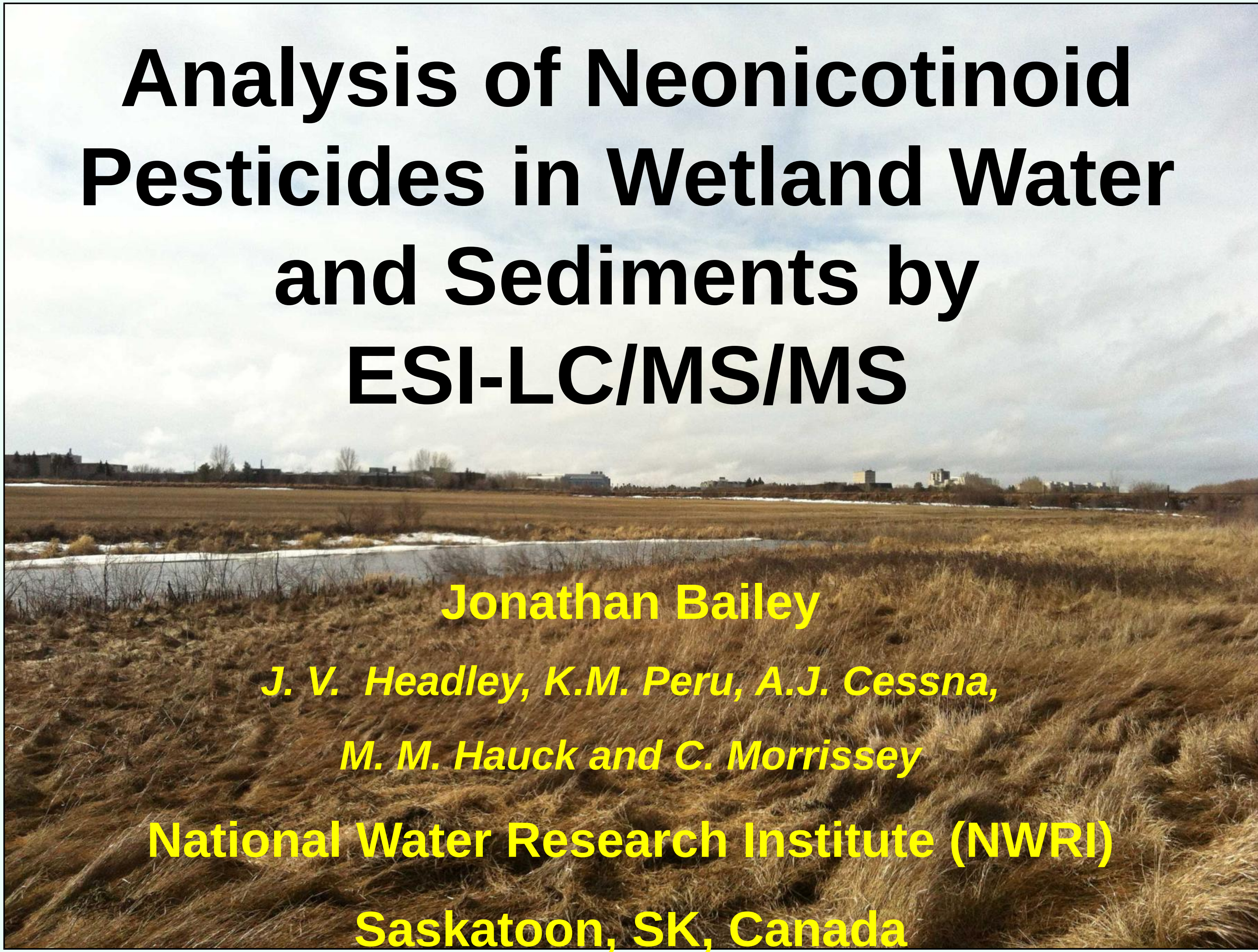

Analysis of Neonicotinoid Pesticides in Wetland Water and Sediments by ESI-LC/MS/MS

Jonathan Bailey

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M. M. Hauck and C. Morrissey

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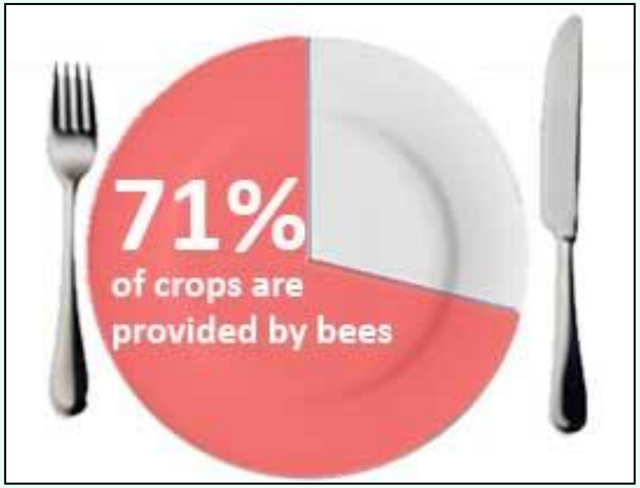
Saskatoon, SK, Canada

Introduction

- Neonicotinoid pesticides are neurotoxins that bind irreversibly to nicotinic acetylcholinesterase receptor
- Used to control aphids, beetles and termites on potato, ginger, garlic and many other fruits and vegetables
- Used for seed-coatings on corn, cotton, canola and cereal crops
- Contributing factor in bee mortality
- Potential for leaching from agricultural lands into surrounding wetlands

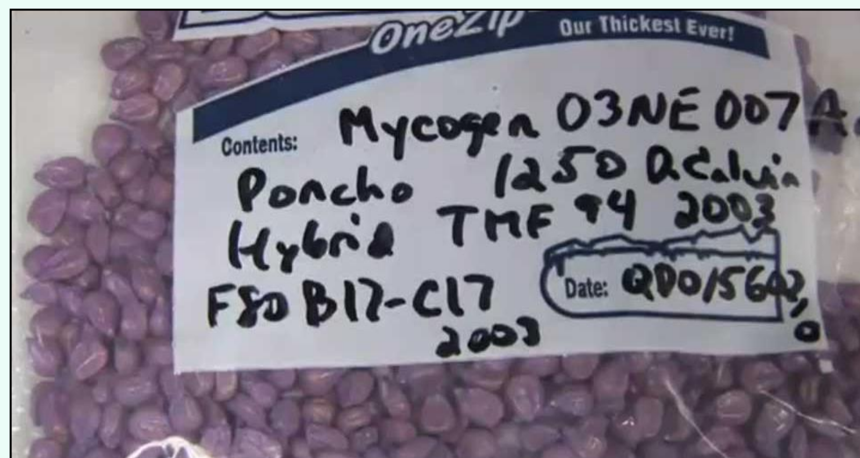
"I may bee small but I play a big role in your life."



- EPA is currently in the process of re-evaluating the registration of clothianidin (EPA-HQ-OPP-2011-0865)
- EFSA issued a 2 year moritorium on the use of clothianidin, imidacloprid and thiamethoxam on crops that are attractive to bees
- Canada’s PMRA is currently reviewing the certification of the neonicotinoids

Neonicotinoids being applied to corn

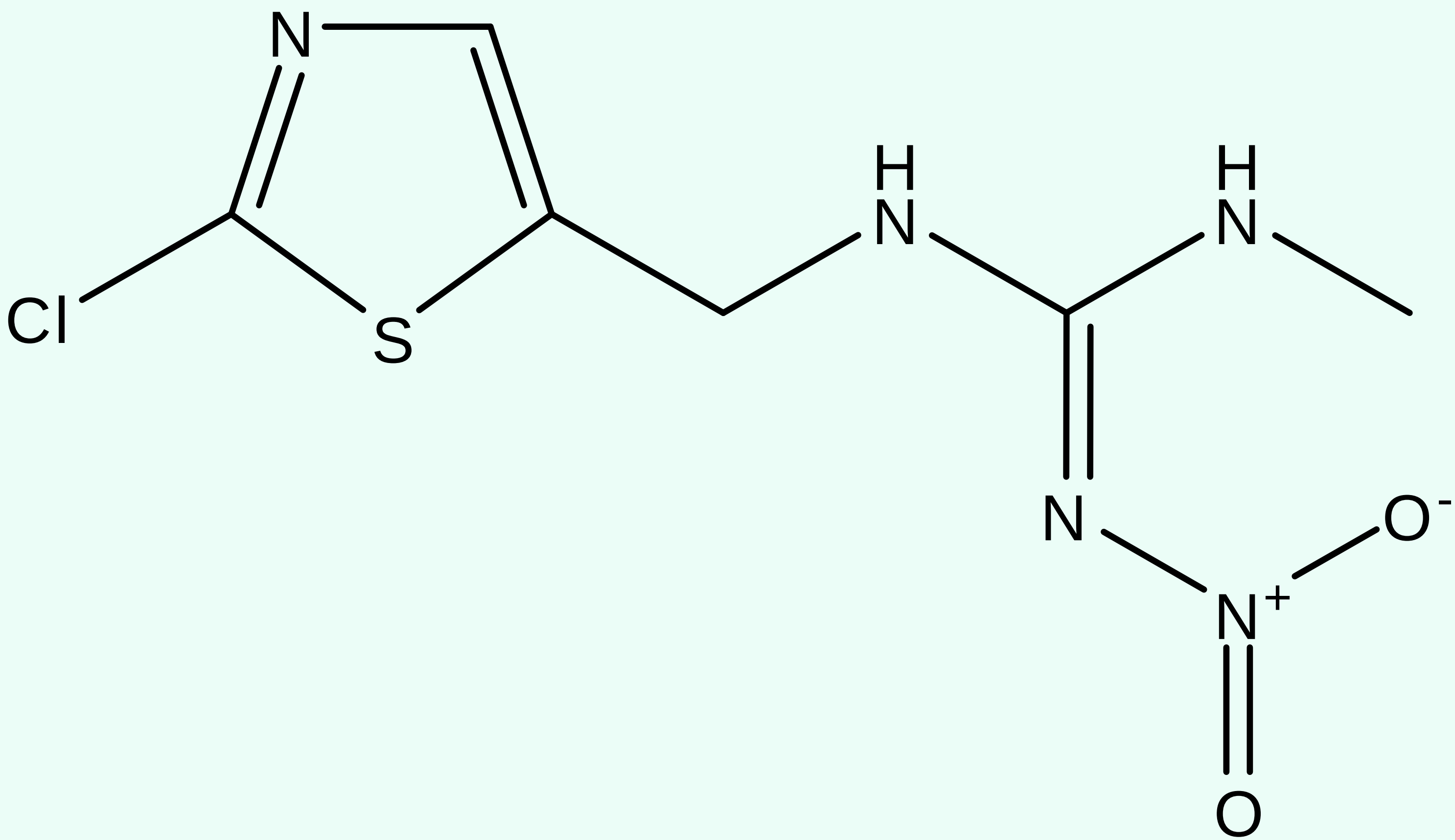
Clothianidin Coated Corn Seeds



Clothianidin
Chemistry & Toxicity

- 3 ng/bee LD50
- Soil half-life is estimated 2-3 years
- Solubility in water: 327 mg/L at 20^{o}C
- Low mammalian toxicity provides alternative to organophosphate, carbamate and pyrethroid pesticides
- Acute risk to honey bees via dust drift from seed treatments

Clothianidin "Poncho"



Should Canada follow the European lead?

