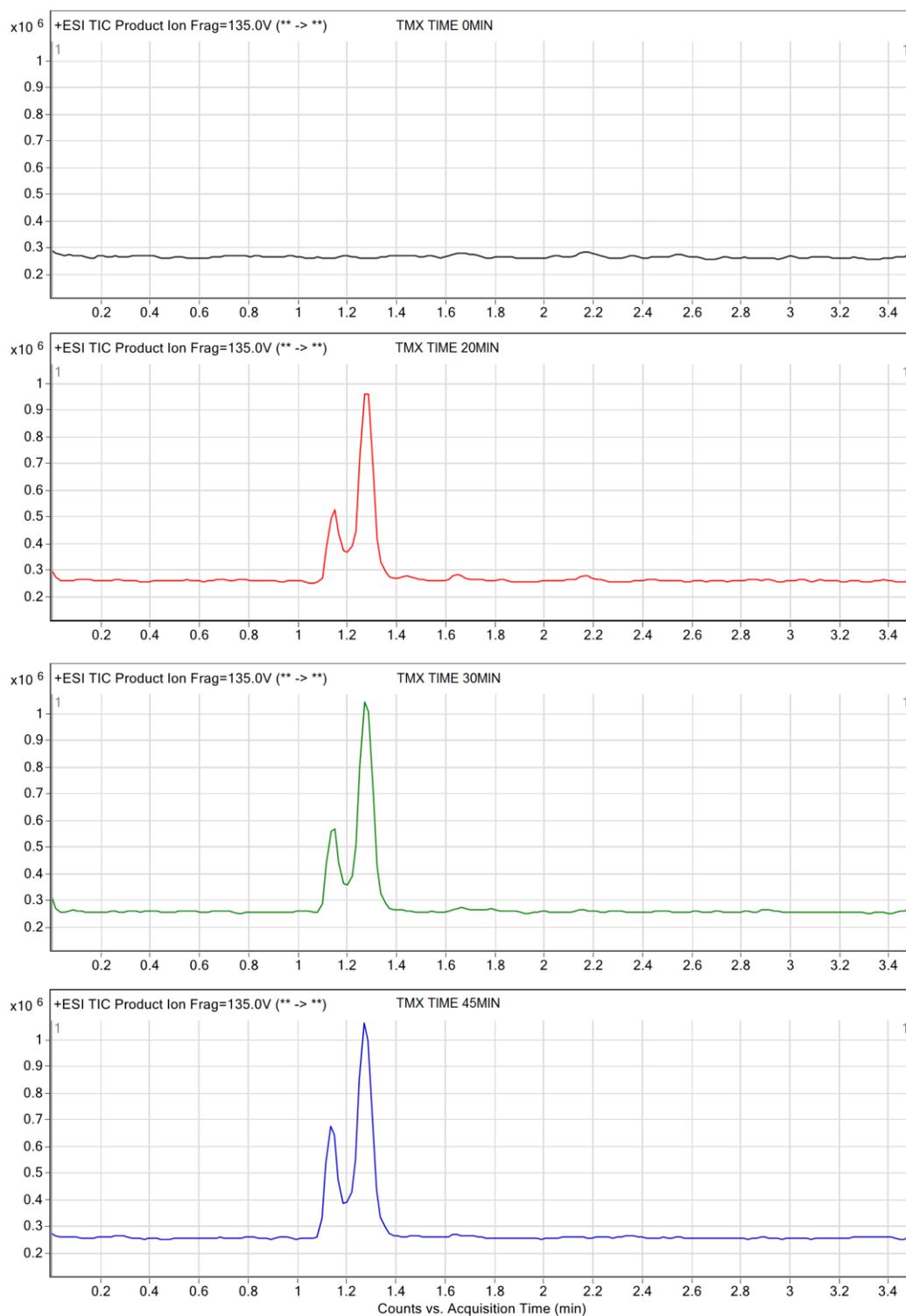
132 ^c Percentages were determined in Excel by sectioning the wavelength distributions into 5 nm regions

133 and integrating the areas under each 5 nm section.

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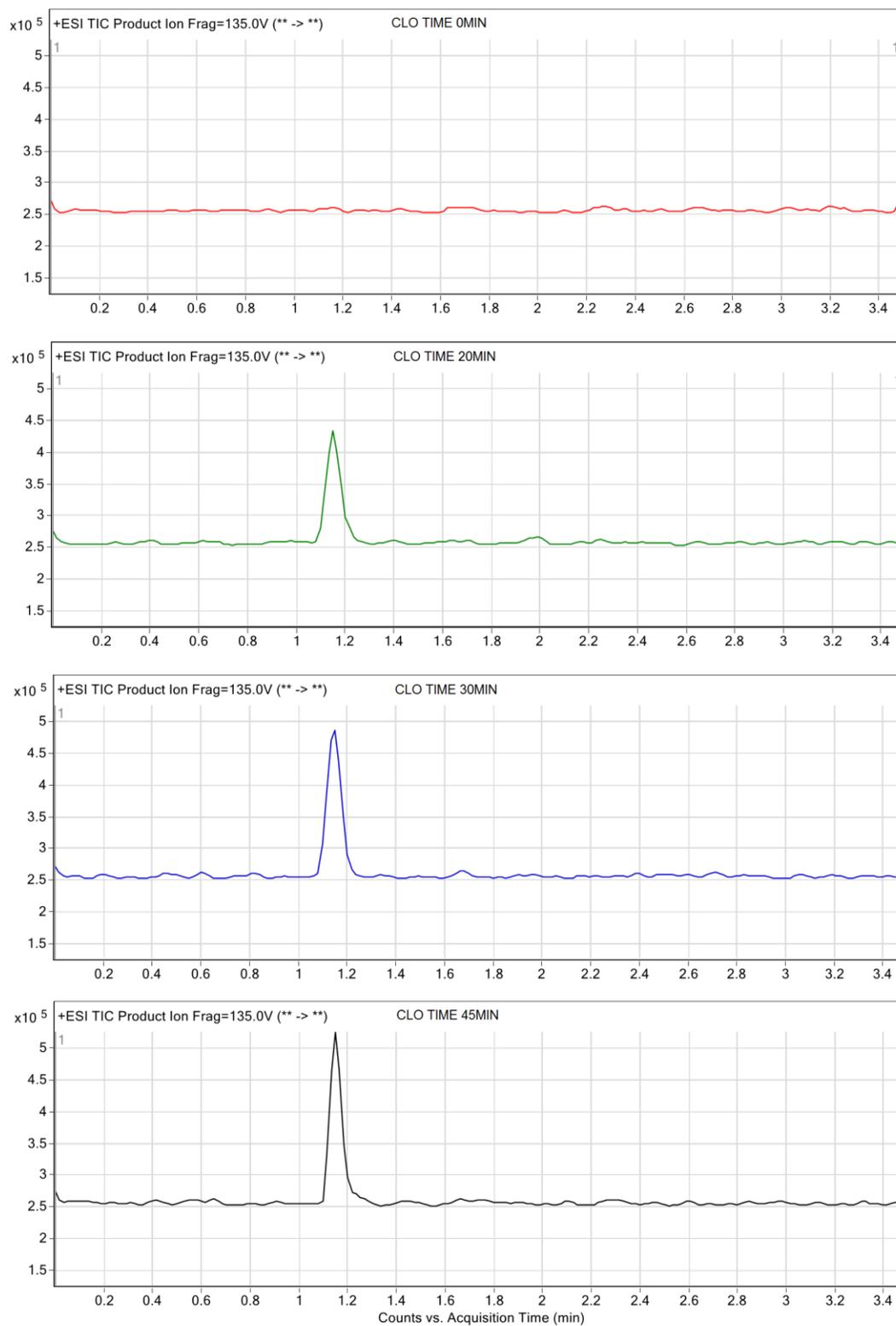
135 **Photoproduct identification**

