Environmental Science & Technology Letters

Letter

pubs.acs.org/journal/estlcu

Agrochemical Mixtures Detected on Wildflowers near Cattle Feed Yards

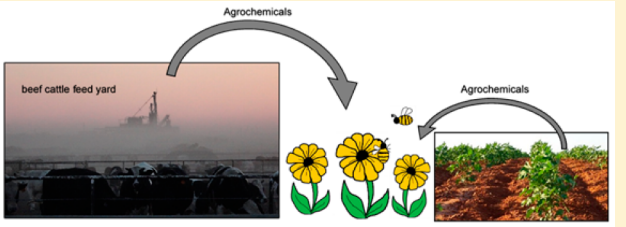
Eric M. Peterson,[†] Kimberly J. Wooten,[†] Seenivasan Subbiah,[†] Todd A. Anderson,[†] Scott Longing,[‡] and Philip N. Smith*[,†]

[†]Department of Environmental Toxicology, Texas Tech University, Lubbock, Texas 79409, United States

[‡]Department of Plant and Soil Sciences, Texas Tech University, Lubbock, Texas 79409, United States

Supporting Information

ABSTRACT: A variety of veterinary pharmaceuticals and pesticides are used on beef cattle feed yards to enhance growth and health of cattle and to control unwanted pests and parasites. Because growth promoters and antibiotics have recently been detected on particulate matter emanating from feed yards, we examined wildflowers collected near feed yards in the Southern Great Plains for the occurrence of antibiotics, β-agonists, other feed yard-related agrochemicals, and neonicotinoids used on regionally grown row crops. Wildflowers contained detectable concentrations of moxidectin, abamectin, monensin, ractopamine, and neonicotinoids (imidacloprid, thiamethoxam, and clothianidin). All wildflower samples contained at least one target analyte, while the majority (82%) contained multiple pharmaceuticals and/or pesticides, including 12% of wildflowers containing moxidectin, monensin, ractopamine, and a neonicotinoid. This preliminary survey demonstrates the potential for insect pollinators occurring near feed yards to become exposed to mixtures of agrochemicals derived from beef cattle feed yards and pesticides from row crop-based agriculture.



INTRODUCTION

Pollinators are ecologically and economically important, facilitating pollination of countless native plants and agricultural crops that contribute an estimated $200 billion per year to the global economy.[1] However, managed honey bee colonies in North America declined by 59% from 1947 to 2005.[2] A recent report from the Center for Biological Diversity suggested that more than 50% of native bee species in North America are in decline and 24% are threatened with extinction.[3] Potential causes of observed declines in honey bees and other anthophilous insects include reduced wildflower availability, monoculture agriculture, parasites, diseases, malnutrition, and pesticides. There is likely not a single cause for observed declines, but rather a combination of factors that have emerged over the past century.

Within the same period, beef, winter wheat, corn, cotton, and sorghum have become predominant agricultural products grown on the Southern Great Plains of North America.[4] Within this region, Texas, Kansas, Oklahoma, Nebraska, and Colorado accounted for 77% of all cattle on confined feeding operations within the United States as of February 1, 2017, representing approximately 8 million head on 1710 feed yards.[5] Significant masses of particulate matter (PM) are generated and emitted daily (234840 kg of PM_{10}/day) from feed yards in these states.[6] Particulate matter is generated as a result of climatic conditions and cattle activity. The Southern Great Plains region is semiarid, generally experiencing declining relative humidity levels and increasing wind speeds as the day progresses.[6,7] Cattle become more active near dusk, facilitating dust production on feed yards by breaking apart dried manure and pen material that contains unmetabolized veterinary pharmaceuticals.[6−8] Feed yard PM is then carried via wind beyond feed yard boundaries. Growth promoters, including steroids, antibiotics, and ractopamine, have recently been detected in fugitive airborne PM collected near beef cattle feed yards, with ractopamine and antibiotics detected most frequently and at the highest concentrations (up to 4730 ng/g of PM).[6,8]