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Re-evaluation Note REV2012-02, Re-evaluation of Neonicotinoid Insecticides

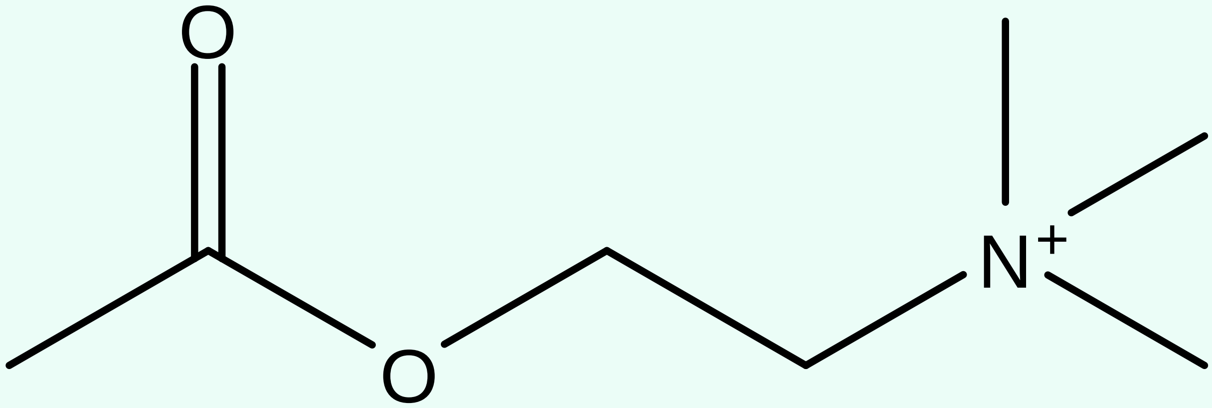
Pest Management Regulatory Agency
12 June 2012
ISSN: 1925-0649 (PDF version)
Catalogue number: H113-5/2012-2E-PDF (PDF version)

Purpose of Study

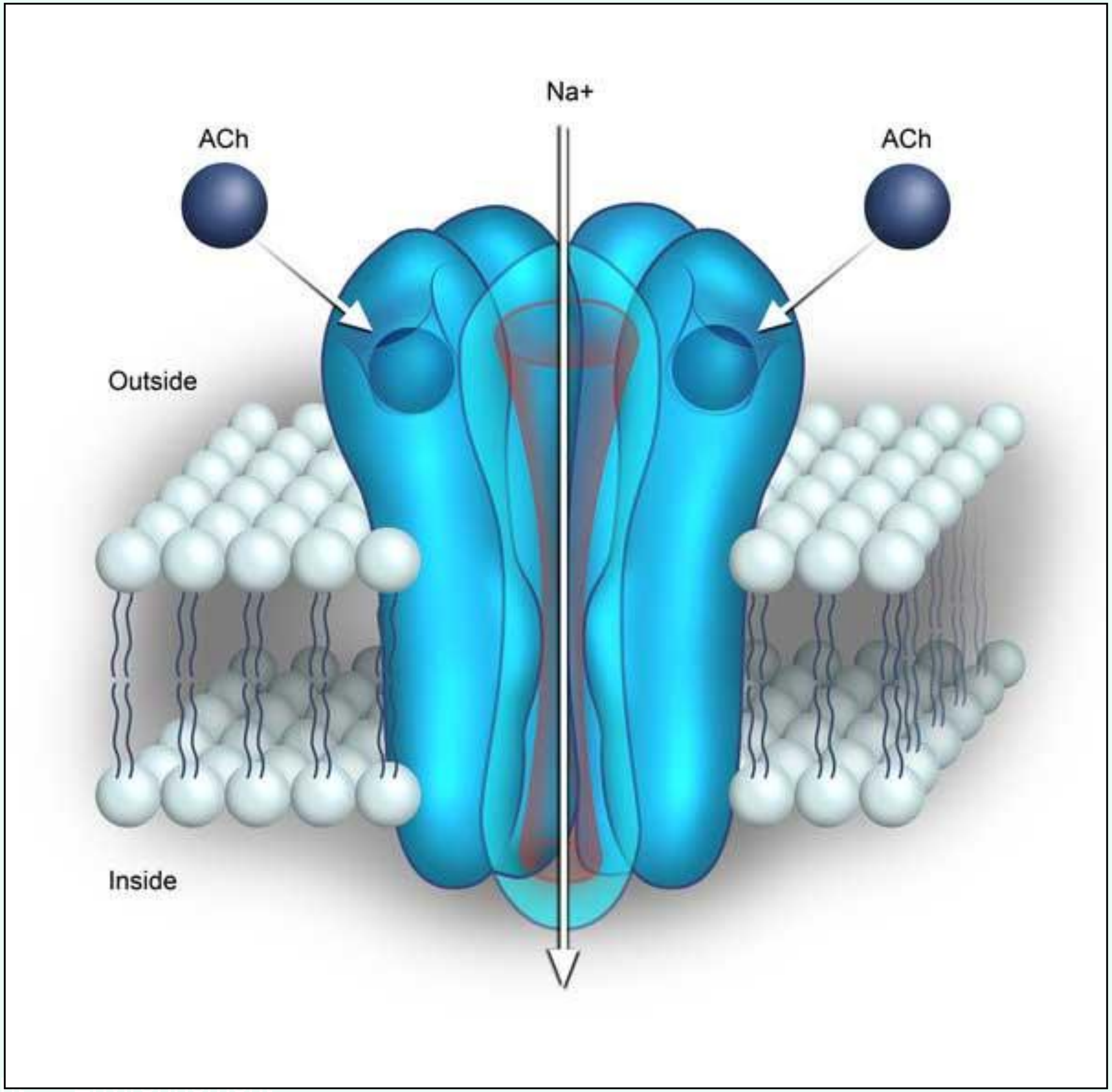
- To determine whether neonicotinoids are being transported to wetlands adjacent to fields where pesticides are applied
- Determine if observed neonicotinoid levels are having an impact on aquatic insects and ecosystems
- Determine if neonicotinoids pose a threat to non-target species such as birds & bees

Why is clothianidin so toxic?

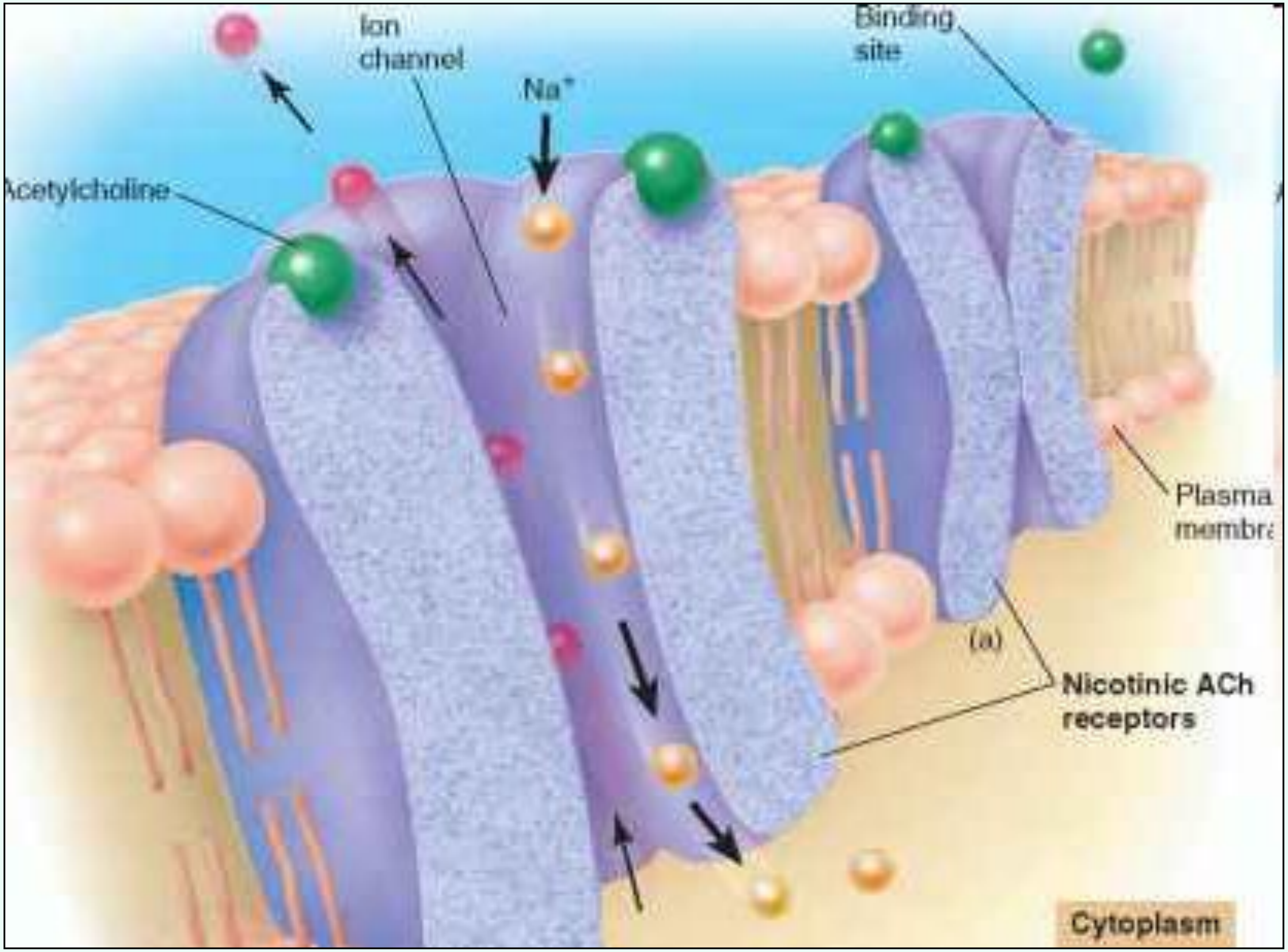
Acetylcholine (ACh)