136 *LC-MS/MS data.*



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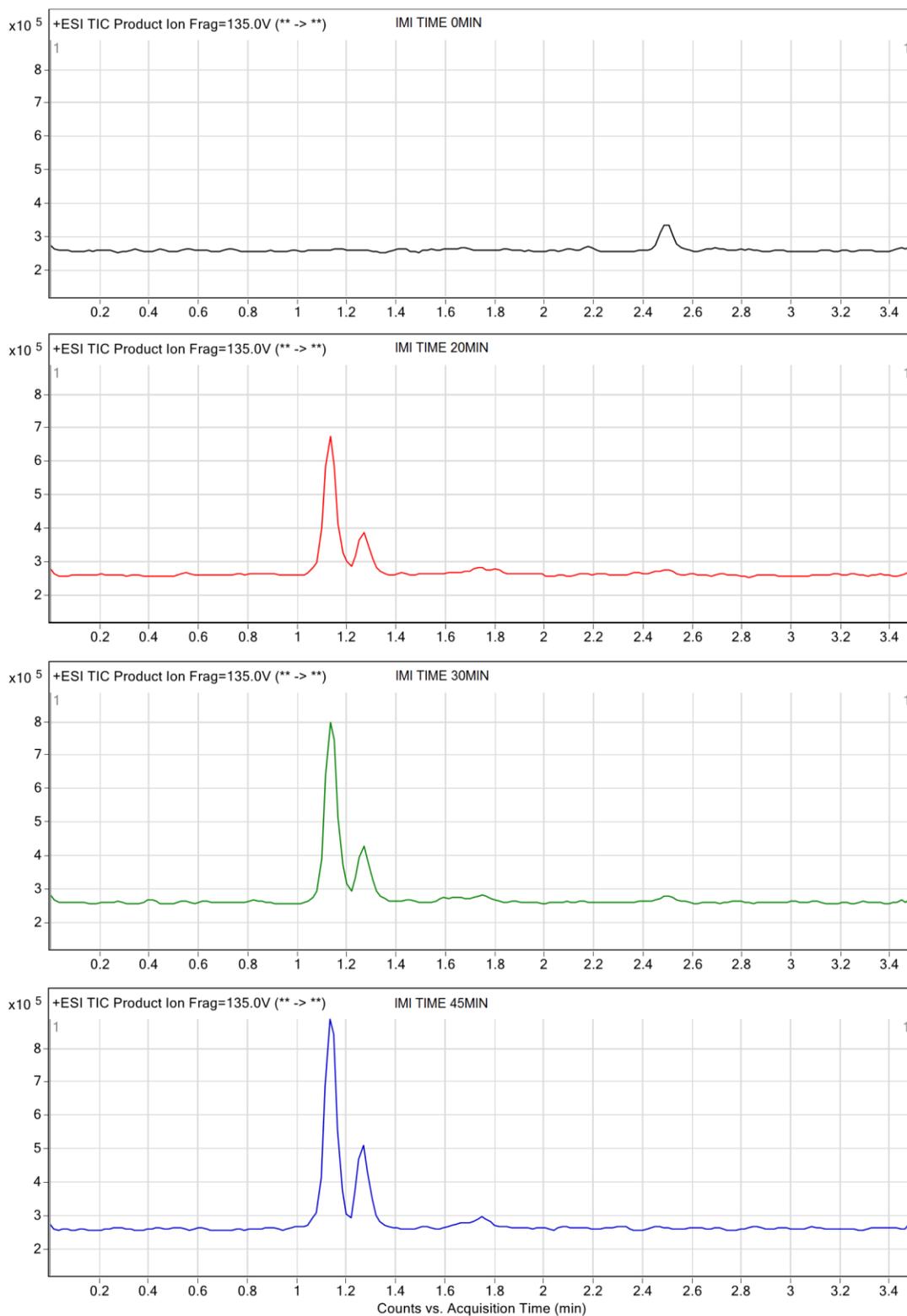
138 **Figure S5:** Total ion chromatograms of thiamethoxam irradiated for 0, 20, 30, and 45 minutes.



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140 **Figure S6:** Total ion chromatograms of clothianidin irradiated for 0, 20, 30, and 45 minutes.