Along with growth-promoting veterinary pharmaceuticals, beef production facilities use considerable amounts of insecticides and parasiticides in the form of spray-ons, powders, feed additives, or injectable formulations. Feed yards (89.6%) in the central United States responding to a survey reported using ivermectin, doramectin, eprinomectin, and moxidectin to control endo- and ectoparasites.[9] These commonly used macrocyclic lactone agrochemicals have similar mechanisms of action and effectively control internal and external parasites, including roundworms, lungworms, horn flies, mites, cattle grubs, and lice. Ivermectin is also toxic to bees[10] and has been used to examine toxic synergism in honey bees upon co-exposure with several compounds routinely encountered by pollinators.[10]

Received: April 7, 2017
Revised: April 27, 2017
Accepted: May 1, 2017
Published: May 1, 2017

DOI: 10.1021/acs.estlett.7b00123
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

Additionally, row crop production in the Southern Great Plains often necessitates the use of insecticides, herbicides, and fungicides to control a wide variety of pests. Among the numerous agrochemicals in use, neonicotinoids have received the most attention as potential contributors to honey bee declines. Neonicotinoids are active ingredients in coatings applied to seeds, including corn, wheat, cotton, and sorghum. When pretreated seeds are planted, a small amount of dust is released (<2%),[11] and concentrations from 1 to 9 ng/g have been detected on field margins.[12−14] Neonicotinoid pesticides may also be absorbed into and translocated systemically throughout plants, thereby increasing the potential for neonicotinoid exposure among nontarget organisms.[15]

Agrochemicals can adversely affect pollinator health, but their occurrence and concentrations in many agroecosystems have not been well characterized.[16,17] Moreover, no published data directly address the question of whether pollinators are exposed to feed yard-derived agrochemicals. Therefore, we hypothesized that mixtures of agrochemicals, including specific antibiotics (monensin, tylosin, chlortetracycline), β-agonists (ractopamine), and macrocyclic lactones (ivermectin, doramectin, abamectin, and moxidectin) that are aerially transported from feed yards on PM and neonicotinoids (thiamethoxam, imidacloprid, and clothianidin) from row crop agriculture, would be detected as mixtures on wildflowers growing near feed yards. To address this hypothesis, wildflowers occurring within 1 km of feed yards and reference wildflowers were collected and subjected to analysis for these select agrochemicals.

MATERIALS AND METHODS

Wildflower Sample Collection. Wildflowers were collected within 1 km of 13 feed yards in the Southern High Plains of Texas on September 23, 2016, between 10:00 a.m. and 5:00 p.m. during optimal weather conditions for PM formation (no rain the preceding week). Wildflowers were cut with scissors into cleaned and sterilized containers and stored on ice until they were returned to the laboratory. Feed yards with numerous wildflower patches allowed for collection of multiple samples from the same area, resulting in 19 distinct wildflower samples. In addition to wildflowers growing near feed yards, samples of similar species were collected from a reference location >11 km from any feed yard and approximately 50 m from the nearest agricultural field. All samples were then transported to our laboratories and stored at −20 °C until the samples were analyzed. Spatial coordinates of all sample collection locations were recorded and used in conjunction with satellite imagery to estimate distance (±1.0 m) from nearest feed yard boundary and row crop boundary.

Quantitation of Agrochemicals. Wildflowers were subjected to analysis for neonicotinoids (thiamethoxam, imidacloprid, and clothianidin), macrocyclic lactones (ivermectin, moxidectin, abamectin, and doramectin), antibiotics (monensin, tylosin, and chlortetracycline), and a β-agonist (ractopamine). Wildflowers included *Lythrum salicaria* (purple loosestrife), *Senecio ampullaceus* (Texas ragwort), *Helianthus annuus* (common sunflower), *Solanum elaeagnifolium* (silverleaf nightshade), *Ipomoea stolonifera* (fiddle-leaf morning glory), and *Cirsium texanum* (Texas thistle). Wildflower tissue (2 ± 1 g) was homogenized with acetonitrile [1:1 (w/v)] and extracted with 6 mL of acetonitrile and 1 g of $MgSO_4$. Following centrifugation, the supernatant was divided for use in macrocyclic lactone or neonicotinoid determination.

For determination of macrocyclic lactones, 1−2 mL of supernatant was evaporated under nitrogen, reconstituted in 50 μL of acetonitrile, derivatized with 1-methylimidazole and trifluoroacetic anhydride, and analyzed via an Agilent 1100 series high-performance liquid chromatography (HPLC) instrument with fluorescence detection (Figure S1). The method, with minor modifications to column and flow rate (Gemini NX-C18 column, 150 mm × 2.0 mm, 3 μm, flow rate of 300 μL/min), was based on U.S. Department of Agriculture FSIS methods.[18] An internal standard [benzo(k)fluoranthene] was added to all samples and standards, along with derivatization reagents to confirm derivatization. Reference composite wildflower samples (n = 3) and solvent blanks were included with each extraction for quality control purposes.