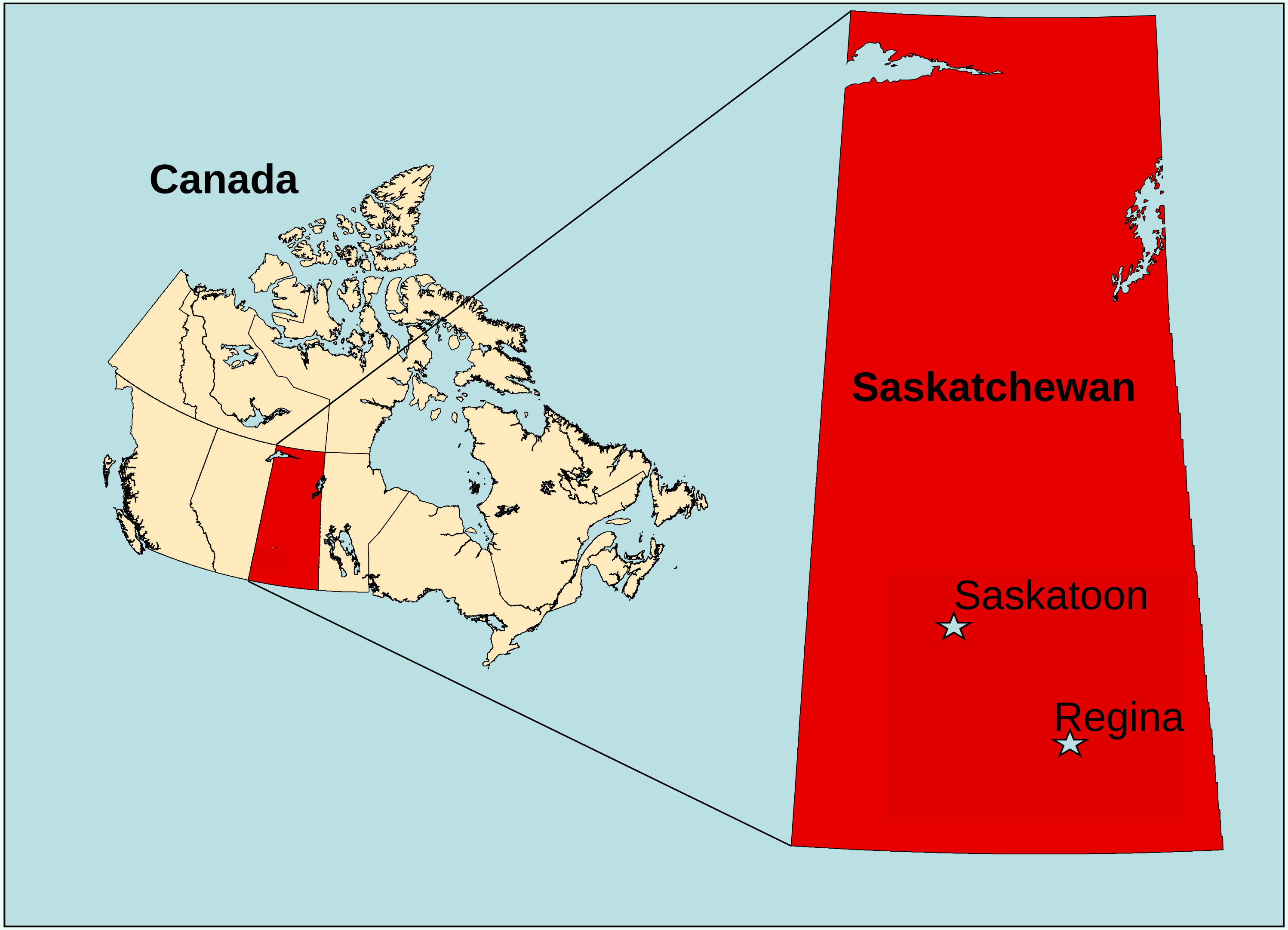


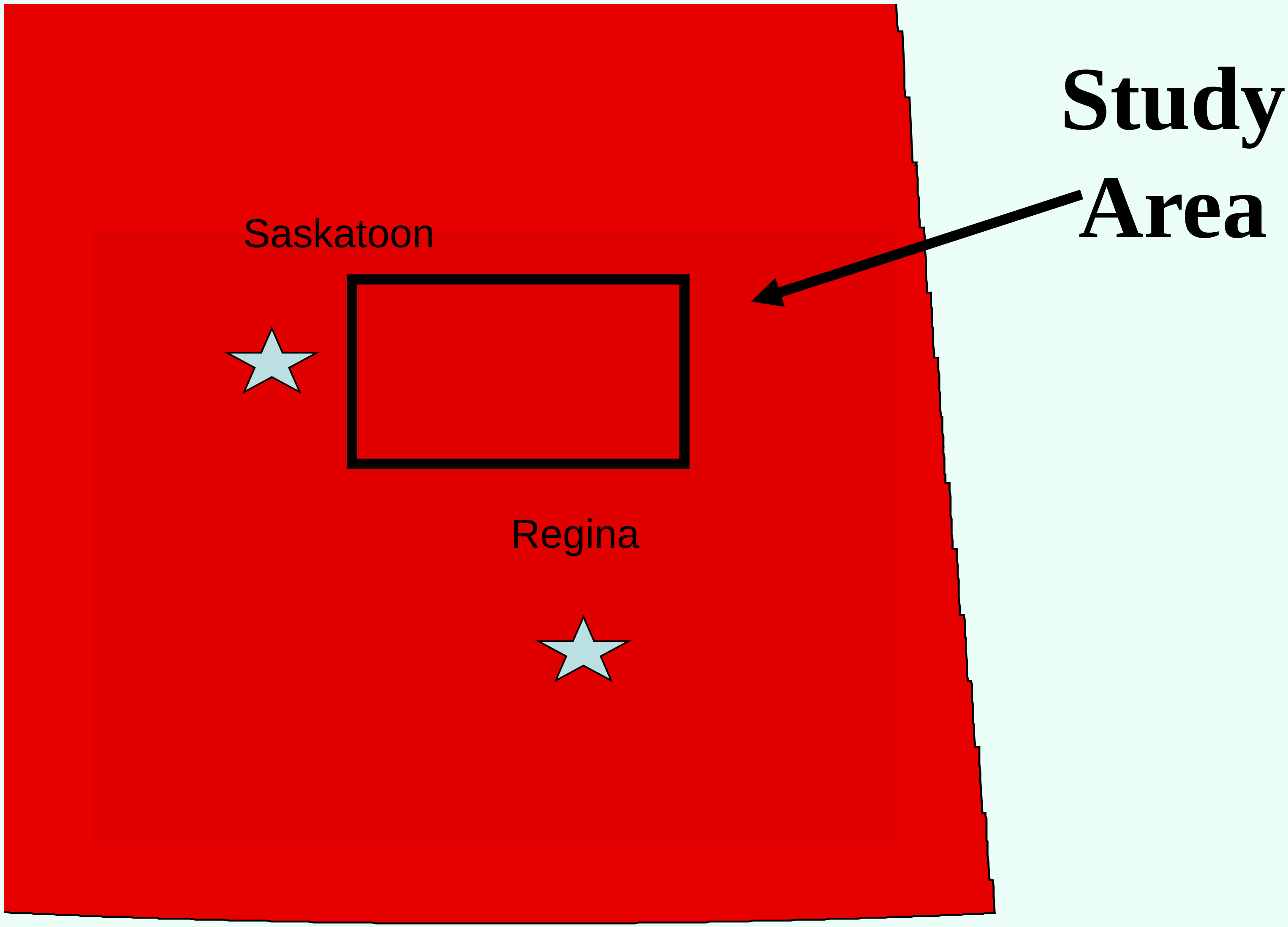
Acetylcholine Gated Ion-Channel



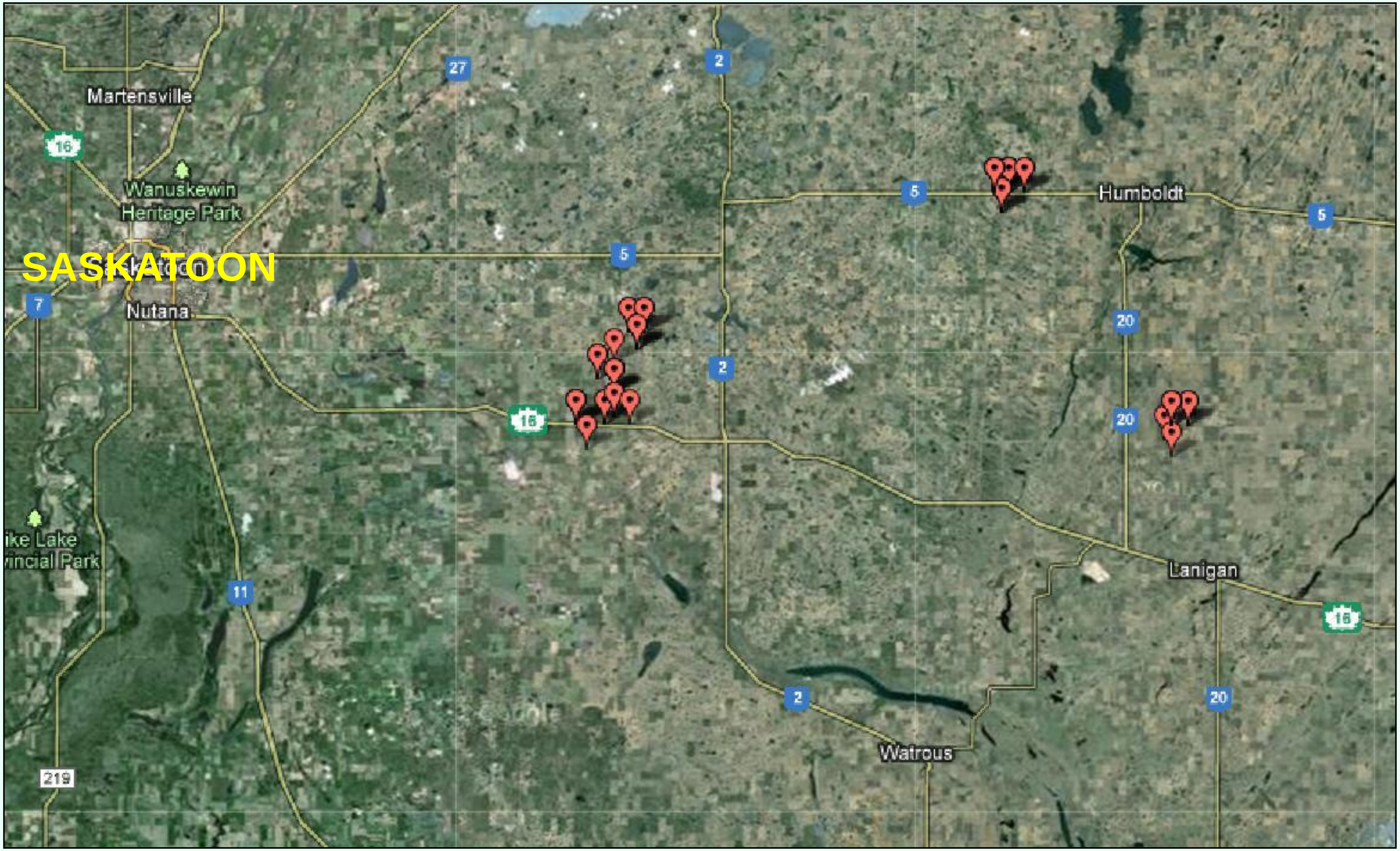
Nicotinic Acetylcholine Receptor




Canada
Saskatchewan
Saskatoon
Regina



Study Area
Saskatoon
Regina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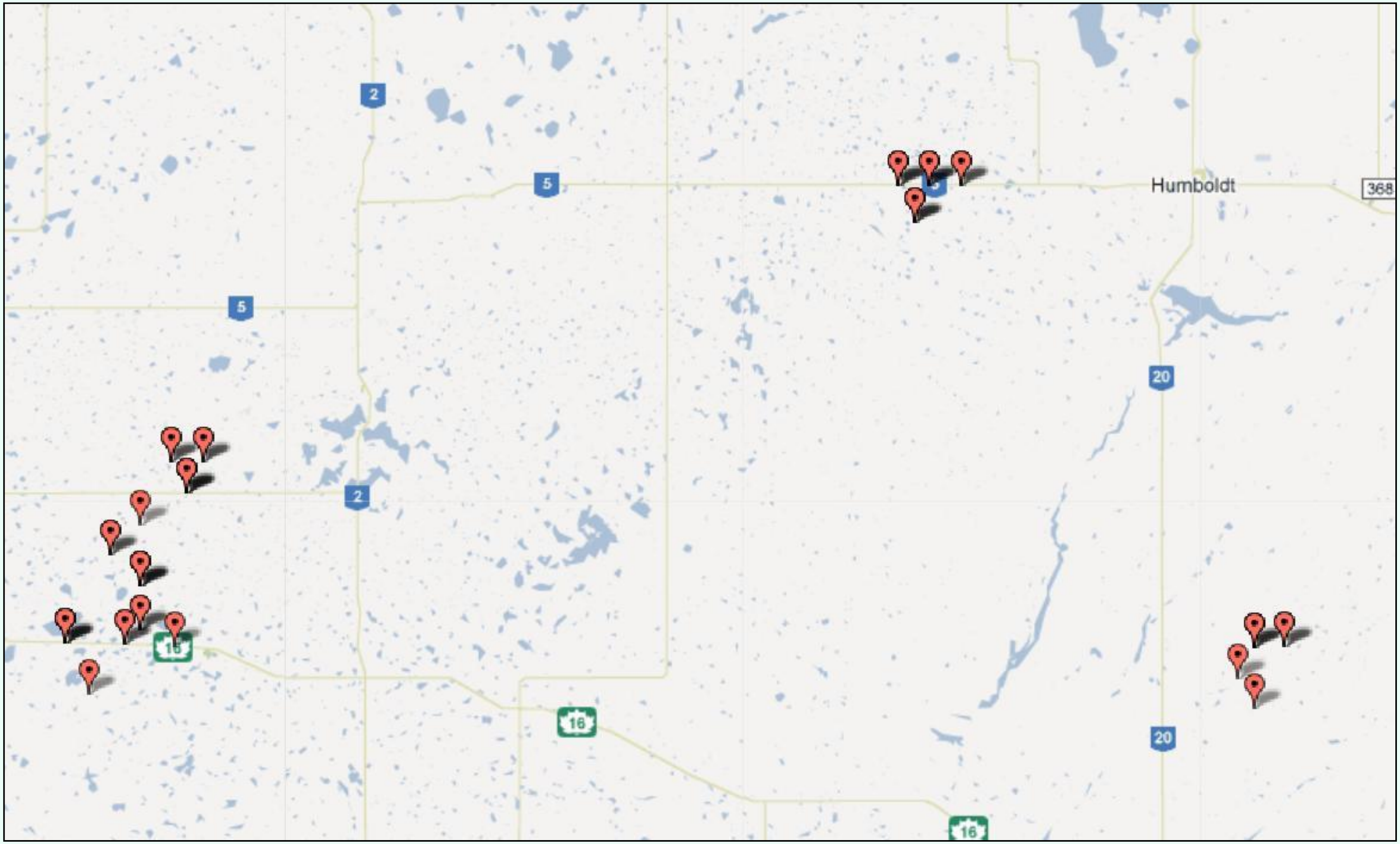