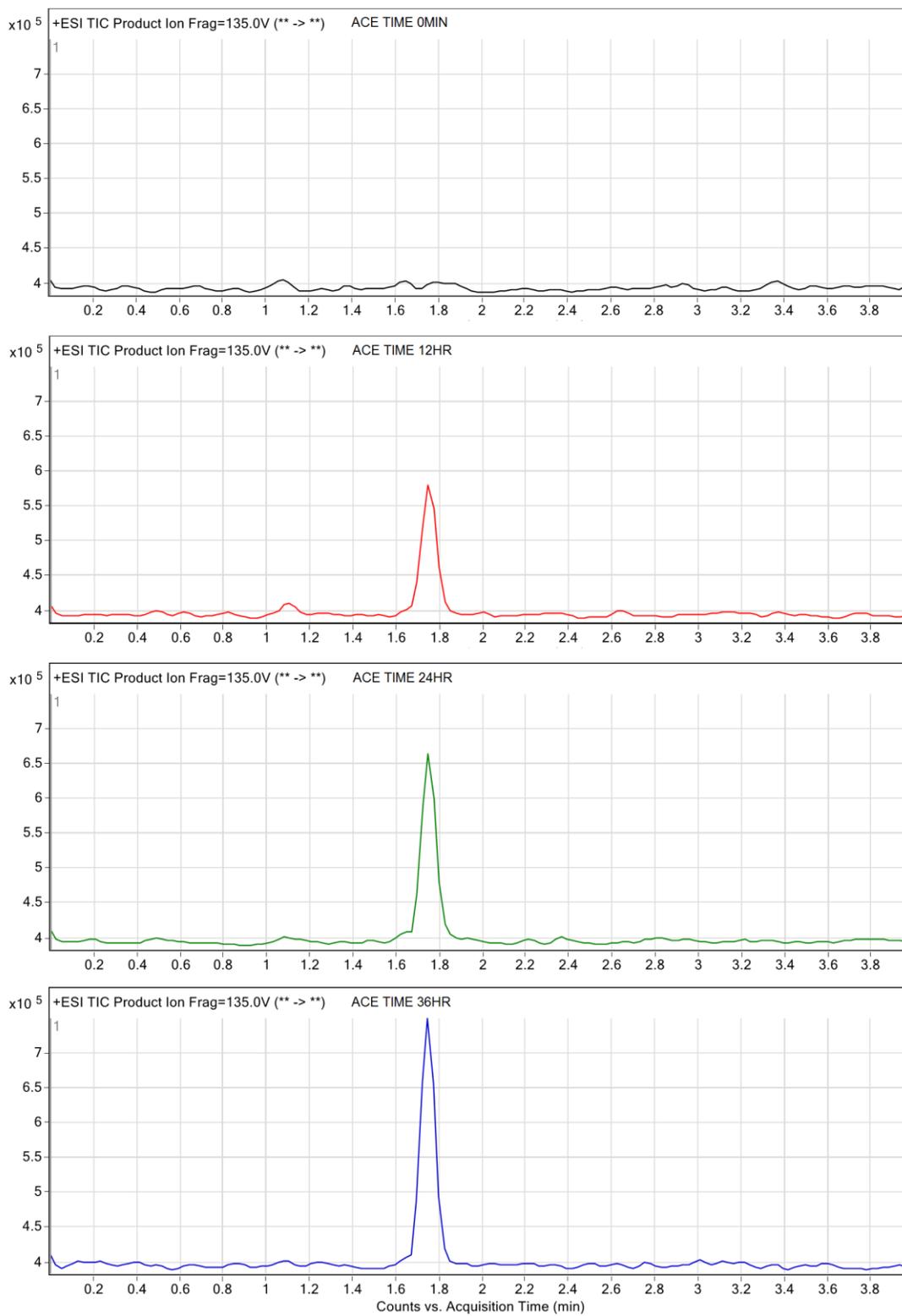
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143 **Figure S7:** Total ion chromatograms of imidacloprid irradiated for 0, 20, 30, and 45 minutes.

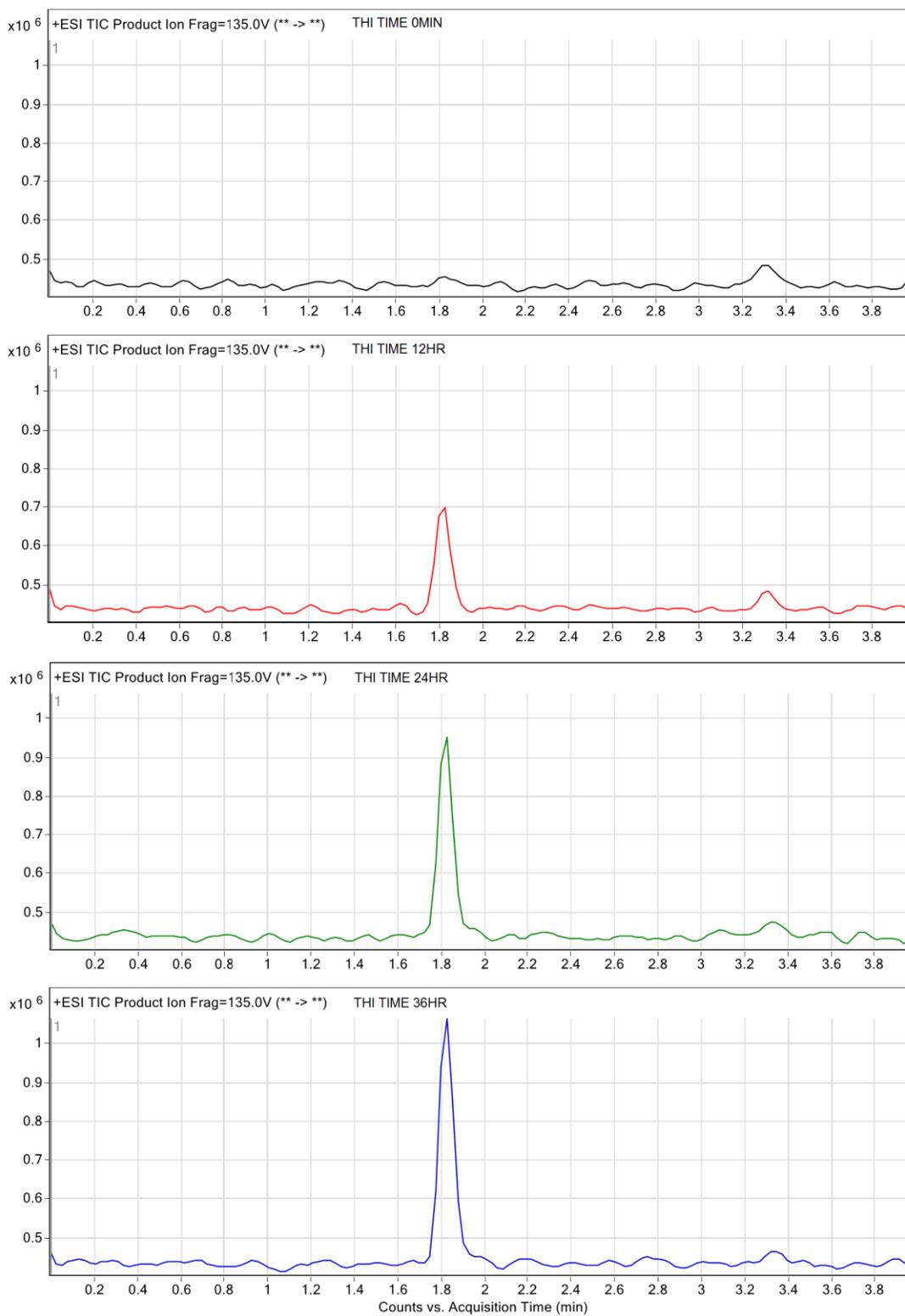
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146 **Figure S8:** Total ion chromatograms of acetamidiprid irradiated for 0, 12, 24, and 36 hours.

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149 **Figure S9:** Total ion chromatograms of thiacloprid irradiated for 0, 12, 24, and 36 hours.

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151 QTOF HRMS data.

152 **Table S3:** Major photolysis products of neonicotinoid insecticides identified by QTOF HRMS.