Neonicotinoid analysis was accomplished using 2 mL of supernatant from the homogenization and centrifugation step described above. The supernatant, along with 2 mL of water, was added to 1 g of QuEChERS salt ($MgSO_4$ and NaCl). The clean aliquot (1.5 mL) was evaporated and reconstituted to 1 mL, which was cleaned with ChloroFiltr (UCT, Bristol, PA). Quantitation of neonicotinoids was achieved via triple-quadrupole liquid chromatography and tandem mass spectrometry (LC−MS/MS) (Thermo TSQ Quantum Access Max, Thermo Scientific, Waltham, MA); electrospray ionization followed chromatography with a Gemini NX-C18 column (150 mm × 2.0 mm, 3 μm; Phenomenex, Torrance, CA) and a water/acetonitrile with 0.1% formic acid gradient elution. Sample extraction was based on standard method EN 15662, with minor modifications to the mobile phase for polar neonicotinoid pesticides.[19]

Wildflower samples remaining after the previous analyses (n = 17) were examined for a subset of beef cattle growth promoters, including monensin, tylosin, chlortetracycline, and ractopamine. Extractions were based on the optimized QuEChERS method published by Chuang et al.[20] with minor modifications. Wildflowers (0.2−1.2 g of wet weight) were homogenized with acetonitrile [1:1 (w/v)] and extracted with an acetonitrile/methanol/Na_2EDTA solution in the presence of NaCl and Na_2SO_4. Additional cleanup was performed on the extracted sample with a d-SPE sorbent mix of C18, PSA, and $MgSO_4$. Quality control samples (blank and spiked wildflower matrix) were extracted along with feed yard wildflowers. LC−MS/MS analysis and quantitation were performed using instrumental methodology described by McEachran et al.[6]

The Shapiro−Wilk test for normality was used to evaluate distributions of analyte concentration data for those with >50% of samples above detection limits, and sample collection distances to nearest feed yard boundary. Normally distributed data were then examined for correlations using the Pearson Product Moment. α was set at 0.05 for all significance interpretations, and all statistics were determined using JMP Pro 12.1.0 (SAS Institute Inc., Cary, NC). All concentration data, from this point forward, are expressed as wet weight.

RESULTS AND DISCUSSION

In this preliminary survey, moxidectin, abamectin, monensin, ractopamine, thiamethoxam, imidacloprid, and clothianidin were detected on wildflowers collected within 1 km of beef cattle feed yards. There were no significant correlations among analytes with detection frequencies above 50% (moxidectin and monensin), and distance from sample location to nearest feed yard boundary (all p values of >0.234), likely because of inherent variability in wind speed, direction, and other climatic

DOI: 10.1021/acs.estlett.7b00123
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

Table 1. LC−MS/MS and HPLC−Fluorescence Analysis of Agrochemicals Detected in Wildflowers Collected within 1 km of 13[a] Separate Feed Yards in the Southern Great Plains[b]

agrochemical	n	LOD[c] (ng/g)	method recovery [% (mean ± SE)]	% >LOD	concentration range[d] (ng/g)
monensin[e]	17	3−11	66 ± 15.1	71	14.9−53.4
ractopamine[e]	17	7.5−27.5	108 ± 14.6	29	37.3−382.2
thiamethoxam[e]	19	0.8−2.4	116[f]	21	0.9−1.5
clothianidin[e]	19	0.8−2.4	84[f]	11	3.6−4.0
imidacloprid[e]	19	0.8−2.4	105[f]	11	1.8−2.1
moxidectin[g]	19	3−10	44 ± 7	100	9.3−83.2
abamectin[g]	19	3−10	44 ± 7	5	26.1

[a]Five feed yards had subsamples, allowing for 19 total wildflower samples analyzed. [b]No analytes of interest were detected in wildflower samples (n = 3) collected from a reference site >11 km from any feed yard and approximately 50 m from the nearest agricultural field. [c]Range due to differences in wildflower mass extracted (N). [d]Only including samples above the LOD (limit of detection). [e]Quantitation by LC−MS/MS. [f]SE not included because n < 3. [g]Quantitation by HPLC fluorescence.

and environmental factors that dictate PM dispersion. No target analyte was detected in wildflower samples from the reference site.