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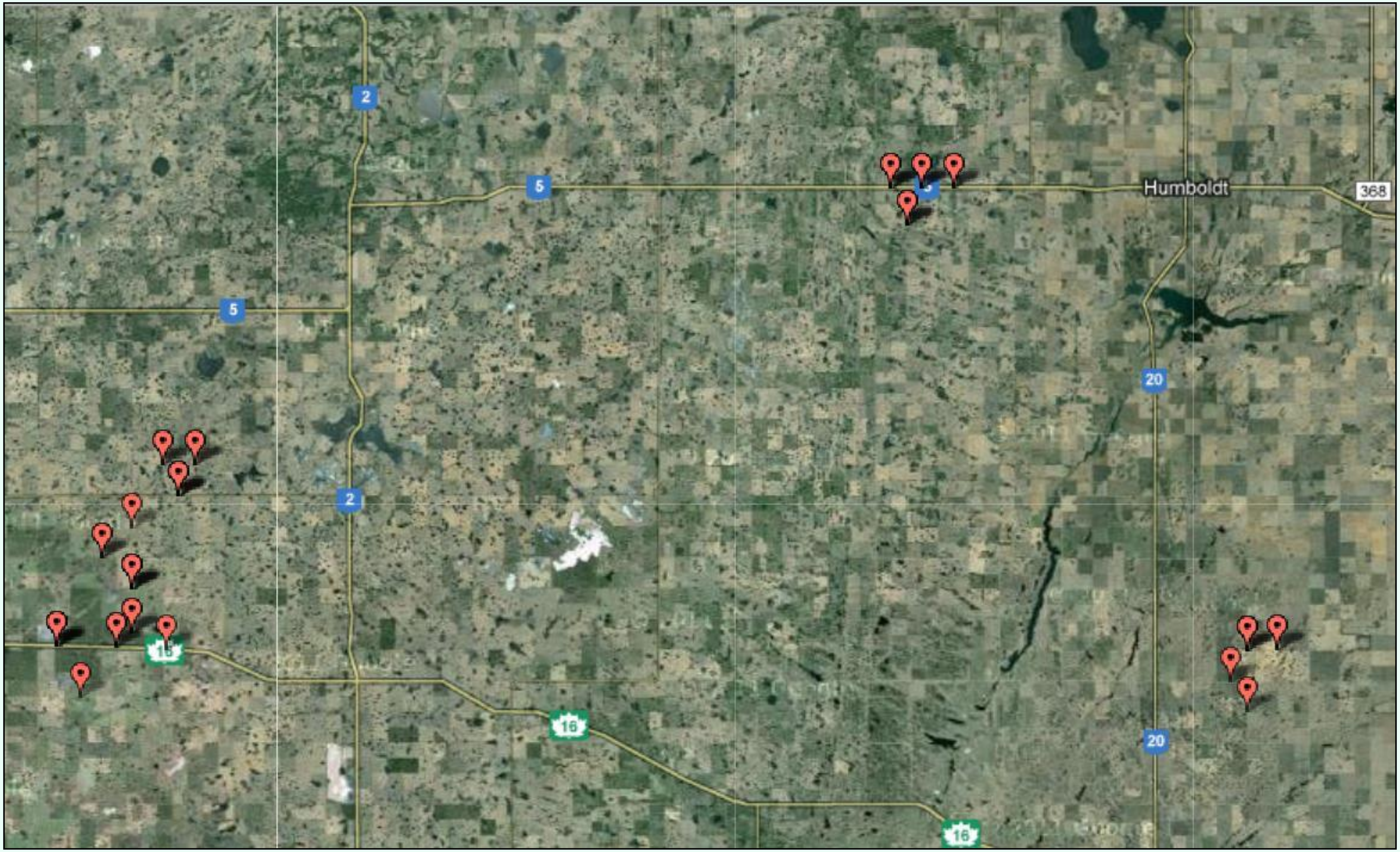
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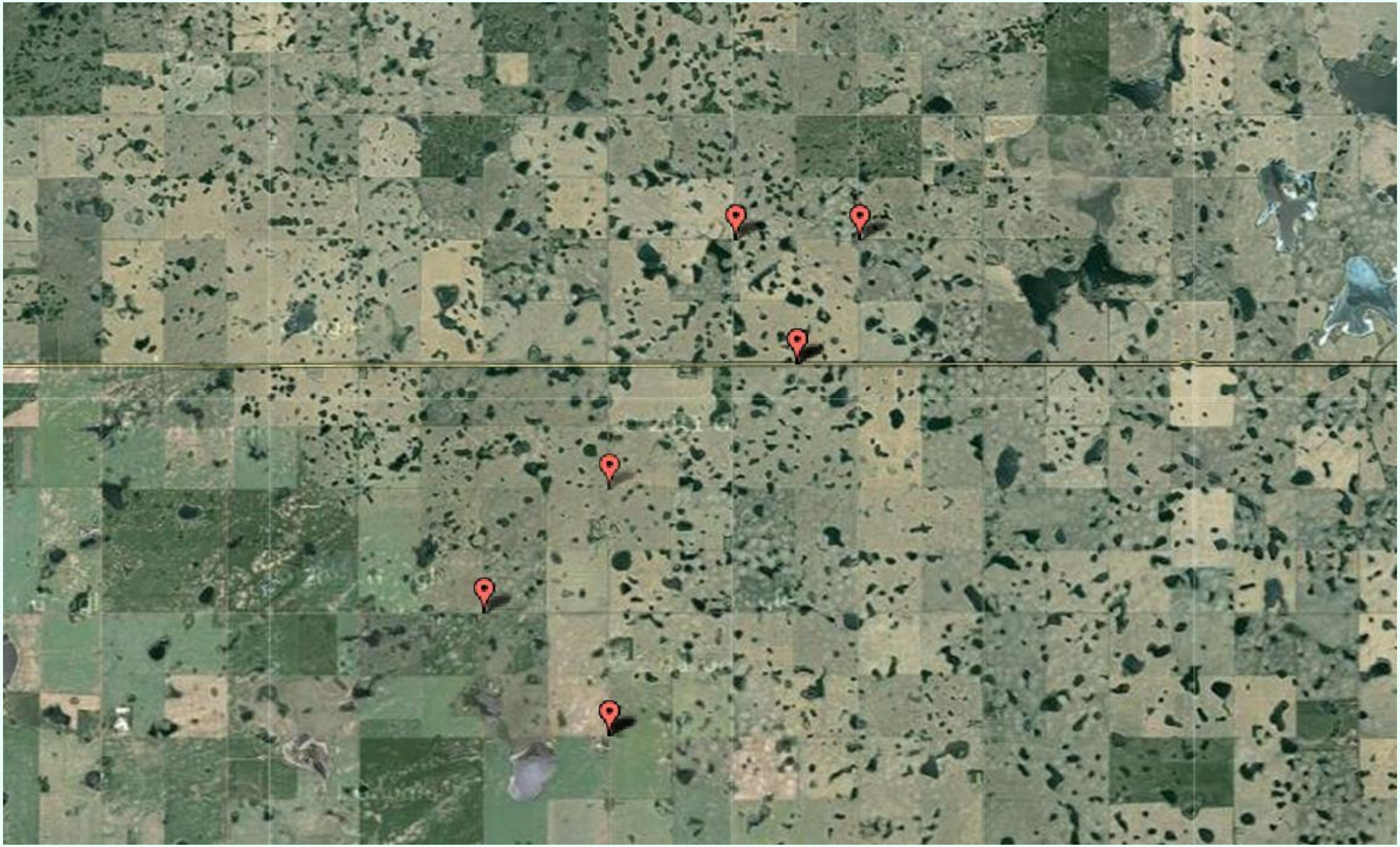
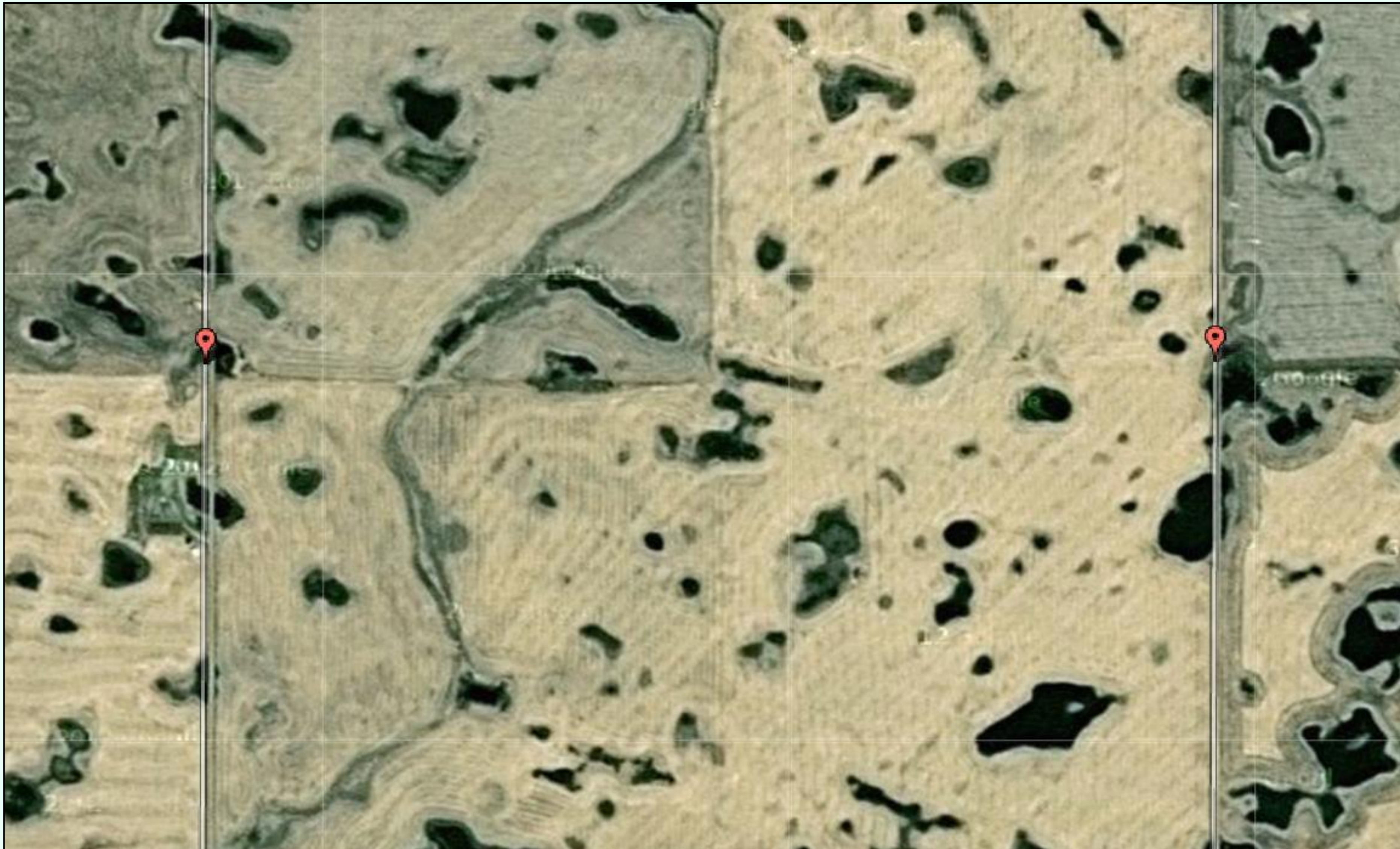




Pre-seeding Wetland Samples collected in April 2012

- 27 clothianidin hits out of 53 wetlands adjacent to fields where canola was grown in 2011
- Range: 2.8-105.5 ng/L
- Average: 22.6 ng/L

Post-seeding Wetland Samples collected in July 2012

- 39 clothianidin hits in 61 wetlands adjacent to fields where canola was sown in the spring of 2012
- Range: 5.1-2280 ng/L
- Average: 162.9 ng/L

Summary of Results

- Clothianidin is the most persistent neonicotinoid residue and was present in wetlands in agricultural fields as a result of either snowmelt run-off or other transport mechanisms
- No neonicotinoid residues were detected in native prairie wetlands
- Imidacloprid, acetamiprid, thiamethoxam were found infrequently and at low levels
- Clothianidin hits occurred primarily near fields where canola had been seeded the previous year
- Wheat, barley, oats, peas and pasture land showed mininmal hits for neonicotinoids