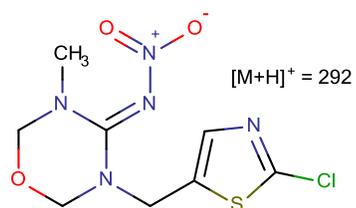
Observed <i>m/z</i>	Chemical formula (<i>m/z</i>)	Error (ppm)	RDB ^a	Literature
Thiamethoxam (292.0272)				
247.0417	C ₈ H ₁₂ N ₄ OSCl (247.0415)	0.9	5	[5]
168.0767	C ₇ H ₁₀ N ₃ O ₂ (168.0768)	-0.3	5	[5]
Clothianidin (250.0155)				
206.0149	C ₆ H ₉ N ₃ OSCl (206.0149)	0.0	4	Not in literature
205.0307	C ₆ H ₁₀ N ₄ SCl (205.0309)	-1.3	4	[6]
Imidacloprid (256.0595)				
212.0586	C ₆ H ₁₁ N ₃ OCl (212.0585)	0.1	6	[7]-[10]
211.0741	C ₉ H ₁₂ N ₄ Cl (211.0745)	-1.9	6	[7]-[10]
189.0769	C ₉ H ₉ N ₄ O (189.0771)	-1.3	8	Not in literature
Acetamiprid (223.0748)				
205.1081	C ₁₀ H ₁₃ N ₄ O (205.1084)	-1.3	7	Not in literature
227.0905	Inconclusive			Not in literature
Thiacloprid (253.0313)				
235.0646	C ₁₀ H ₁₁ N ₄ OS (235.0648)	-0.8	8	Not in literature
257.0469	Inconclusive			Not in literature

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154 ^aRDB = ring double bond equivalent

Thiamethoxam (TMX)

TMX parent ion: m/z 292.0272



TMX photoproduct 1: m/z 247.0417

$C_8H_{12}N_4OSCl$ (247.0415); error = 0.9 ppm; RDB = 5

Urzedo et al., 2007⁵

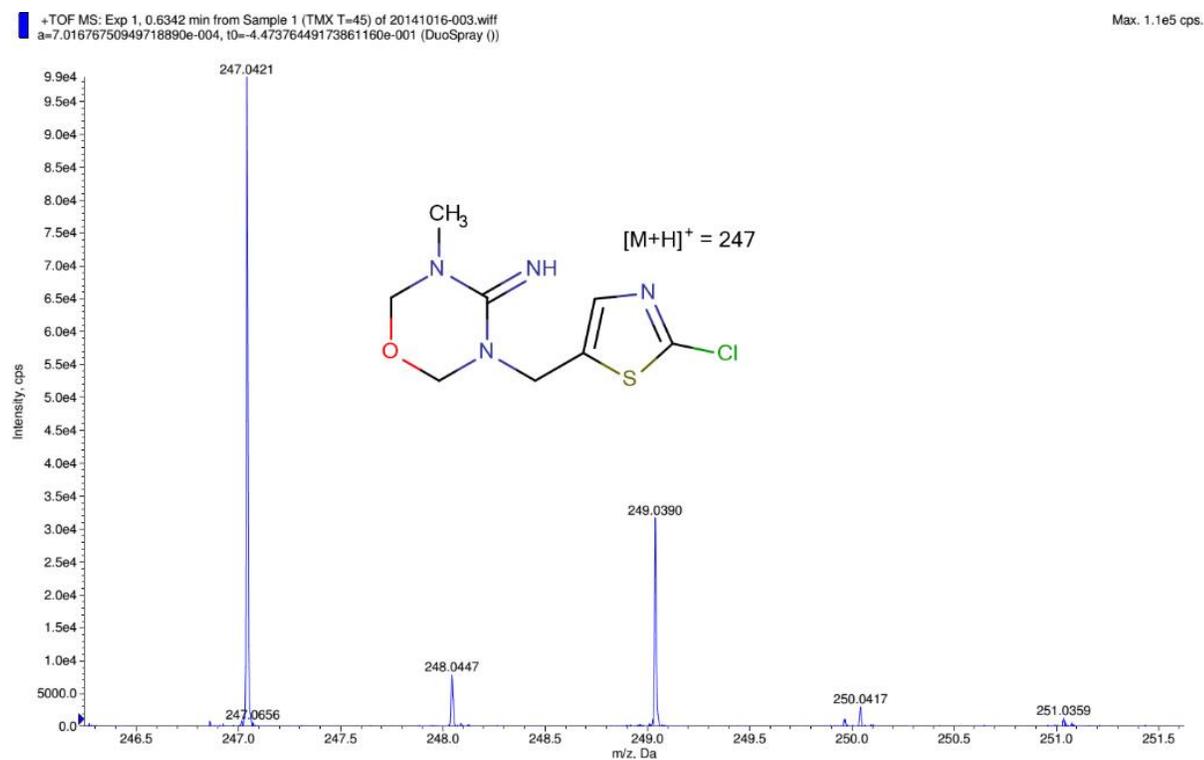


Figure S10: Mass spectrum and proposed structure of TMX photoproduct $m/z = 247$

TMX photoproduct 2: m/z 168.0767

$C_7H_{10}N_3O_2$ (168.0768); error = -0.3 ppm; RDB = 5

Urzedo et al., 2007⁵

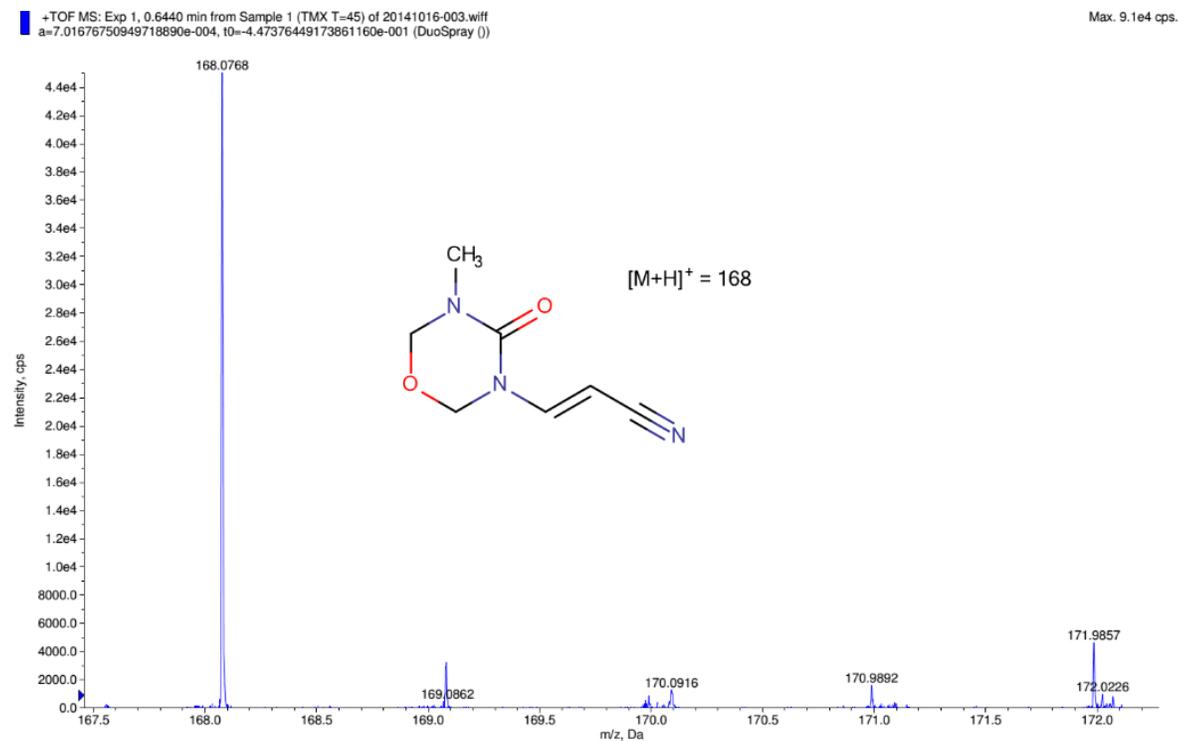
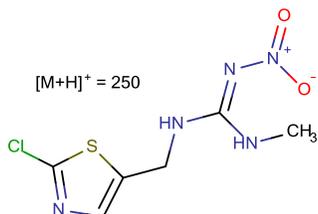