All wildflower samples [100% (Table 1)] collected near feed yards contained moxidectin at concentrations ranging from 9.3 to 83.2 ng/g with a mean [±standard error (SE)] of 45.1 ± 5.1 ng/g, and one wildflower sample contained abamectin (26.1 ng/g). Ivermectin and doramectin were not detected in any wildflower samples [limit of detection (LOD) of 3−10 ng/g]. There are no readily available toxicity data for moxidectin and pollinators. However, avermectins are highly toxic to honey bees, particularly abamectin [LD_{50} = 2 ng/bee (contact) and 9 ng/bee (oral)].[21] Further, Guseman and colleagues recently reported a 24 h LC_{50} of 1.57 μg/mL for honey bees exposed to ivermectin in a sucrose solution.[10] Ivermectin (0.05 ng/bee) applied topically to the thorax resulted in decreased long-term olfactory retention among honey bees.[22]

Avermectins (ivermectin, abamectin, and doramectin) and milbemycins (moxidectin) have similar chemical structures (a 16-carbon macrocyclic lactone ring) but are differentiated by the absence of a sugar from the structural backbone of milbemycins.[23] Moxidectin and ivermectin are effective against the same parasites because of similarities in modes of action.[24] Both ivermectin and moxidectin cause an influx of chloride ions into insect neurons leading to paralysis and death.[24−26] However, the insecticidal activity of ivermectin is potentiated upon co-administration with antibiotics and fungicides regularly encountered by bees.[10] Ivermectin-associated synergistic toxicity occurs as a result of decreased P-glycoprotein activity, which allows for greater absorption and higher concentrations of ivermectin at neuronal targets.[10,23,27] Individual chemical toxicity test data may therefore underestimate the potential for synergistic toxic effects among pollinators exposed to agrochemical mixtures containing macrocyclic lactones.

The ionophore antibiotic monensin was detected on 71% of wildflowers, with concentrations ranging from below limits of detection to 53.4 ng/g. Neither tylosin nor chlortetracycline was detected on wildflowers (LOD of 3−11 or 7.5−27.5 ng/g, respectively). Monensin and other antibiotics are administered to beef cattle to enhance growth and/or for disease prophylaxis. Though some antibiotics are routinely applied in apicultural settings to control bee brood diseases, recent data suggest that tetracycline (detected in airborne feed yard PM by McEachran et al.[6]) can have long-lasting, severe effects on the honey bee gut microbiome.[6,28] Many eusocial insects, including managed honey bees and bumble bees, rely on gut microbiota for protection against parasites, and horizontal transmission of beneficial gut bacteria has been shown to be an important process for host immune systems in social bees.[29,30] Therefore, antibiotic-induced dysbiosis may increase the susceptibility of bees to opportunistic pathogens and reduce survivorship.[28]

The β-adrenergic agonist ractopamine was detected on wildflowers less frequently (29%) than moxidectin (100%) and monensin (71%) were, but concentrations were among the highest observed in this survey (37.3−382 ng/g). Ractopamine is administered to livestock as a feed additive but has been banned in most countries, some notable exceptions being the United States, Canada, and Mexico. Mechanistically, ractopamine stimulates $TAAR_1$, $Beta_1$, and $Beta_2$ receptors, thereby increasing the rate of protein synthesis.[8] Although scant data exist, invertebrates appear to be sensitive to ractopamine exposure. A study using the nematode *Caenorhabditis elegans* revealed altered locomotive behavior at 10 ng/L and reduced brood size at 100 ng/L among individuals exposed to ractopamine as developing larvae.[31] On the basis of limited available invertebrate toxicity data and concentrations observed on wildflowers in this study, we can speculate that bees exposed to ractopamine via wildflowers near feed yards may exhibit altered locomotor activity that could, in turn, adversely affect communication, foraging efficiency, and other critical behaviors.