Environment
Canada

Steps used to diminish Matrix Effects and Enhance Accuracy

- Fortify river, wetland and de-ionized water to determine SPE recovery efficiency
- Run 1 fortified QC sample (50ng/L) within every set of 9 real samples
- Use Imidacloprid-d_4 to compensate for variability in electrospray ionization efficiency
- Run calibration standards before and after each set of 10 analytical samples to minimize variability in instrument sensitivity
- Insert "solvent blank" after 1st set of calibration standards to monitor and eliminate carry-over
- Use 5, 10, 25, 50 ug/L calibration curve to minimize carry-over and fit concentration range of the majority of the sample hits

Solid Phase Extraction

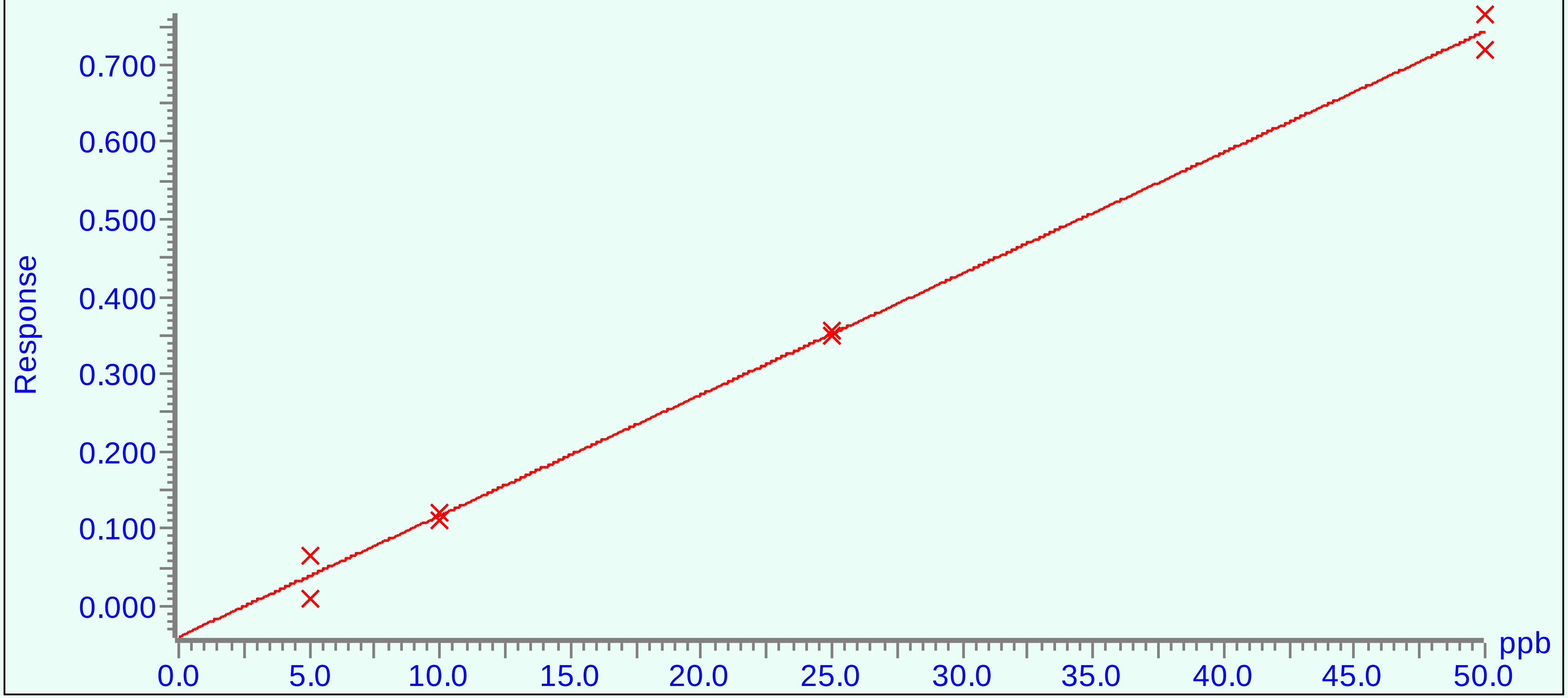
- Waters Oasis HLB (225mg)
- Conditioning with 10mL MeOH & 10mL H_2O
- 500mL water sample in graduated cylinder
- Rate of addition: ~1mL/minute
- Wash cartridge with 5mL of Milli-Q H_2O
- Dried on house vaccum for 5 minutes
- Elution with 10mL MeOH
- Evaporation under N_2 gas in water bath
- Reconstitution in 500µL Milli-Q water