Figure S11: Mass spectrum and proposed structure of TMX photoproduct $m/z = 168$

Clothianidin (CLO)

CLO parent ion: m/z 250.0155



CLO photoproduct 1: m/z 206.0149

$C_6H_9N_3OSCl$ (206.0149); error = 0.0 ppm; RDB = 4

Gong et al., 2012⁶

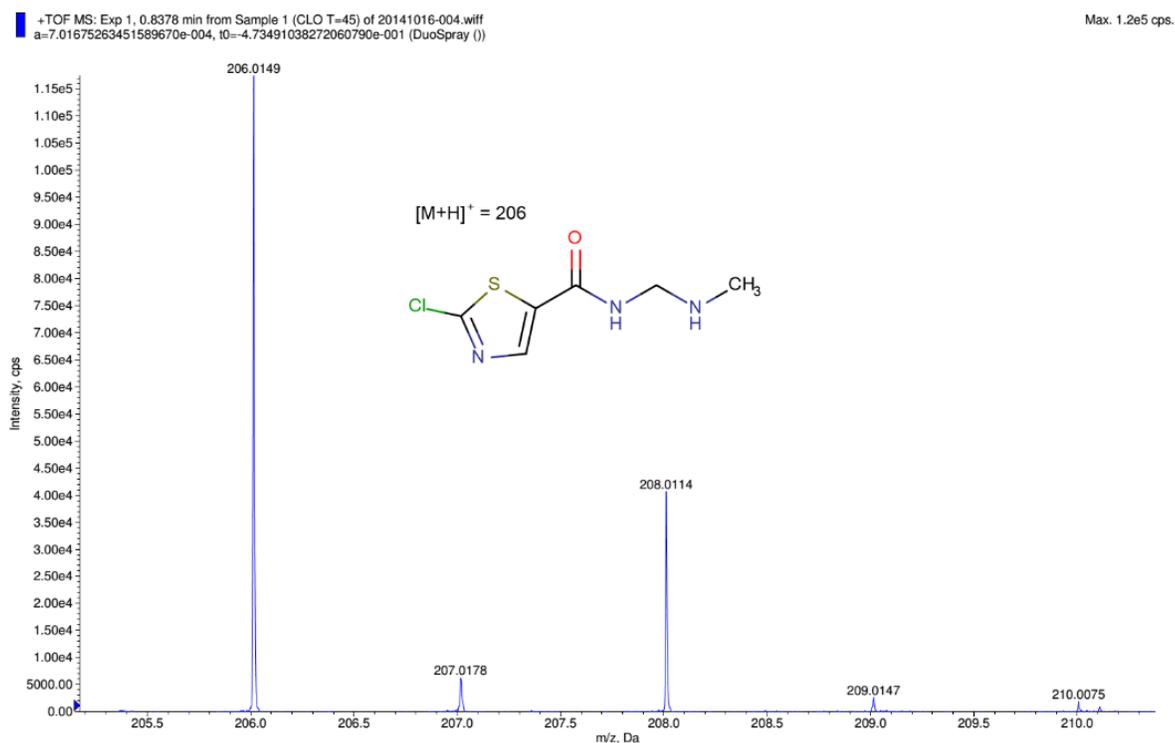
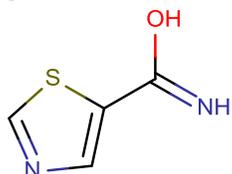


Figure S12: Mass spectrum and proposed structure of CLO photoproduct $m/z = 206$

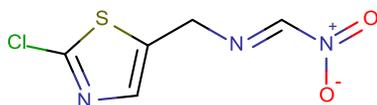
TOF-MS/MS experiment with the photoproduct $m/z = 206$ (spectrum not shown) shows a fragmentation ion at $m/z = 113$ which supports the formation of the McLafferty rearrangement product plus the loss of Cl from the 5-membered ring (shown below). The presence of this fragment further supports our proposed structure for $m/z = 206$.

$[M+H]^+ = 113$



Our proposed structure for the photoproduct $m/z = 206$ (above) differs from the structure proposed by Gong et al., 2012.⁶ Their proposed structure (shown below) does not match the empirical data generated by our HRMS data.