Each of the neonicotinoids included in our analysis was detected on wildflowers [8 of 19 (42%)], at low concentrations, and no wildflower sample contained more than one neonicotinoid. Thiamethoxam was detected in four wildflower samples, while clothianidin and imidacloprid were each detected in two wildflower samples. Concentrations of neonicotinoids ranged from 0.9 ng/g (thiamethoxam) to 4.0 ng/g (clothianidin). Wildflower samples were collected in September, a month in which little or no planting activities occur on the Southern High Plains. Therefore, the occurrence of neonicotinoids in wildflowers did not likely result from seed dust distribution.

The literature is replete with toxicity data pertaining to neonicotinoids and pollinators, and it has been thoroughly reviewed.[32,33] It appears that environmentally relevant concentrations of neonicotinoids, such as those observed in this study, are not acutely toxic to honey bees, and thus, neonicotinoids may not be solely responsible for recent honey bee declines.[33,34] While it is clear that neonicotinoid insecticides are associated with adverse effects in bees,[35] synergistic effects upon co-administration with other chemicals (e.g., fungicides) have only recently been reported.[36]

Studies that focus on toxic responses of pollinators to individual agrichemical moieties (e.g., neonicotinoids) may not

DOI: 10.1021/acs.estlett.7b00123
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

adequately reflect complex real world exposure dynamics and resulting cumulative stress loads. The extent of adverse effects among pollinators that result from exposure to an individual toxicant depends largely on the degree of simultaneous exposure to other toxicants and pathogens, nutritional status of exposed individuals, and many other factors.[37] Clearly, each of the agrochemicals described above has potential to adversely impact pollinator biochemical function, development, behavior, and indeed survival following exposure to sufficiently high concentrations, but exposure to these individual chemicals and classes (and in general) is thought to occur at levels that do not result in quantifiable or observable effects.

However, flowering crops as well as wildflowers are often contaminated with a broad range of agrochemicals.[38] Thus, pollinators are rarely exposed to individual agrochemicals, rather, mixtures of natural chemical compounds and xenobiotics used for a wide variety of agriculture-related purposes.[37] Although pollinator exposure cannot be directly inferred from occurrence data and/or concentrations of agrochemicals on wildflowers, it is important to note that among the wildflower samples examined in this study, 12% contained all four targeted drug/chemical classes (macrocyclic lactones, antibiotics, β-agonists, and neonicotinoids), 53% contained three or more, and 82% contained two or more. All wildflowers collected near feed yards contained at least one target analyte.

More than 50% of wildflower samples contained only agrochemicals used on feed yards (moxidectin, monensin, and/or ractopamine), whereas no wildflower sample contained only row crop pesticides (thiamethoxam, clothianidin, and/or imidacloprid). As hypothesized, 42% of wildflower samples contained mixtures of both feed yard-derived agrochemicals and row crop pesticides. There are few to no data available detailing the toxicity of feed yard-derived agrochemicals among pollinators, and while toxicity information about synergistic effects of some pesticides has recently come under investigation, the potentially toxic synergisms arising from the exposure of pollinators to feed yard and row crop agrochemical mixtures have not. A likely reason for this apparent data gap is that, until now, there has been no indication that pollinators could become exposed to these types of agrochemical mixtures because there was no known pathway for exposure to occur. Recent discoveries indicating that these compounds are environmentally disseminated via windblown PM have raised the specter of exposure and potential adverse effects among managed bees and native pollinators.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.7b00123.

Chromatograms of standards, reference wildflowers, and wildflowers with positive detections of macrocyclic lactones (Figure S1), veterinary pharmaceuticals (Figures S2−S6), and neonicotinoids (Figures S7−S10) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: phil.smith@ttu.edu. Phone: (806) 834-6180.

ORCID

Eric M. Peterson: 0000-0003-4606-597X

Kimberly J. Wooten: 0000-0002-4558-2185

Todd A. Anderson: 0000-0001-8176-6339

Philip N. Smith: 0000-0002-7557-7563

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Gallai, N.; Salles, J. M.; Settele, J.; Vaissiere, B. E. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* **2009**, *68*, 810−821.

(2) Goulson, D.; Nicholls, E.; Botias, C.; Rotheray, E. L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **2015**, *347*, 6229.

(3) Kopec, K. Pollinators in Peril: A systematic status review of North American and Hawaiian native bees. Technical Report; Center for Biological Diversity: Tucson, AZ, 2017.