SPE Recoveries

Neonicotinoid	HLB Spike (10 ng L^{-1}) River Water % Recovery & (RSD) (n=8)	HLB Spike (50 ng L^{-1}) Matrix samples % Recovery & (RSD) (n=8)	HLB Spike (10 ng L^{-1}) Milli-Q H2O % Recovery & (RSD) (n=8)
Imidacloprid	84.5 (7.5)	86.2 (4.4)	94.8 (2.1)
Thiamethoxam	89.5 (5.5)	92.4 (2.3)	94.3 (3.7)
Acetamiprid	95.1 (4.4)	92.2 (4.6)	89.0 (3.1)
Clothianidin	87.5 (7.2)	75.8 (5.1)	78.0 (8.5)

Calibration Curve

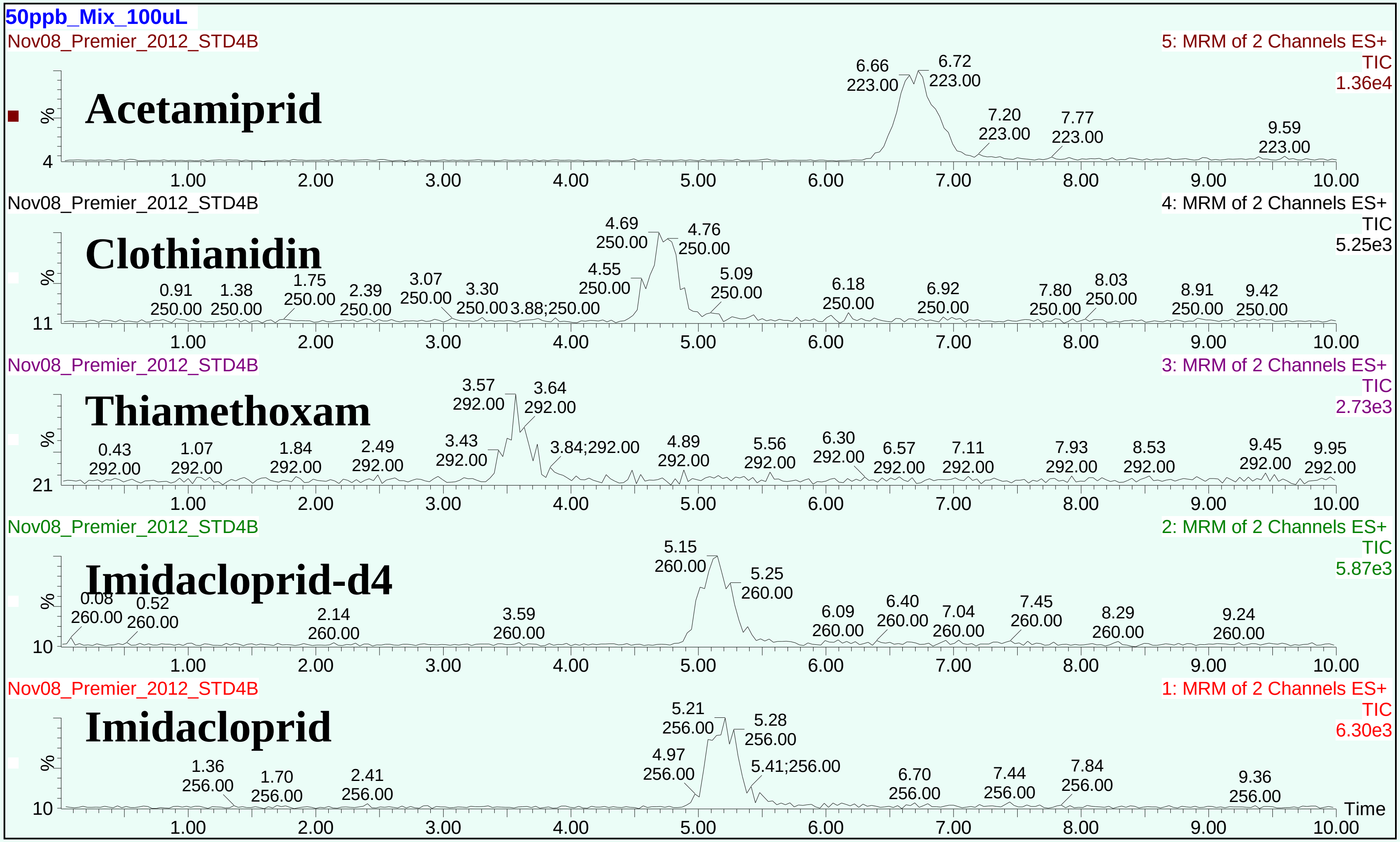
Compound name: Clothianidin(TIC)
Correlation coefficient: r = 0.997681, r^2 = 0.995367
Calibration curve: 0.0156513 * x + -0.0395516
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None



Liquid Chromatographic Parameters

- LC Instrument: Waters 2695 Alliance HPLC System
- Column: Waters Xterra: C8: 2.1mm x 100mm
- Particle size: 3.5 µm
- Column temp: 30 ^{o}C
- Solvent A: 100% (H_2O)
- Solvent B: 90:10 (Acetonitrile / H_2O)
- Modifier: 0.1 % formic acid
- Flow rate: 200 µL/min
- Separation mode: Isocratic (80%A / 20%B)
- Injection volume: 20 µL

Chromatogram of 50 µg/L Standard



MS/MS Parameters

- Instrument: Micromass Quattro Premier
- Source temp: 90°C
- Electrospray Interface: ESI(+)
- Cone Voltage: 30 V
- Capillary voltage setting: 3.00 kV
- Hexapole 1: 7.9 V
- Hexapole 2: 0 V
- Aperture Voltage: 0 V
- Desolvation temp: 220°C

MS/MS Parameters Cont.

- Desolvation gas: N_2 @ 488 L h^{-1}
- Cone gas: N_2 @ 154 L h^{-1}
- Quadrupole 1: unit mass resolution
- Quadrupole 3: ~2.0 m/z resolution
- Photomultiplier voltage: 689 V
- Collision gas: Argon
- Collision gas pressure: 3.04×10^{-4} mbar
- Collision energy: 15 eV

ESI(+) Collision-induced Dissociation

Neonicotinoid Pesticides	MRM Transition Monitored (m/z)	Cone Voltage (V)	Collision Energy (eV)	Retention Time (min)
Imidacloprid	256.0 > 209.3	27	18	5.2
	256.0 > 175.2	27	18	5.2
D4-Imidacloprid	260.1 > 213.1	27	18	5.2
	260.1 > 179.2	27	18	5.2
Thiamethoxam	292.1 > 211.0	27	18	3.6
	292.1 > 181.0	27	18	3.6

ESI(+) Collision-induced Dissociation

Neonicotinoid Pesticides	MRM Transition Monitored (m/z)	Cone Voltage (V)	Collision Energy (eV)	Retention Time (min)
Clothianidin	250.1 > 169.0	19	18	4.7
	250.1 > 131.9	19	18	4.7
Acetamiprid	223.1 > 126.1	27	18	6.7
	223.1 > 56.0	27	18	6.7

Conclusions

- ESI-LC/MS/MS along with SPE provide an excellent analytical tool for detecting neonicotinoid insecticide residues in prairie wetlands
- Canola seeds treated with clothianidin account for majority of hits found in wetlands adjacent to agricultural fields

Future Work

- Wetlands sampled in 2012 will again be sampled in 2013
- Pre & post-seeding samples will be analyzed using the existing analytical methodology – unless??
- Improve methodology for the sampling and analysis of sediments

Acknowledgements: Literature

1. “Determination of Neonicotinoid Pesticides residues in agricultural samples by solid-phase extraction combined with liquid chromatography-tandem mass spectrometry ” by Wen Xie et.al.; Journal of Chromatography A vol. **1218** (2011) pp. 4426–4433
2. “Thiamethoxam is a neonicotonoid precursor converted to clothianidin in insects and plants” by Ralf Nauen et.al.; Pesticide Biochemistry and Physiology, vol. **76** (2003) pp. 55-69
3. “Liquid chromatography-mass spectrometry identification of imidacloprid photolysis products” by Tao Ding et.al ; Microchemical Journal, vol. **99**, (2011) pp. 535-541

Allan Cessna

Matthew Hauck

Christy Morrissey

Kerry Peru

Dr. John Headley

Acknowledgements

- Anson Main, PhD student in School of Environment and Sustainability, U. of S. for selecting and sampling wetlands
- WCTOW organizing committee for giving me the opportunity to speak
- You – for your patience and attention!