$[M+H]^+ = 206$



$C_5H_5N_3O_2S$ Cl; Gong et al., 2012⁶

CLO photoproduct 2: m/z 205.0307

$C_6H_{10}N_4SCl$ (205.0309); error = -1.3 ppm; RDB = 4

Gong et al., 2012⁶

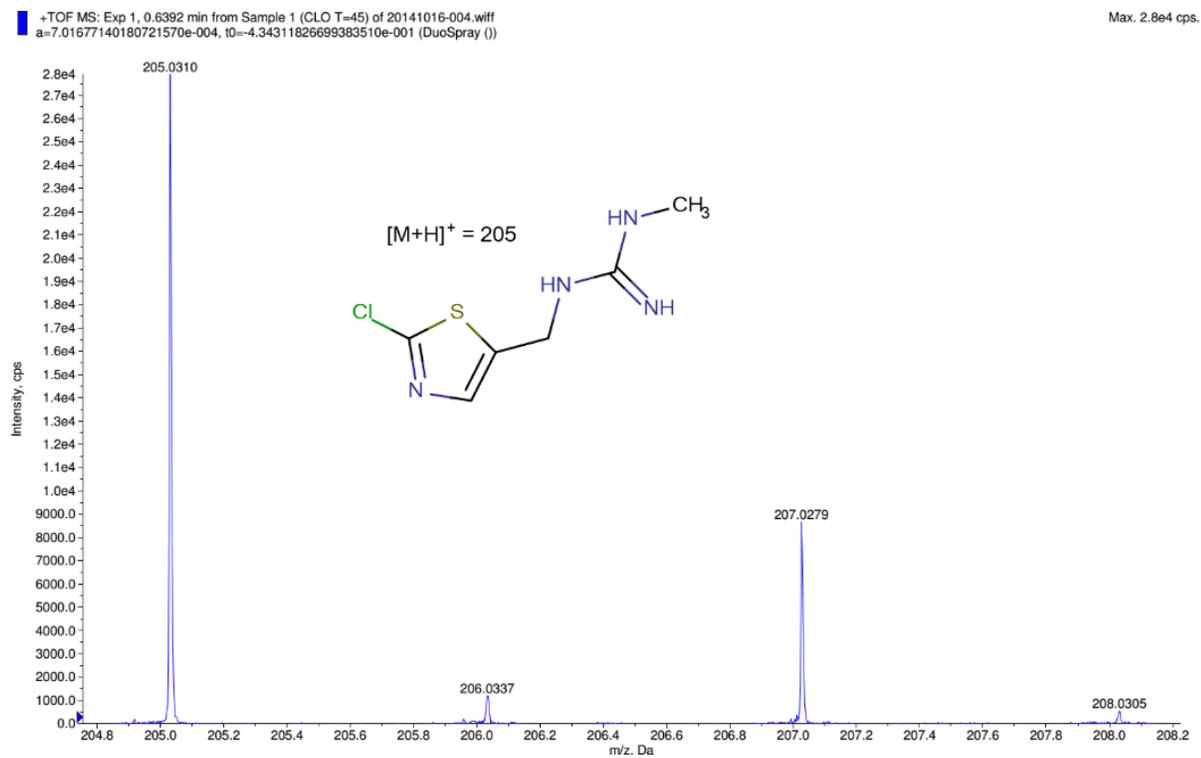
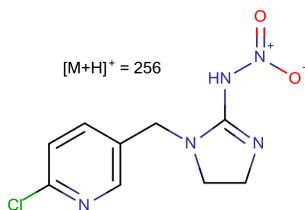


Figure S13: Mass spectrum and proposed structure of CLO photoproduct $m/z = 205$

Imidacloprid (IMI)

IMI parent ion: m/z 256.0595



IMI photoproduct 1: m/z 212.0586

$C_6H_{11}N_3OCl$ (212.0585); error = 0.1 ppm; RDB = 6

Wamhoff and Schneider, 1999;⁷ Redlich et al., 2007;⁸ Schippers and Schwack, 2008 and 2010^{9,10}