(4) U.S. Department of Agriculture. NASS Texas County Estimates (March 2017). https://www.nass.usda.gov/Statistics_by_State/Texas/Publications/County_Estimates/ce_pdf/index.php.

(5) U.S. Department of Agriculture. NASS Cattle on Feed (Feb 2017). http://usda.mannlib.cornell.edu/usda/current/CattOnFe/CattOnFe-02-24-2017.pdf.

(6) McEachran, A. D.; Blackwell, B. R.; Hanson, J. D.; Wooten, K. J.; Mayer, G. D.; Cox, S. B.; Smith, P. N. Antibiotics, Bacteria, and Antibiotic Resistance Genes: Aerial Transport from Cattle Feed Yards via Particulate Matter. *Environ. Health Perspect.* **2015**, *123*, 337−343.

(7) Blackwell, B. R.; Wooten, K. J.; Buser, M. D.; Johnson, B. J.; Cobb, G. P.; Smith, P. N. Occurrence and Characterization of Steroid Growth Promoters Associated with Particulate Matter Originating from Beef Cattle Feedyards. *Environ. Sci. Technol.* **2015**, *49*, 8796−8803.

(8) Wooten, K. J.; Smith, P. N. Ractopamine associated with airborne particulate matter emitted from beef cattle feed yards (manuscript submitted for publication).

(9) Feedlot, U. Part IV: Health and Health Management on US Feedlots with a capacity of 1,000 or more head. Technical Report; USDA-APHIS-VS-CEAH-NAHMS: Fort Collins, CO, 2011.

(10) Guseman, A. J.; Miller, K.; Kunkle, G.; Dively, G. P.; Pettis, J. S.; Evans, J. D.; vanEngelsdorp, D.; Hawthorne, D. J. Multi-Drug Resistance Transporters and a Mechanism-Based Strategy for Assessing Risks of Pesticide Combinations to Honey Bees. *PLoS One* **2016**, *11*, e0148242.

(11) Tapparo, A.; Marton, D.; Giorio, C.; Zanella, A.; Solda, L.; Marzaro, M.; Vivan, L.; Girolami, V. Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds. *Environ. Sci. Technol.* **2012**, *46*, 2592−2599.

(12) Krupke, C. H.; Hunt, G. J.; Eitzer, B. D.; Andino, G.; Given, K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* **2012**, *7*, e29268.

(13) Marzaro, M.; Vivan, L.; Targa, A.; Mazzon, L.; Mori, N.; Greatti, M.; Toffolo, E. P.; Di Bernardo, A.; Giorio, C.; Marton, D.; Tapparo, A.; Girolami, V. Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. *Bulletin of Insectology* **2011**, *64*, 119−126.

(14) Stewart, S. D.; Lorenz, G. M.; Catchot, A. L.; Gore, J.; Cook, D.; Skinner, J.; Mueller, T. C.; Johnson, D. R.; Zawislak, J.; Barber, J. Potential Exposure of Pollinators to Neonicotinoid Insecticides from the Use of Insecticide Seed Treatments in the Mid-Southern United States. *Environ. Sci. Technol.* **2014**, *48*, 9762−9769.

(15) Schaafsma, A.; Limay-Rios, V.; Baute, T.; Smith, J.; Xue, Y. Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in southwestern Ontario. *PLoS One* **2015**, *10*, e0118139.

(16) Arena, M.; Sgolastra, F. A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology* **2014**, *23*, 324−334.

(17) Lundin, O.; Rundlof, M.; Smith, H. G.; Fries, I.; Bommarco, R. Neonicotinoid Insecticides and Their Impacts on Bees: A Systematic Review of Research Approaches and Identification of Knowledge Gaps. *PLoS One* **2015**, *10*, e0136928.