THE END

Sediment Method Extraction Parameters

- Sample size: 5.0g sediment sample glass centrifuge tube
- Extraction solvent: 20mL ACN (2x 10mL)
- Sonicated for 30 minutes with 10mL ACN
- Centrifuged for 15 minutes (5000 RPM)
- Drawed off supernatant to labeled graduated test tube
- Repeated sonication with another 10mL ACN followed by centrifuge
- Dried test tube to ~ 0.5mL using N_2 gas in water bath
- After dry down, added other 10mL ACN from extraction and dried again to ~0.5mL
- Added 500uL H2O to test tubes and vortex mixed
- Added to 2mL volumetric flask
- Added 500uL H2O to test tubes and vortex mixed
- This was added to 2mL volumetric and brought to volume with DI H2O
- Spike 20uL 5 ppm Internal Standard + mixed flask
- Transferred to HPLC vial +Analyzed on LC/MS/MS

Sediment QC Sample Recoveries

Neonicotinoid (transitions used for Quantification purposes)	ACN Solvent Extraction Spike (0.02 mg kg^{-1}) n = 18 % Recovery + RSD
Imidacloprid (256 > 209) + (256 > 175)	73.5 (+/-) 9.63
Thiamethoxam (292 > 211) + (292 > 181)	73.6 (+/-) 7.03
Acetamiprid (223 >126) + (223 > 56)	74.5 (+/-) 7.93
Clothianidin (250 >169) + (250 >132)	72.3 (+/-) 9.66

RESULTS: June Sediment Samples

Crop Type	Imidacloprid # of positive hit (% of total) Concentration Range (mean) µg/kg wet weight	Thiamethoxam # of positive hit (% of total) Concentration Range (mean) µg/kg wet weight	Clothianidin # of positive hit (% of total) Concentration Range (mean) µg/kg wet weight	Acetamiprid # of positive hit (% of total) Concentration Range (mean) µg/kg wet weight	# of samples
Barley	0 (0%) NA	0 (0%) NA	1 (5.6%) 2.0 µg/kg X = 2.0	0 (0%) NA	n = 18
Canola	1 (1.6%) 13.8 µg/kg X = 13.8	1 (1.6%) 15.8 µg/kg X = 15.8	2 (3.3%) 2.2 - 3.0 µg/kg X = 2.6	0 (0%) NA	n = 61
Oats	0 (0%) NA	0 (0%) NA	0 (0%) NA	0 (0%) NA	n = 3
Peas	0 (0%) NA	0 (0%) NA	1 (12.5%) 3.44 µg/kg X = 3.44	0 (0%) NA	n = 8
Pasture/ Prairie	0 (0%) NA	0 (0%) NA	0 (0%) NA	0 (0%) NA	n = 15
Wheat	0 (0%) NA	0 (0%) NA	2 (6.9%) 1.7 - 2.6 µg/kg X = 2.2	0 (0%) NA	n = 29

Ban threatens production?

that might threaten agriculture," said Dr Jeroen van der Sluijs at Utrecht University. "This substance should be phased out internationally as soon as possible." The pollution was so bad in some places that the ditch water in fields could have been used as an effective pesticide, he said.

Van der Sluijs added that half the 20,000 tonnes of the imidacloprid produced each year is not affected by the EU ban. It is used not to treat crops, but to combat fleas and other pests in cattle, dogs and cats. "All this imidacloprid ends up in surface water," he said.

The Dutch Experience

Van der Sluijs said the imidacloprid pollution appeared to break existing EU law: "In my view the present use of imidacloprid is not consistent with what the law says: that the product should not have unnacceptable impacts on non-target organisms."

He blamed the underlying problem on imidacloprid's extreme potency in killing invertebrates and its long persistence in soil and water. He said there was also a "system error" in the way that pesticides are authorised in the EU, which, for example, assesses only their effect in individual crops, not any cumulative impact. A recent

Evidence of Serious Environment Risk

The scientists found several cases of extreme pollution, with imidacloprid levels 25,000 times the limit. "The water contained so much insecticide that it could actually be used directly as a lice-control pesticide," van der Sluijs said. "A bee or bumblebee drinking that water would die within a day." The extreme cases were all found close to greenhouses, in which imidacloprid is addded to the water used to water the plants.

The EU standard for imidacloprid pollution is five times higher than the Dutch limit - 67 nanogram per litre versus 13 ng/l - but even water meeting this standard proved

Van der Sluijs

Van der Sluijs said it was highly likely the insecticide was causing the invertebrate die-offs, because imidacloprid was already known to be acutely toxic to these species and is by far the greatest pollutant in the waters. "Of all the chemicals, it is one of the prime suspects and when you look at the level of exceedence - often 100 times above national limits - it is suspect number one," he said.

The scientists found several cases of extreme pollution, with imidacloprid levels 25,000 times the limit. "The water contained so much insecticide that it could actually be

Surface Water Contamination

The research combined results from wildlife and water pollution surveys at 700 sites across the Netherlands conducted between 1998 and 2009. It found a very strong correlation between high levels of imidacloprid pollution and low numbers of invertebrates. In water exceeding the Dutch national pollution limit, just 17 species were found on average, whereas 52 species were found in cleaner water.

Van der Sluijs said it was highly likely the insecticide was causing the invertebrate die-offs, because imidacloprid was already known to be acutely toxic to these species and

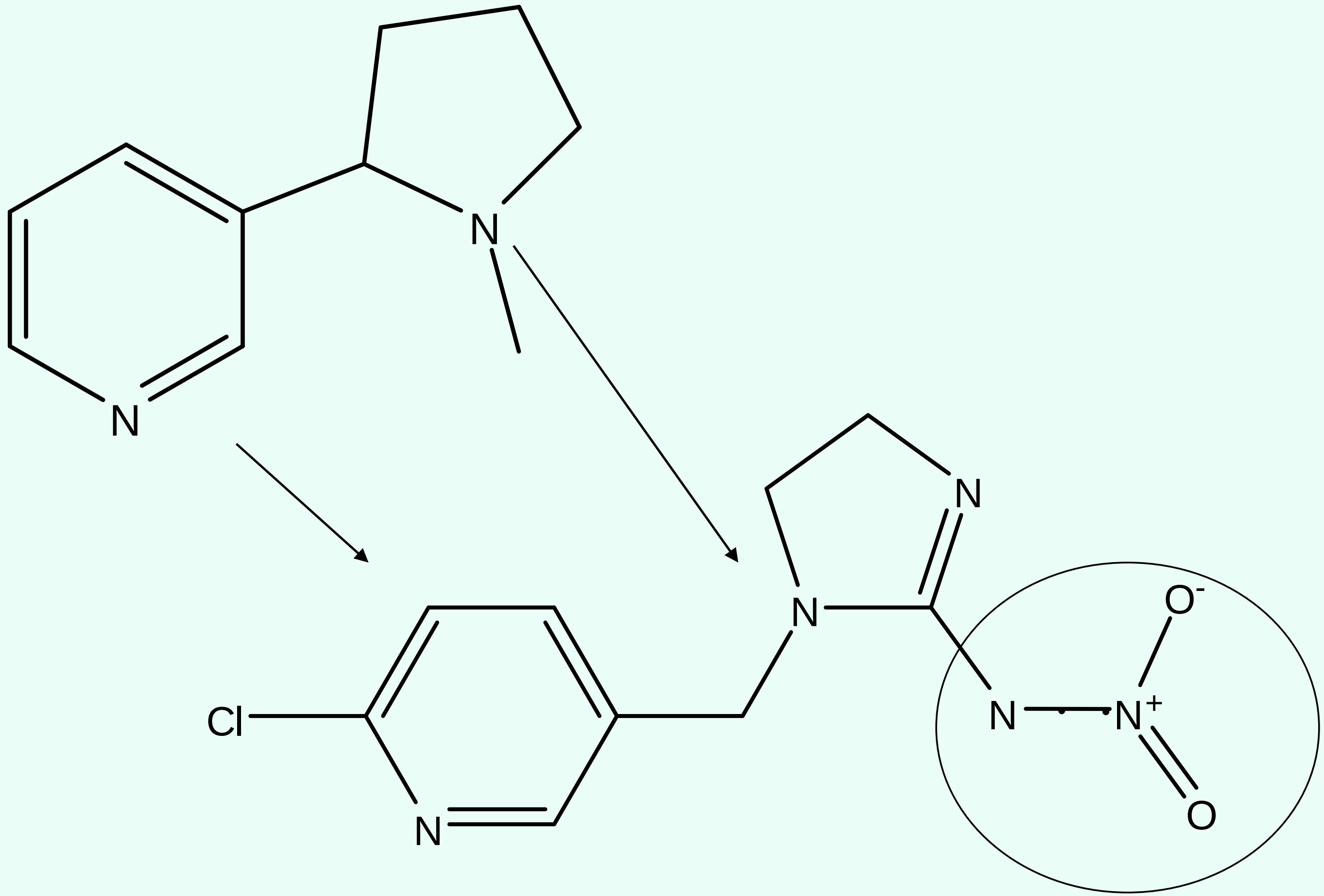
Industry Acknowledges Potential Harm

Julian Little, spokesman for Bayer Cropscience, which manufactures imidacloprid, said: "There doesn't appear to be anything hugely surprising in this article. It shows the presence of high levels of insecticide in water can have effects on aquatic insects and other invertebrates. Should we have strong stewardship of insecticides to minimise any contamination of water? Yes we should and yes we do."

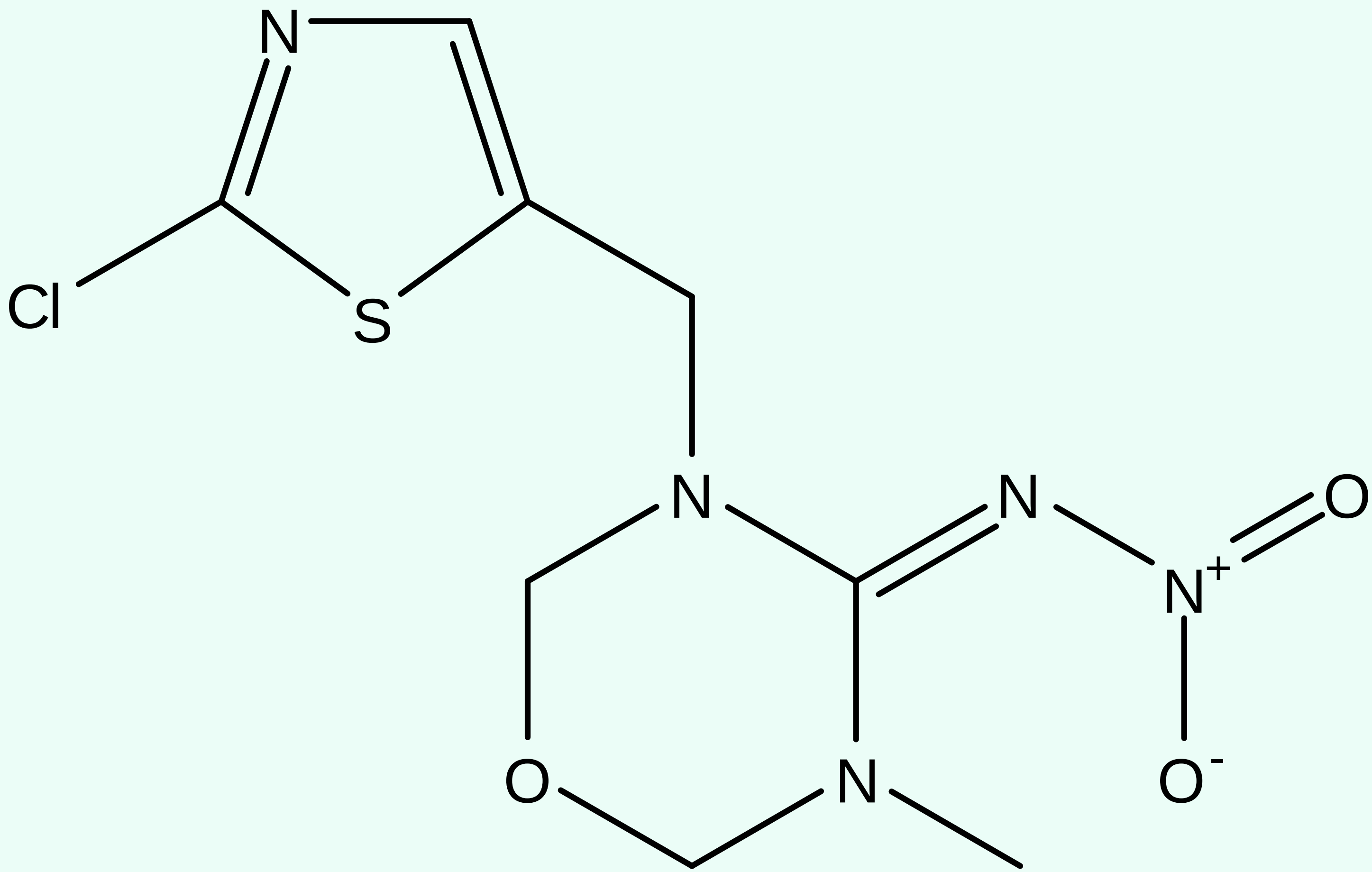
Neonics toxic to invertebrates

The research, published in the peer-reviewed journal PLOS One, found that 70% less invertebrate species were found in water polluted with the insecticide compared to clean water. There were also far fewer individuals of each species in the polluted water. "This is the first study to show this happens in the field," van der Sluijs said. As well as killing mayflies, midges and molluscs, the pollution could have a knock-on effect on birds such as swallows that rely on flying insects for food, he added.

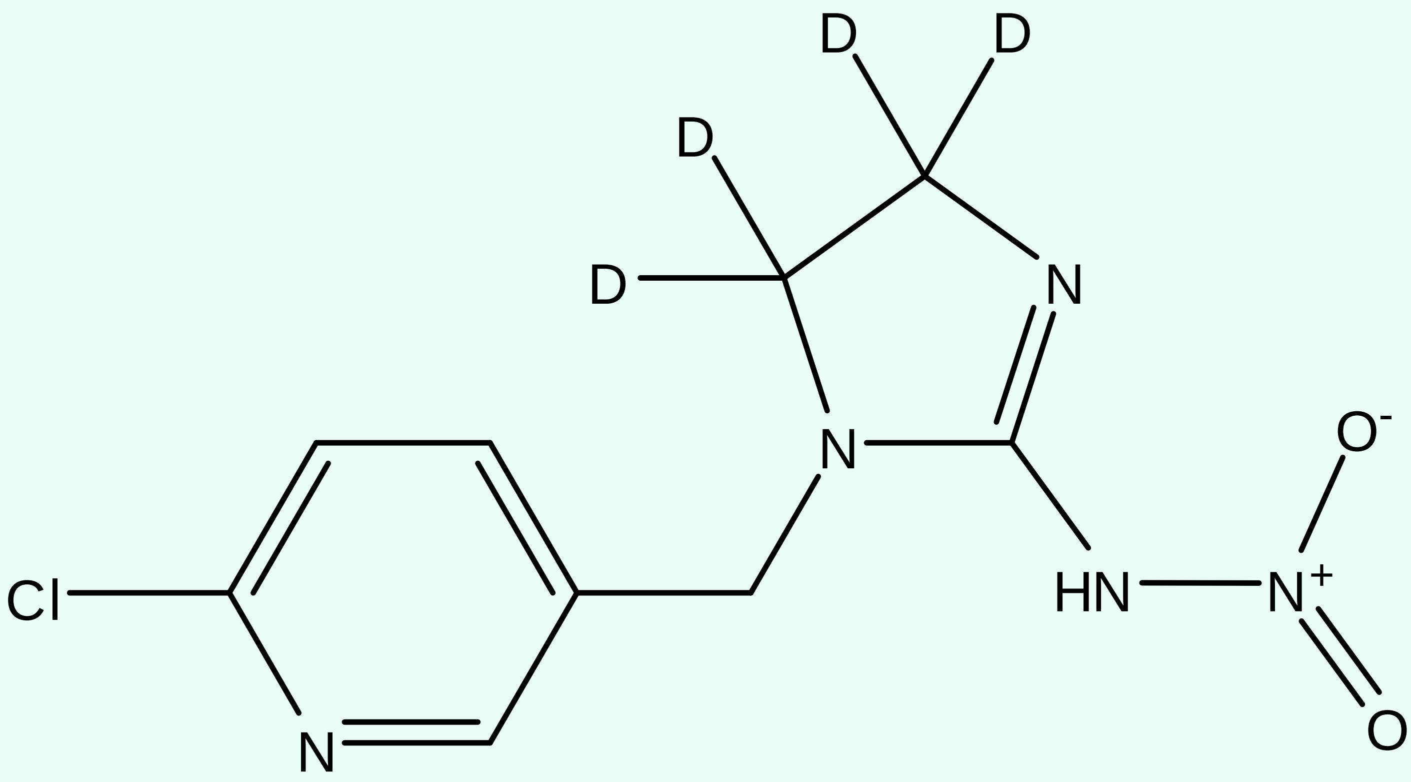
Similarities between Nicotine and Neonicotinoid "Imidacloprid"

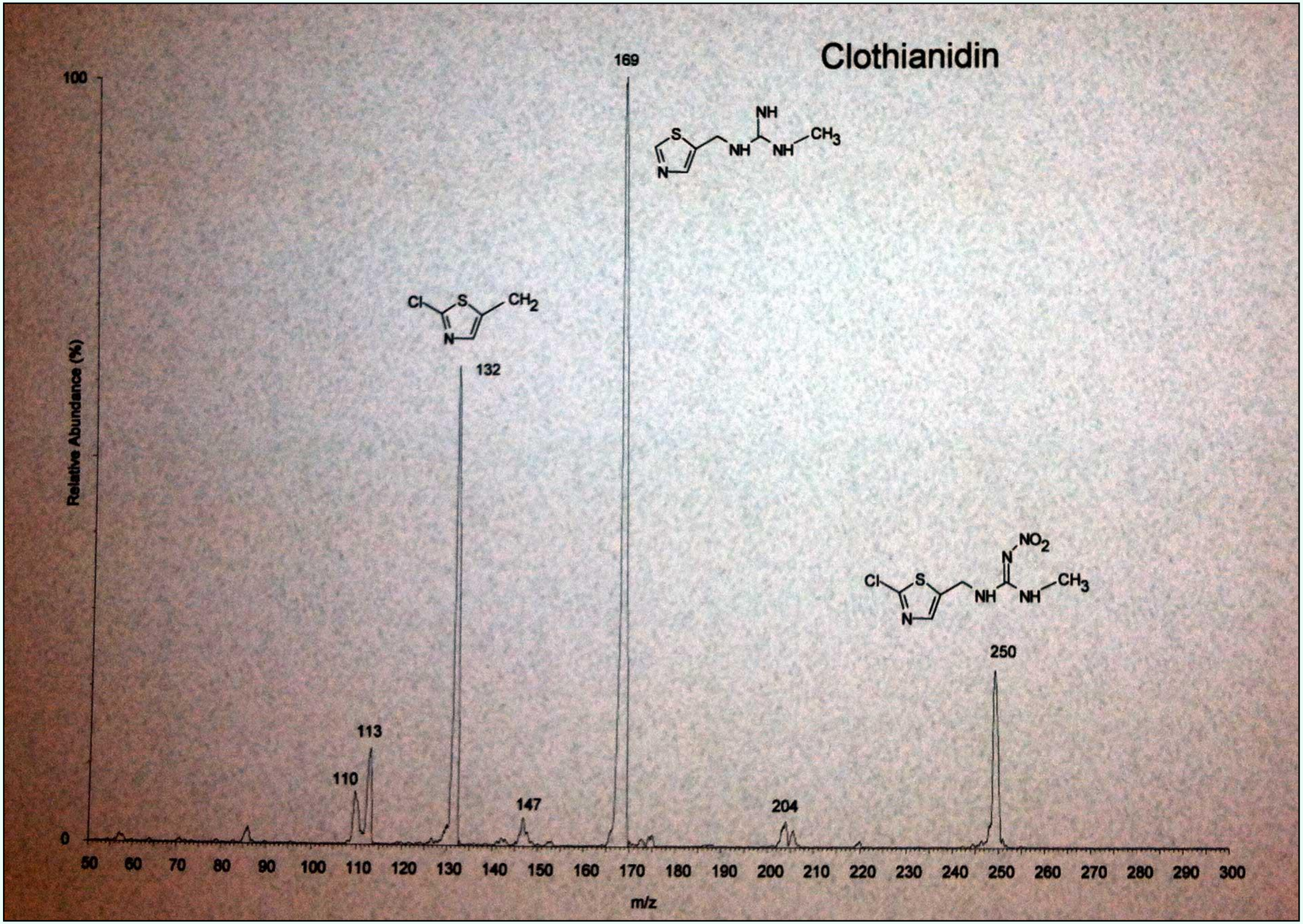


Thiamethoxam



Internal Standard Imidacloprid-d_4




Clothianidin
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Relative Abundance (%)
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250
113
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147
204
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CH3
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