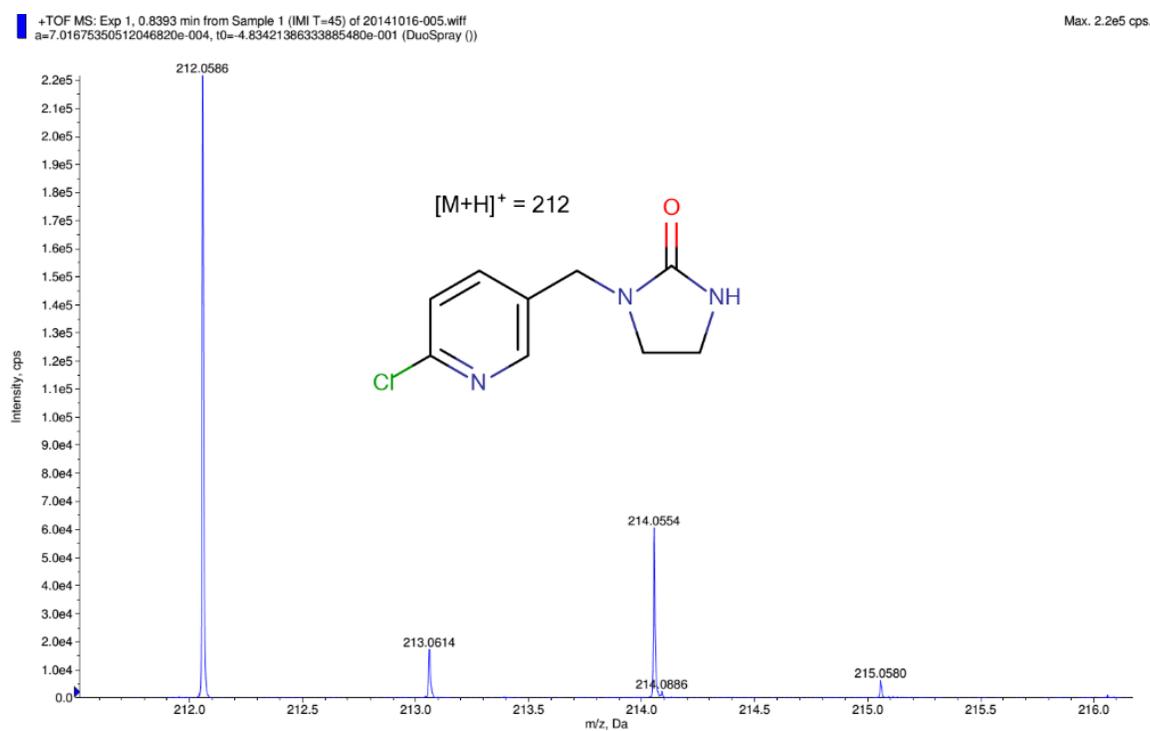


Figure S14: Mass spectrum and proposed structure of IMI photoproduct $m/z = 212$

IMI photoproduct 2: m/z 211.0741

$C_9H_{12}N_4Cl$ (211.0745); error = -1.9 ppm; RDB = 6

Wamhoff and Schneider, 1999;⁷ Schippers and Schwack, 2008 and 2010^{9,10}

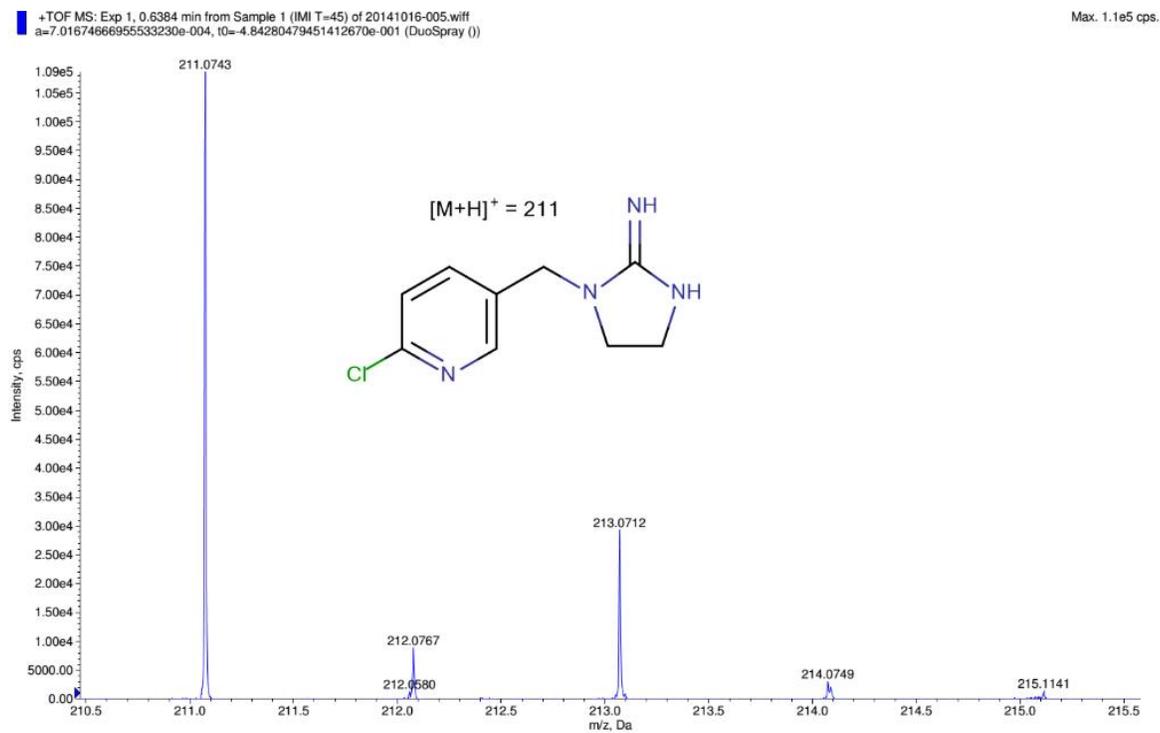


Figure S15: Mass spectrum and proposed structure of IMI photoproduct $m/z = 211$

IMI photoproduct 3: m/z 189.0769

$C_9H_9N_4O$ (189.0771); error = -1.3 ppm; RDB = 8

Photoproduct not found in literature. A proposed structure could not be determined with the available data.

Spectrum from 20141016-005.wiff (sample 1) - IMI T=45, Experiment 4, +TOF MS² (30 - 1000) from 0.502 min
Precursor: 189.1 Da, CE: 35.0

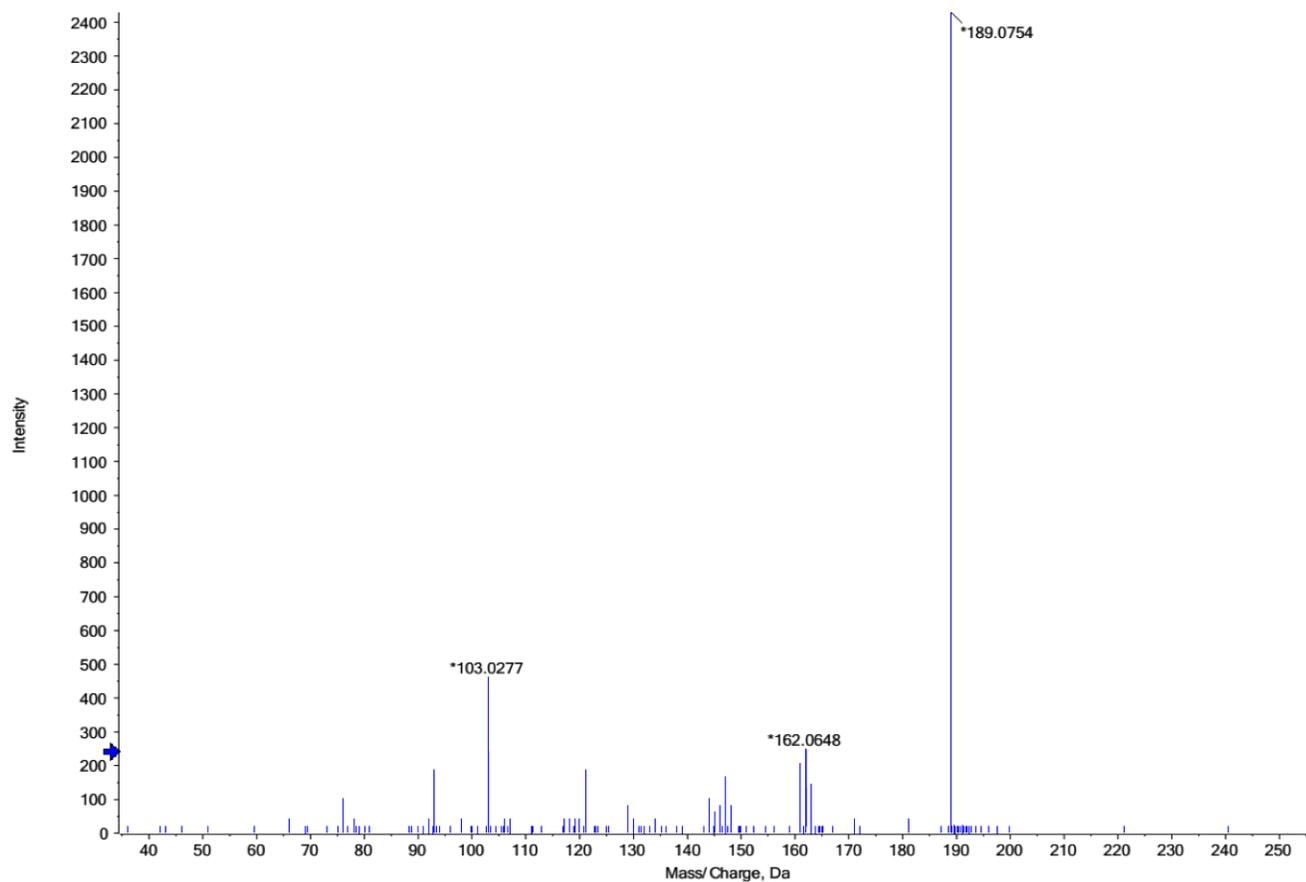
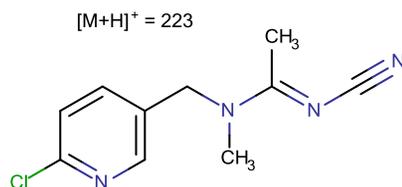


Figure S16: Mass spectrum of IMI photoproduct $m/z = 189$

Acetamiprid (ACE)

ACE parent ion; 223.0748



ACE photoproduct 1: *m/z* 205.1081

C₁₀H₁₃N₄O (205.1084); error = -1.3 ppm; RDB = 7

Photoproduct not found in literature

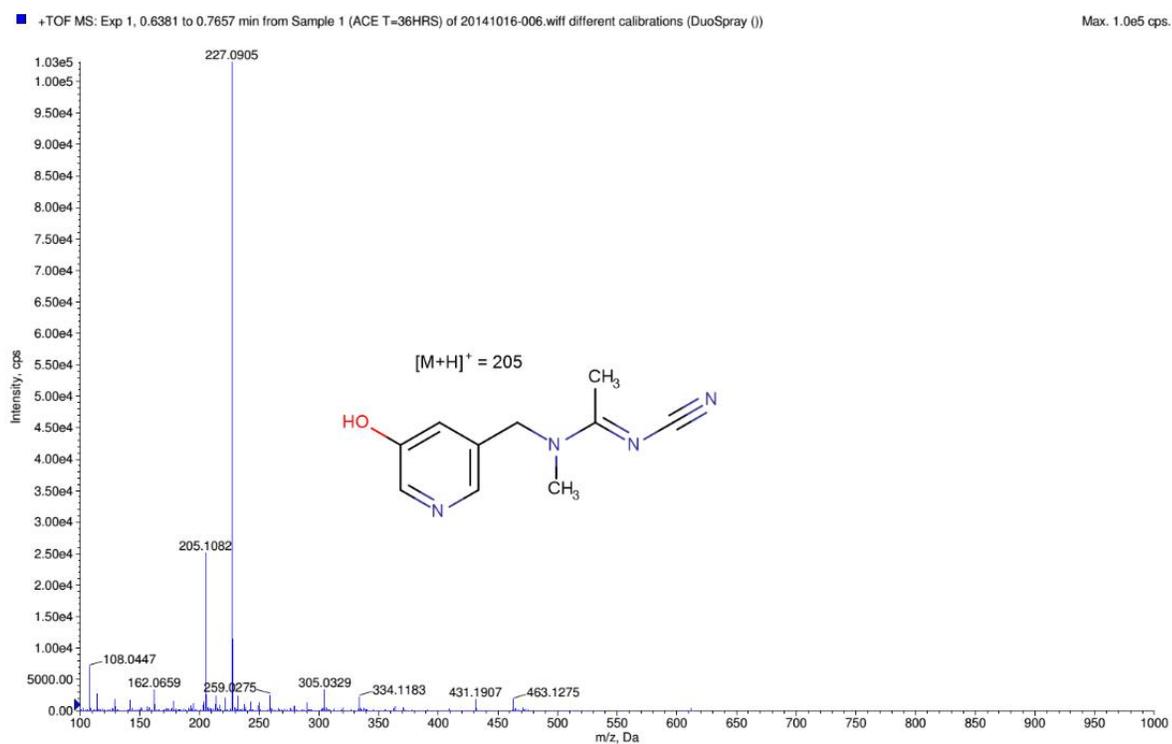
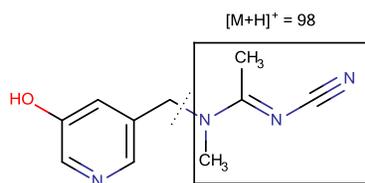


Figure S17: Mass spectrum and proposed structure of ACE photoproduct *m/z* = 205

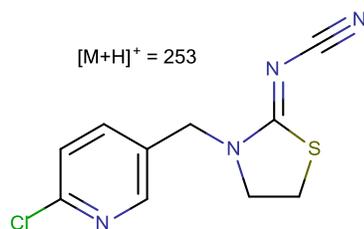
TOF-MS/MS experiment with the photoproduct $m/z = 205$ (spectrum not shown) shows a fragmentation ion at $m/z = 98$. This is a plausible fragment of the photoproduct $m/z = 205$, and supports the proposed structure.



$m/z = 227.0905$ was also observed in large abundances only in the irradiated samples, however a logical structure could not be deduced from the HRMS empirical data.

Thiacloprid (THI)

THI parent ion: m/z 253.0313



THI photoproduct 1: m/z 235.0646

$C_{10}H_{11}N_4OS$ (235.0648); error = -0.8 ppm; RDB = 8

Photoproduct not found in literature

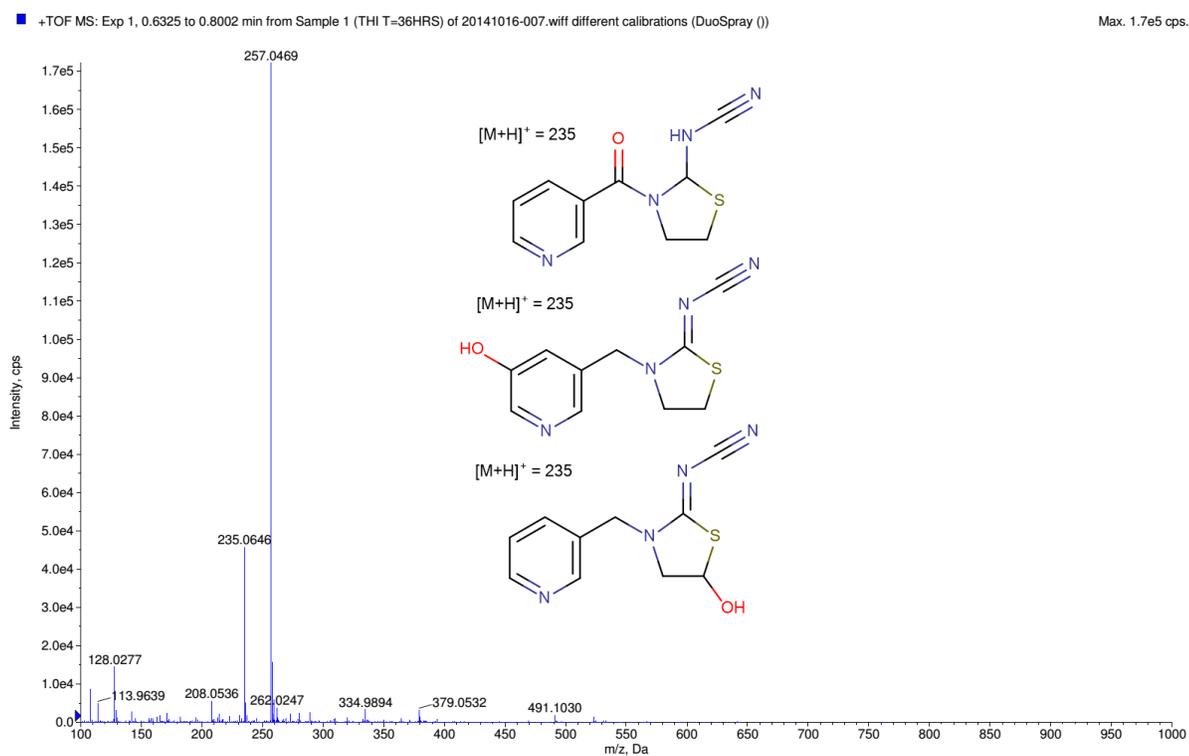


Figure S18: Mass spectrum and proposed structures of THI photoproduct $m/z = 235$

TOF-MS/MS spectra (not shown) of the photoproduct $m/z = 235$ was inconclusive in determining between the three proposed structures. Given the structural similarity between the three, the mass fragments at $m/z = 80$, 128, and 108 could have corresponded to either structure.

$m/z = 257.0469$ was also observed in large abundances only in the irradiated samples, however a logical structure could not be deduced from the HRMS empirical data.

References

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