DOI: 10.1021/acs.estlett.7b00123
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

(18) Determination of Ivermectin, doramectin, and moxidectin by HPLC. USDA Report FSIS 2011 (https://www.fsis.usda.gov/wps/wcm/connect/87680e50-d76b-407b-9d94-d2ecc37b3cd0/CLG_AVR_04.pdf?MOD=AJPERES).
(19) EN, B. 15662:2008. Foods of plant origin-determination of pesticide residues using GC-MS and; or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE-QuEChERS-Method. http://esearch.cen.eu/esearch; 2008.
(20) Chuang, Y. H.; Zhang, Y. J.; Zhang, W.; Boyd, S. A.; Li, H. Comparison of accelerated solvent extraction and quick, easy, cheap, effective, rugged and safe method for extraction and determination of pharmaceuticals in vegetables. *J. Chromatogr., A* **2015**, *1404*, 1−9.
(21) Wislocki, P.; Grosso, L.; Dybas, R. Environmental aspects of abamectin use in crop protection. In *Ivermectin and abamectin*; Springer: Dordrecht, The Netherlands, 1989; pp 182−200.
(22) El Hassani, A. K.; Giurfa, M.; Gauthier, M.; Armengaud, C. Inhibitory neurotransmission and olfactory memory in honeybees. *Neurobiol. Learn. Mem.* **2008**, *90*, 589−595.
(23) Lespine, A. Lipid-like properties and pharmacology of the anthelmintic macrocyclic lactones. *Expert Opin. Drug Metab. Toxicol.* **2013**, *9*, 1581−1595.
(24) Cobb, R.; Boeckh, A. Moxidectin: a review of chemistry, pharmacokinetics and use in horses. *Parasites Vectors* **2009**, *2*, S5.
(25) Conder, G. A.; Thompson, D. P.; Johnson, S. S. Demonstration of co-resistance of Haemonchus contortus to ivermectin and moxidectin. *Vet. Rec.* **1993**, *132*, 651−652.
(26) Prichard, R.; Menez, C.; Lespine, A. Moxidectin and the avermectins: Consanguinity but not identity. *Int. J. Parasitol.: Drugs Drug Resist.* **2012**, *2*, 134−153.
(27) Sangare, A. K.; Rolain, J. M.; Gaudart, J.; Weber, P.; Raoult, D. Synergistic activity of antibiotics combined with ivermectin to kill body lice. *Int. J. Antimicrob. Agents* **2016**, *47*, 217−223.
(28) Raymann, K.; Shaffer, Z.; Moran, N. A. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biol.* **2017**, *15*, e2001861.
(29) Koch, H.; Schmid-Hempel, P. Socially transmitted gut microbiota protect bumble bees against and intestinal parasite. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 19288−19292.
(30) Engel, P.; Martinson, V. G.; Moran, N. A. Functional diversity within simple gut microbiota of the honey bee. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 11002−11007.
(31) Zhuang, Z. H.; Zhao, Y. L.; Wu, Q. L.; Li, M.; Liu, H. C.; Sun, L. M.; Gao, W.; Wang, D. Y. Adverse Effects from Clenbuterol and Ractopamine on Nematode *Caenorhabditis elegans* and the Underlying Mechanism. *PLoS One* **2014**, *9*, e85482.
(32) Chauzat, M. P.; Faucon, J. P.; Martel, A. C.; Lachaize, J.; Cougoule, N.; Aubert, M. A survey of pesticide residues in pollen loads collected by honey bees in France. *J. Econ. Entomol.* **2006**, *99*, 253−262.
(33) Goulson, D. REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* **2013**, *50*, 977−987.
(34) Dively, G. P.; Embrey, M. S.; Kamel, A.; Hawthorne, D. J.; Pettis, J. S. Assessment of Chronic Sublethal Effects of Imidacloprid on Honey Bee Colony Health. *PLoS One* **2015**, *10*, e0126043.
(35) Van der Sluijs, J.; Simon-Delso, N.; Goulson, D.; Maxim, L.; Bonmatin, J.-M.; Belzunces, L. P. Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr. Opin. Environ. Sustain.* **2013**, *5*, 293−305.
(36) Thompson, H. M.; Fryday, S. L.; Harkin, S.; Milner, S. Potential impacts of synergism in honeybees (*Apis mellifera*) of exposure to neonicotinoids and sprayed fungicides in crops. *Apidologie* **2014**, *45*, 545−553.
(37) Johnson, R. M. Honey bee toxicology. *Annu. Rev. Entomol.* **2015**, *60*, 415−434.
(38) David, A.; Botías, C.; Abdul-Sada, A.; Nicholls, E.; Rotheray, E. L.; Hill, E. M.; Goulson, D. Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environ. Int.* **2016**, *88*, 169−178.

DOI: 10.1021/acs.estlett.7b00123
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX