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Hummingbirds and Bumble Bees Exposed to Neonicotinoid and Organophosphate Insecticides in the Fraser Valley, British Columbia, Canada

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Abstract: To measure exposure to neonicotinoid and other pesticides in avian pollinators, we made novel use of cloacal fluid and fecal pellets from rufous (*Selasphorus rufus*) and Anna's (*Calypte anna*) hummingbirds living near blueberry fields in the Fraser River Valley and Vancouver Island, British Columbia, Canada. To examine on-farm exposure to pesticides in invertebrate pollinators, we also collected bumble bees native to Canada (*Bombus mixtus*, *Bombus flavifrons*, and *Bombus melanopygus*), their pollen, and blueberry leaves and flowers from within conventionally sprayed and organic blueberry farms. By sites and sample type, the results reported in the present study represent pooled samples ($n = 1$). In 2015 to 2016, the combined concentration of the neonicotinoid insecticides imidacloprid, thiamethoxam, and clothianidin detected in hummingbird cloacal fluid from sites near conventionally sprayed blueberry fields was 3.63 ng/mL (ppb). Among the 18 compounds measured in fecal pellets, including one neonicotinoid (imidacloprid), only piperonyl butoxide was detected (1.47–5.96 ng/g). Piperonyl butoxide is a cytochrome P450 inhibitor applied with some insecticides to increase their toxic efficacy. Only diazinon was detected in bumble bees (0.197 ng/g), whereas diazinon (1.54–1.7 ng/g) and imidacloprid (up to 18.4 ng/g) were detected in pollen collected from bumble bees including the bees from organic sites located near conventionally sprayed blueberry farms. Imidacloprid was also detected at 5.16 ng/g in blueberry flowers collected 1 yr post spray from 1 of 6 conventionally sprayed blueberry farms. *Environ Toxicol Chem* 2018;37:2143–2152. © 2018 SETAC

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INTRODUCTION

Since the 1990s, neonicotinoid insecticides have rapidly become among the best-selling insecticides globally, which is partially due to their low acute toxicity to mammals, their systemic capabilities, and their efficacy (Goulson 2013; Simon-Delso et al. 2015). Concern over loss of the pollination services provided by bees to ecosystems and agriculture has arisen where neonicotinoid insecticides are used (Blacquière et al. 2012; Goulson 2015), but exposure and effects in other warm-blooded pollinators such as hummingbirds have not received the same attention, even though the geographic ranges and migration routes of hummingbirds overlap with intensive agricultural areas in western North America (Williamson 2001; US Department of Agriculture 2012; Natural Resources Canada 2017).

Rufous (*Selasphorus rufus*) hummingbirds overwinter in Mexico and the Gulf States of the United States, and nest from southern California to Alaska, traveling farther north than any other hummingbird, with the core of the rufous hummingbird breeding range occurring in British Columbia, Canada, including the Fraser River watershed (Sibley 2016). Rufous hummingbirds are noted as a species of conservation concern by Partners in Flight, and their populations are estimated to have declined by 60% between 1970 and 2014 (Rosenberg et al. 2016). In contrast, Anna's hummingbirds (*Calypte anna*) live predominantly in the west of North America, breeding from southern California to southern British Columbia; their geographic range has been expanding annually, and they are now common in coastal British Columbia (Sibley 2016).

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Greenhouses and vegetable production have long been important components of the agricultural economy in the Fraser Valley; recently berry production has become a valuable cash crop, and the planted acreage of berries has been increasing in both Canada and the United States (Brazelton and Strik 2007; World Atlas 2017). Insecticides, including neonicotinoids, are used in all these agricultural activities (British Columbia Ministry of Agriculture 2009, 2012, 2017, 2018), as well as in residential areas of the Fraser Valley (Pest Management Regulatory Agency, Government of Canada 2016a, 2016b). In areas of Canada where farms are integrated into an urban and suburban landscape such as the Fraser Valley in British Columbia, wildlife interactions with farmland are inevitable, and where wildlife such as birds use hedgerows or farmland proper as habitat, the potential for pesticide exposure exists (Thompson 1996). Hummingbird exposure to pesticide use has not yet been documented, but the exposure of bees and other nontarget invertebrates to agricultural use of neonicotinoid insecticides (Blacquière et al. 2012) has led to a recent review of their use in Canada (Pest Management Regulatory Agency, Government of Canada 2016a, 2016b). Neonicotinoids and carbamate insecticides in seed dressings are also a current conservation concern due to sublethal neurotoxic effects, including migratory disorientation, in songbirds at environmentally relevant exposures (Eng et al. 2017).

The primary objective of the present study was to determine pesticide exposure, with a focus on neonicotinoid compounds, in pollinators living in or near blueberry-growing areas of the Fraser River Valley of British Columbia, Canada. We made novel use of cloacal fluid and fecal pellets sampled from wild hummingbirds living near blueberry fields and suburban and rural areas. Rufous and Anna's hummingbirds overlap in their occurrence in British Columbia (Sibley 2016), and therefore we sampled both of them at sites within 0.5 or 1 km or more from blueberry fields. To further understand pesticide exposure in pollinators living in or near blueberry fields, we also collected bumble bees native to Canada (*Bombus mixtus*, *Bombus flavifrons*, and *Bombus melanopygus*; Williams et al. 2014), their pollen, and blueberry leaves and flowers from conventionally sprayed and organic blueberry farms in the Fraser Valley.

METHODS

Study sites and sample periods

Cloacal fluid and fecal pellets from hummingbirds were collected in mid-April, mid-May, and mid-June in 2015 and 2016. It was necessary to pool samples from hummingbird species together by type (fluid or pellets), by sex, by site, and by year to attain sufficient volume of fluid (300 μL) or biosolid mass (≥1 g) for the chemical extraction methods we used (Table 1).

Our reference sites for the hummingbird study were located ≥1 km from a conventionally sprayed blueberry field in the Fraser River Valley and >10 km from agricultural fields on Vancouver Island (BC, Canada; Table 1). Our pesticide-exposed sites were ≤0.5 km from conventionally sprayed blueberry fields (Table 1).

For cloacal fluid from hummingbirds in 2015, we sampled 2 reference sites and combined those samples into one pooled sample for analysis to attain a minimum volume of 300 μL for analysis. One reference site was within the Fraser Valley (reference site 1; Figure 1) and was located ≥1 km from a blueberry field; the second site was on Vancouver Island in a watershed without blueberry fields and was >10 km from agricultural fields (reference site 2; Table 1 and Figure 1). Samples to determine pesticide exposure in agricultural areas were collected from hummingbirds trapped ≤0.5 km from conventionally sprayed blueberry fields (Table 1 and Figure 1).

TABLE 1: Neonicotinoid insecticides detected in cloacal fluid samples from rufous (*Selasphorus rufus*) and Anna's (*Calypte anna*) hummingbirds from Fraser Valley and Vancouver Island (BC, Canada) in April, May, and June of 2015 and 2016 (ng/mL)[a]

Year sampled (no. of sites in pooled sample) and sample location	Site type	Imidacloprid	Clothianidin	Thiamethoxam	No. of individual hummingbird cloacal fluid samples in pooled analysis
2015 (2) Fraser Valley (reference site 1); Vancouver Island (reference site 2)	≥1 km from CSBF	0.086	0.659	ND	36
2015 (5) Fraser Valley	≤0.5 km from CSBF	0.197	1.96	1.47	56
2016 (1) Vancouver Island (reference site 2)	No blueberry fields within watershed and ≥10 km from agricultural sites	ND	ND	ND	15
2016 (1) Vancouver Island (reference site 3)	No blueberry fields within watershed and ≥10 km from agricultural sites	ND	ND	ND	20
2016 (2) Fraser Valley	≤0.5 km from CSBF	0.081	ND	ND	11
2016 (1) Fraser Valley	≤0.5 km from CSBF	0.068	ND	ND	46
2016 (1) Fraser Valley	≤0.5 km from CSBF	0.184	ND	ND	10
2016 (1) Fraser Valley	≤0.5 km from CSBF	0.452	ND	ND	13

[a]The following compounds were measured (ng/mL) but were below the minimum detection limit (MDL): acetamiprid (MDL = 0.0033); thiocloprid (MDL = 0.20).
CSBF = conventionally sprayed blueberry field; ND = below minimum detection limit.

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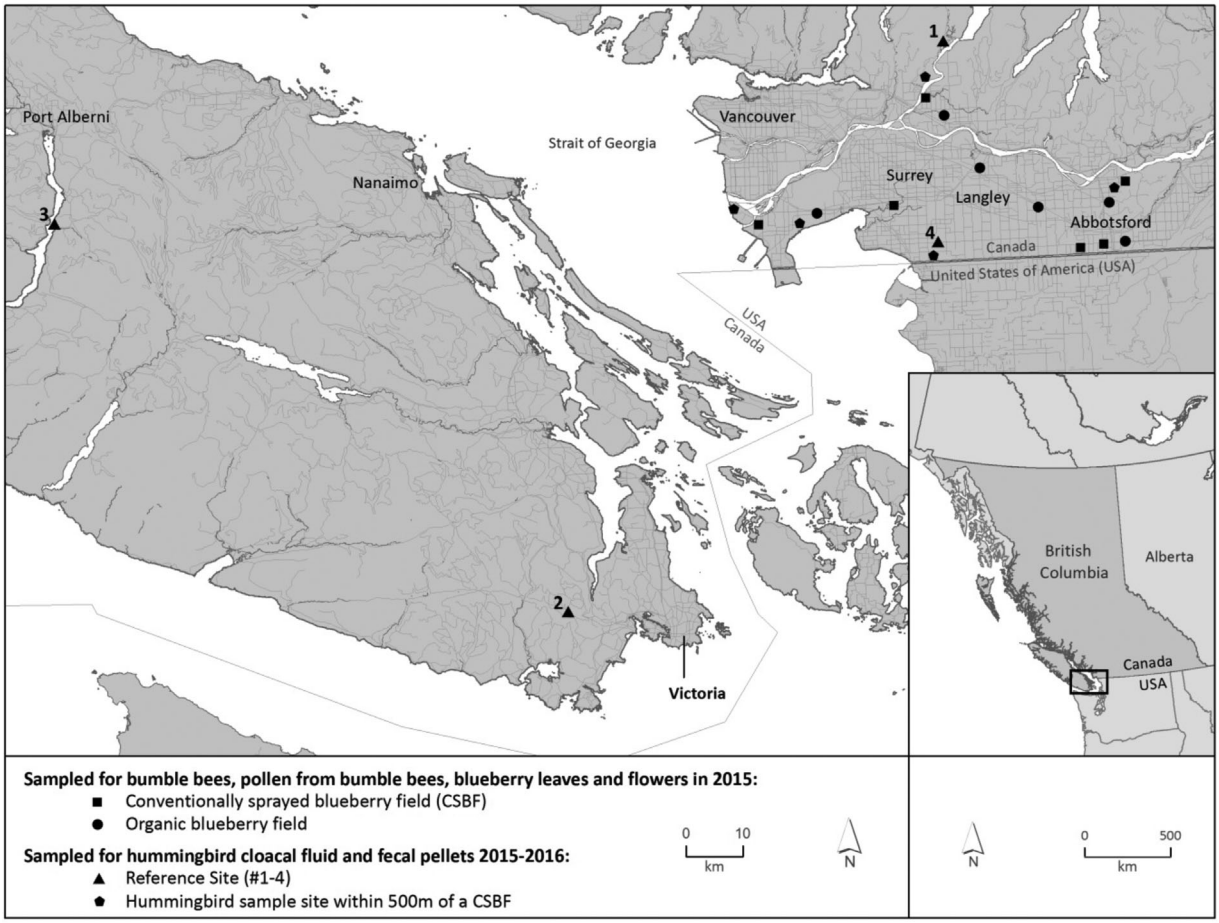

FIGURE 1: Sites in British Columbia, Canada sampled to determine pesticide concentrations in hummingbird cloacal fluid and fecal pellets, bumble bees, pollen from bumble bees, and blueberry leaves and flowers (2015–2016).

For cloacal fluid in 2016, we sampled the same reference site used on Vancouver Island in 2015 (reference site 2) as well as a second reference site on Vancouver Island (reference site 3; Figure 1). We analyzed the samples from each site separately (Table 1). We also resampled the same sites sampled in 2015, located ≤0.5 km from conventionally sprayed blueberry fields within the Fraser Valley (Table 1 and Figure 1). From 2 of those sites we had to pool the samples to attain the 300 μL volume (Table 1).

For fecal pellets, samples were collected in 2015 and 2016, from the same sites and many of the same birds that provided the cloacal fluid; one additional site was sampled near a conventionally sprayed blueberry field in the Fraser Valley (Table 2). However, the pooling scenarios were different. Our target was to analyze pools of fecal pellets by site and by year, but because a mass of >1 g was necessary for analytical purposes, fecal pellet samples from both species of hummingbirds were pooled into 3 samples for analysis (Table 2). Three

TABLE 2: Compounds (ng/mL) detected in pooled rufous (*Selasphorus rufus*) and Anna's hummingbird (*Calypte anna*) fecal pellet samples from Fraser Valley (BC, Canada; samples collected in April, May, and June 2015 and 2016)[a]

Site type	Piperonylbutoxide (detection limit = 0.153 ng/g)	No. of sites in pooled sample (no. of individual hummingbird fecal samples in pooled analysis)	Year
3 sites ≤0.5 km from CSBF + 1 site ≥1 km from CSBF (reference site 4) Fraser Valley	1.47	4 (31)	2015, 2016
One site: ≤0.5 km from CSBF Fraser Valley	5.96	1 (18)	2015, 2016
No blueberry fields within watershed and ≥10 km from agricultural site on Vancouver Island (reference sites 2 and 3)	ND	2 (16)	2016

[a]Eighteen compounds were measured: aldicarb, 3-hydroxycarbofuran, aldicarb sulfone, imidacloprid, aldicarb sulfoxide, methiocarb, aminocarb methomyl, bendiocarb mexacarbate, bendiocarb mexacarbate, carbaryl oxamyl, carbofuran, chlorpyrifox, pirimicarb, diazinon, promecarb, dioxcarb, propoxur, and piperonyl butoxide. Detection limits ranged from 0.153 to 3.06 ng/g. Specifically, the detection limit for piperonyl butoxide was 0.153 ng/g.
CSBF = conventionally sprayed blueberry field; ND = below minimum detection limit.

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TABLE 3: Insecticides (ng/g) detected in bumble bees (*Bombus mixtus*, *B. flavifrons*, and *B. melanopygus*) and pollen collected from bumble bees from the Fraser Valley (BC, Canada) in 2015[a]

Site type and location	Sample type	Diazinon	Imidacloprid	No. of sites in pooled sample (no. of pooled samples analyzed)
Conventionally sprayed blueberry field, Fraser Valley	Bumble bee pollen	1.54	4.96	6 (1)
Organic blueberry field, Fraser Valley	Bumble bee pollen	1.70	18.4	6 (1)
Organic blueberry field, Fraser Valley	Bumble bee	ND (≤~2 ng/g)	ND (≤~2 ng/g)	6 (1)
Conventionally sprayed blueberry field, Fraser Valley	Bumble bee	0.197	ND (≤~2 ng/g)	6 (1)

[a]Eighteen compounds were measured: aldicarb, 3-hydroxycarbofuran, aldicarb sulfhone, imidacloprid, aldicarb sulfhoxide, methiocarb, aminocarb methomyl, bendiocarb mexacarbate, bendiocarb mexacarbate, carbaryl oxamyl, carbofuran, chlorpyrifox, pirimicarb, diazinon, promecarb, dioxcarb, propoxur, and piperonyl butoxide. Detection limits ranged from 0.153 to 3.06 ng/g. Specific detection limits: 3.06 ng/g for imidacloprid; 0.153 ng/g for diazinon.
ND = below minimum detection limit.

pooled samples were analyzed: 1) the reference site sample was a pooled sample of the 2 Vancouver Island sites (reference sites 2 and 3; Figure 1 and Table 2); 2) 4 sites (≤0.5 km from a conventionally sprayed blueberry field) in the Fraser Valley; and 3) a single site (≤0.5 km from a conventionally sprayed blueberry field) in the Fraser Valley where we had collected enough fecal samples to allow for analysis of that site as an individual pool (Table 2).

Blueberry flowers and leaves, bumble bees, and pollen from bumble bees were collected from within 6 conventionally sprayed blueberry fields and 6 organic blueberry farms in 2015 and analyzed as pools by site and sample type (Tables 3 and 4 and Figure 1). These sites were not the same as those where hummingbirds were trapped and sampled, but they were also in the Fraser Valley (Figure 1).

Hummingbird sample collection

In 2015 and 2016, we used a noninvasive method to collect cloacal fluid and fecal pellet samples from male and female hummingbirds. Rufous and Anna's hummingbirds were captured using a modified Hall trap and hummingbird feeder (Russell and Russell 2001). Each sampling period was 3 h per site conducted during 0700 to 1100 h on a single day per site in mid-April, mid-May, and mid-June in both years. One trap per site was used.

The hummingbird feeders contained white retail-purchased sugar and water solution (1:4; Figure 2). For neonicotinoid analysis in 2016, we analyzed 2 pooled samples of sugar water from feeders. We collected approximately 1 mL of sugar water from the feeders used at each of 5 sample sites in the Fraser Valley (4 sites located ≤0.5 km from a conventionally sprayed blueberry field and a reference site ≥1 km from a conventionally sprayed blueberry field [reference site 4]) and from one feeder at a sample site on Vancouver Island (reference site 2; Figure 1).

Hummingbirds were removed from the Hall trap by hand and gently restrained by wrapping an 8- × 4-cm disposable cloth around their wings that was secured with a mini-alligator clip (Figure 3). A micropipettor (100 μL) was used to capture any and only cloacal fluid or any fecal pellets that were spontaneously produced by the bird during handling when the birds were being measured and banded for a related study. Pipettor tips were changed between collections of fluid and pellets from the same bird and between sampled birds.

Some birds voluntarily produced approximately 5 to 60 μL of cloacal fluid/bird. If fluid or fecal pellets were not spontaneously produced, no samples were collected from the bird. Anna's hummingbirds often produced up to 30 to 60 μL/bird whereas rufous hummingbirds, when they produced cloacal fluid, tended to produce 5 to 30 μL/bird. Samples were stored in 1-mL vials, placed on ice packs and in the dark within 2 min of collection, and stored at –5 °C within

TABLE 4: Insecticides detected in prespray blueberry flowers (*Vaccinium corymbosum*) and blueberry leaves post spray with imidacloprid from Fraser Valley (BC, Canada) in 2015 (ng/g)[a]

Site type and location	Sample type	Time of sample collection	Imidacloprid	No. (pooled samples)
Conventionally sprayed blueberry field, Fraser Valley	Blueberry flowers	Collected in 2015, ~1 yr post spray of imidacloprid period in 2014 on these farms	5.16 from one farm; samples from all other farms were ND (≤~3.06 ng/g)	6 (1 pooled sample of flowers from each of 6 farms)
Organic blueberry field, Fraser Valley	Blueberry flowers	Collected in 2015, ~1 yr post spray of imidacloprid period on conventionally sprayed blueberry fields nearby in 2014	All samples ND	6 (1 pooled sample of flowers from each of 6 farms)
Conventionally sprayed blueberry field, Fraser Valley	Blueberry leaves	Collected in 2015, 1 wk post spray of imidacloprid on these farms in 2015	1770; 1990	2 (1 pooled sample of leaves from each of 2 farms)
Conventionally sprayed blueberry field, Fraser Valley	Blueberry leaves	Collected in 2015, 1 mo post spray of imidacloprid on these farms in 2015	14.5; 508; 103	3 (1 pooled sample of leaves from each of 3 farms)

[a]Detection limit for imidacloprid was ≤~3.06 ng/g. Seventeen other pesticide and related compounds were also measured, and concentrations were below detection limits of 3.06–0.153 ng/g.
ND = below minimum detection limit.

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FIGURE 2: Hummingbirds were captured with a Hall trap suspended over a hummingbird feeder containing white sugar and water solution (1:4). Photograph credit: Christina Lam.

5 h of collection until they were thawed to be pooled by treatment or site (Table 1), using a micropipettor, into Eppendorf microcentrifuge vials. Rufous and Anna's hummingbird samples were combined by site (Table 1). The samples were refrozen at –5 °C and shipped on dry ice to laboratories for analysis. The cloacal fluid was shipped to and analyzed at the Environment and Climate Change Canada federal laboratory at the National Wildlife Research Centre (NWRC; Ottawa, ON, Canada). Fecal pellets were shipped on dry ice and analyzed at SGS AXYS Enviro (Sidney, BC, Canada). When samples were to be extracted, they were thawed at room temperature immediately prior to extraction.

FIGURE 3: Hummingbirds were gently restrained by wrapping an 8- × 4-cm disposable cloth around their wings, which was secured with a mini-alligator clip. A micropipettor (100 μL) was used to capture cloacal fluid or any fecal pellets that were spontaneously produced by the bird during handling when the birds were being measured and banded for a related study. Photograph credit: Christine Bishop.

Bumble bee and pollen collection

The blueberry bloom period in the Fraser Valley lasts for approximately 3 wk; it can be as early as the last week of April and can extend to the end of May (British Columbia Ministry of Agriculture 2012). We conducted our field collections of bumble bees for pesticide analysis (pollen removed from corbiculae before analysis) within a 12-d period in early May 2015, which was the middle of the blooming season before imidacloprid was sprayed for the season. Worker bumble bees of the 3 most abundant species (*B. mixtus*, *B. flavifrons*, and *B. melanopygus*) were net-collected during 10 d in early May 2015 from 12 blueberry farms (6 conventional and 6 organic; Figure 1 and Table 3) and pooled by site. The range in mass of bumble bee samples collected per site was 0.52 to 2.9 g at conventionally sprayed and 0.86 to 2.31 g at organic blueberry farms (7–10 bees/site).

To collect bumble bees for analysis, we visited both conventionally sprayed and organic blueberry farms within the same day. Surveys were limited to days with mostly to partly sun, temperatures above 13 °C, and nonwindy conditions, which are conducive to invertebrate pollinator foraging within the farms. Bees were stored in vials, placed on ice packs in the dark immediately after collection at the farm site, and then stored at –80 °C until they were transferred onto dry ice and shipped to the SGS AXYS laboratory for analysis.

Pollen was collected from different individuals than the bumble bee samples but in the same manner and from the same sites. The bumble bees for pollen collection were sampled 5 times from mid-April to the end of June, which was during and after blueberry bloom. The pollen was removed from bumble bees in the laboratory and then stored at –80 °C in the dark. Because total pollen mass collected was low per site, we pooled pollen by site type: one sample for conventionally sprayed blueberry fields and one sample for organic farms (pooled sample from conventionally sprayed blueberry fields = 0.5 g; from organic farms = 0.7 g).

Flowers and leaves

In the Fraser Valley (British Columbia Ministry of Agriculture 2012) and based on our communications with the farm operators of the fields, imidacloprid was known to be sprayed after blossom fall once a year on the conventionally sprayed blueberry fields. Therefore, flower samples were collected in May 2015 during full bloom period assuming that this was approximately 1 yr after the 2014 spray and before the 2015 imidacloprid spray on those fields (Table 4). Leaf samples were collected at 2 time intervals after an imidacloprid spray in 2015: 1 wk post spray and

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1 mo post spray (Table 4). All flowers and leaves were collected at the same blueberry fields at 2 distances from the field edge (25 and 50 m). Leaves and flowers were taken from approximately 10 blueberry bushes at each distance and pooled together to comprise one sample per farm. Leaves and flowers were placed on ice packs in the dark immediately after collection in the field, then stored at −10 °C before analysis, and then shipped to SGS AXYS under the same conditions used for bees and pollen.

Chemical analysis

In 2015 and 2016, cloacal fluid samples were analyzed for neonicotinoids by Laboratory Services, NWRC (National Wildlife Research Centre 2015, 2016), following a method adapted from Main et al. (2014).

An aliquot of 200 μL of each cloacal fluid pool was spiked with 50 μL of acetonitrile containing the internal standards. The sample was then vortexed and transferred to a 2-mL autosampler vial containing a 250-μL micro-insert. A 50-μL aliquot was injected into the Agilent 1200 high-performance liquid chromatography (LC) system equipped with a Waters X-Terra® mass spectrometer (MS) C8 (3.5 μm; 2.1 × 100 mm) column maintained at 40 °C. The analytes were separated using isocratic conditions (80:20; 0.1% formic acid in reverse osmosis water:0.1% formic acid in acetonitrile) at a flow rate of 0.5 mL/min. Under these analytical conditions, all compounds eluted in under 5 min. The neonicotinoids were detected using the API 5000 Triple Quadropole Mass Spectrometer (AB Sciex) with the TurboSpray ion source in positive polarity. Multiple reaction monitoring transitions for each neonicotinoid and triple quadropole settings were used (Supplemental Data, Tables S1 and S2).

Neonicotinoids were quantified using the internal standard method. A calibration curve was built using 8 levels ranging from 0.25 to 100 ppb with $R > 0.995$ (linear regression, no weighting). The analytical standards and internal standards were from Sigma-Aldrich.

Solvent blanks (water:acetonitrile 80:20) were injected at the beginning and the end of each set of samples to monitor injection cross-contamination. A sample blank (reverse osmosis water and/or nectar) spiked with internal standards was analyzed with each set of samples to detect possible contamination. None of the blanks had detectable amounts of neonicotinoids. Method precision was evaluated by analyzing one random sample per set in duplicate. All duplicate results above the minimum reporting level (3× the minimum detectable limit) were <15%. Method accuracy was quantified by spiking a clean cloacal fluid pool with a mixed neonicotinoid standard and calculating the recoveries; all values were between 85 and 115%. To monitor quantification accuracy, we analyzed a second source standard (commercially prepared solution; ChemService) against a calibration on a daily basis. The calculated concentrations were within 85 to 115% of the expected concentrations.

Furthermore, during method development, matrix effect was evaluated by diluting a cloacal fluid pool in various proportions with reverse osmosis water. We were able to observe that ion suppression was minimal in the undiluted cloacal fluid pool. Recoveries ranged (average of 6 replicates at 2 levels) from 95% to 117% for acetamiprid, clothianidin, imidacloprid, thiamethoxam, and thiacloprid (Supplemental Data, Table S3). Because of an important interference at the dinotefuran retention time peak, this compound could not be measured. The detection limits ranged from 0.02 to 0.063 ng/mL for the measured compounds: imidacloprid (0.051), clothianidin (0.042), thiamethoxam (0.063), acetamiprid (0.033), and thiocloprid (0.20; Table 1).

Fecal pellets, leaves, flowers, bumble bees, and pollen were extracted and analyzed by SGS AXYS following their method MLA-047 for biosolids. The liquid–solid extraction was conducted using ultrasonic agitation with aqueous acetonitrile. Analyte stability during extraction was maintained with acetate buffer at pH = 4. Extracts were cleaned up using aminopropyl solid-phase extraction cartridges. Analytes were separated using a Water SunFire LC column (C18, 3.5 μm, 4.6 × 30 mm). Analysis was conducted using LC–MS/MS in the (+) electrospray ionization mode. Data were acquired in the multiple reaction monitoring mode. The isotope dilution/internal standard method of quantification was applied. Five isotope labeled surrogate standards and one labeled recovery standard were used. Each analysis batch included a laboratory blank, a spiked blank, and a duplicate sample. Percentage recoveries ranged from 81.2 to 110%. The 18 compounds measured in the AXYS standard panel pesticide testing were aldicarb, 3-hydroxycarbofuran, aldicarb sulfone, imidacloprid, aldicarb sulfoxide, methiocarb, aminocarb methomyl, bendiocarb mexacarbate, bendiocarb mexacarbate, carbaryl oxamyl, carbofuran, chlorpyrifox, pirimicarb, diazinon, promecarb, dioxcarb, propoxur, and piperonyl butoxide. Detection limits ranged from 0.153 to 3.06 ng/g. The detection limits for the 3 compounds detected in our samples were 3.06 ng/g for imidacloprid, 0.153 ng/g for diazinon, and 0.153 ng/g for piperonyl butoxide.

RESULTS

We detected 3 neonicotinoid compounds in the pooled hummingbird cloacal fluid samples. Imidacloprid was present in cloacal fluid samples from all samples from the Fraser Valley in 2015 and 2016. The Vancouver Island samples from 2016 did not contain detectable concentrations of any neonicotinoid compound (Table 1). Neonicotinoids were not detected in the sugar water samples from the hummingbird feeders in the Fraser Valley or Vancouver Island.

In 2015, the combined concentration of thiamethoxam, clothianidin, and imidacloprid detected in cloacal fluid from sites near conventionally sprayed blueberry fields was 3.63 ng/mL (ppb; Table 1). In 2015, the pooled sample of 2 reference sites, 1 site in the Fraser Valley and 1 site on Vancouver Island, contained imidacloprid but at a lower concentration of 0.086 ng/mL (Table 1).

In 2016, only imidacloprid was detected in cloacal fluid, with concentrations ranging from 0.068 to 0.452 ng/mL among 4 sites located near conventionally sprayed blueberry fields (mean [standard deviation (SD)] = 0.196 [0.178]; Table 1). The

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maximum concentration in 2016 of imidacloprid was 0.452 ng/mL, twice that of the 0.197 ng/mL detected in 2015 in the pooled sample collected near the same conventionally sprayed blueberry field (Table 1).

Among the 18 compounds in the analytical screen of fecal pellets, only piperonyl butoxide was detected (Table 2). Piperonyl butoxide was present in 2 fecal pellet samples from the Fraser Valley, which were both collected from sites near conventionally sprayed blueberry fields. The concentration in the sample from a single site was 4 times higher compared with results in a pooled sample comprised of fecal pellets from 4 sites (Table 2). Piperonyl butoxide is not a pesticide but rather a cytochrome P450 inhibitor that can be applied with insecticides such as synthetic pyrethroids to increase their toxic efficacy (Brooks and Harrison, 1964). No compounds were detected in the fecal pellets from the reference site on Vancouver Island (Table 2).

Only diazinon was detected in bumble bees collected in May 2015, whereas diazinon and imidacloprid were detected in corbicular pollen from bumble bees collected from April through June 2015 (Table 3). Imidacloprid in pollen from organic farms was detected at 18.4 ng/g, which was 3 times higher than in pollen from bees collected from conventionally sprayed blueberry farms (Table 3). Pollen from organic farms also contained concentrations of diazinon similar to those from conventionally sprayed blueberry fields.

Imidacloprid was detected at concentrations ranging from 1770 to 1990 ng/g in blueberry leaves collected 1 wk post spray (mean [SD] = 1880 [155.6]) and concentrations declined to between 14.5 and 508 ng/g within 1 mo post spray (mean [SD] = 208.5 [263.1]; Table 4). At 1 yr post spray, imidacloprid was detected in 1 of 6 blueberry flower samples from conventionally sprayed blueberry fields (mean [SD] = 2.13 [1.48]; mean calculated using half the detection limit for the 5 samples with nondetected values of <3.06 ng/g; Table 4). No other pesticides were detected.

DISCUSSION

We detected pesticides and related compounds in cloacal fluid and fecal pellets of hummingbirds, revealing pesticide exposures of multiple types, a finding that has not been documented previously. Bumble bees, their pollen, and blueberry flowers also contained pesticides, with the highest concentration of imidacloprid in pollen from organic farms. Imidacloprid remained present on blueberry leaves 1 mo post spray. By sampling a variety of sites in the Fraser Valley and Vancouver Island and examining organic and conventionally sprayed farms, our findings extend beyond simple detection of pesticides in hummingbirds and in bumble bees and their pollen and indicate that pesticide exposure routes may be widespread and complex.

The lethal and sublethal effects of neonicotinoids and other pesticides to bee species have been widely researched and documented (Woodcock et al. 2017). There are, in contrast, no published studies of exposure or toxicity of any pesticide on hummingbirds. Passerine birds have a relatively high oral acute toxicity threshold to neonicotinoids (oral median lethal dose: 25–50 mg/kg; Gibbons et al. 2014). However, environmentally relevant exposures to neonicotinoids induce sublethal effects on the ability of white-crowned sparrows (*Zonotrichia leucophrys*) to retain body mass, and also cause migratory disorientation (Eng et al. 2017). We do not know the health implications of 3.63 ng/mL of neonicotinoids in hummingbird cloacal fluid, or whether this represents exposure in just a few or many birds, or whether this concentration is a dilution effect of many unexposed birds and some highly exposed birds. Because hummingbirds have a high metabolism and are metabolically very different from passerines (McNab 1988; Suarez and Gass 2002), the toxicity of pesticides in passerines may not be representative. The only comparable data available on neonicotinoids in fluids in vertebrates are in humans (*Homo sapiens*), in whom concentrations of neonicotinoid insecticides have been reported in urine and blood (Taira et al. 2013; Ueyama et al. 2015). The toxicity associated with neonicotinoid concentrations in human urine is unknown; however, in blood, 2.05 to 84.9 ng/mL of acetamiprid is associated with severe to lethal neurotoxic and cardiac symptoms (Tamura et al. 2002; Proenca et al. 2005).

For hummingbirds, our analytical and field methods could provide valuable tools to examine health effects in these birds. There was consistency between years in analytical results, and the variation in concentration of neonicotinoids in the cloacal fluid among sites also demonstrates that site-specific differences are detectable. The limitations are that individual bird exposure was not measured due to the volume of the sample required for analysis. We collected as much sample as the birds produced individually, but to attain appropriate volumes for analysis we pooled samples from rufous and Anna's hummingbirds. We found that Anna's hummingbird cloacal fluid volumes were at least twice that of rufous hummingbirds, and, therefore, the results may represent a bias toward exposure in Anna's hummingbirds. The higher volumes of fluid from Anna's hummingbirds may be attributed to their limited ability to concentrate urine (Casotti et al. 1998). Given the declining population trends in the rufous hummingbird, there is a need to sample this species alone, and when low volumes (5 μL) are obtained per bird the sampling protocol would require that at least 30 birds be sampled for a single analysis of cloacal fluid per site. For fecal pellets, we found that 15 samples of pellets from either species of hummingbird was sufficient for approximately 1 g of mass for analysis.

Given that hummingbirds feed on nectar and invertebrates (Yanega 2007), pesticide exposure detected in fluid was most likely during nectar feeding, and fecal samples may primarily represent insect sources. This aspect of hummingbird excretion in which fluids and feces are well separated, unlike in most birds, offers an opportunity to examine chemical partitioning. Nectar is metabolized quickly in hummingbirds, and excretion of fluids occurs within ≤1 h (Bakken et al. 2004). If pesticides are metabolized similarly, detections in cloacal fluid may represent exposure in the previous several hours or less if the exposure route is primarily through nectar consumption. In quail (*Coturnix* japonica), clearance of imidacloprid from plasma is rapid (with a single dose, elimination half-lives were estimated to be 1–7 h

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[low dose] and 1–5 h [high dose]; Bean et al. 2017). Imidacloprid in plasma was only detectable in 2 of 16 quail 24 h post exposure. Concentrations of the metabolites 5-OH imidacloprid and imidacloprid olefin were 6 to 10 and 4 to 5 times greater than the parent compound in liver and plasma, respectively, but 1 order of magnitude lower than imidacloprid in brain. Excretion of the parent compound in feces (and urates combined) was 37 to 78 times less than that of these 2 metabolites. Concentrations per gram of feces were 1 to 2 orders of magnitude greater than concentrations per gram of tissue. Thus, it appears that imidacloprid is rapidly cleared from quail as polar metabolites (Bean et al. 2017). This is similar to results for neonicotinoids fed to rats in which the administered doses of methylene-^{14}C imidacloprid and imadizolidine 4,5-^{14}C imidacloprid were rapidly absorbed (peak plasma concentrations at 1–2.5 h and 1–4 h post dosing, respectively), and 90% was eliminated within 24 h following oral exposure. Urinary excretion is a major route of elimination (70–91% of the administered dose), with only 7 to 25% eliminated in feces (Pest Management Regulatory Agency, Government of Canada 2016a). Metabolites of imidacloprid such as desnitro-imidacloprid, which have active toxicological effects, are also detectable in mouse feces (Pest Management Regulatory Agency, Government of Canada 2016a) but were not measured in our hummingbirds.

Our finding that imidacloprid was detectable in blueberry flowers collected prespray in 2015 from one of 6 farms suggests that hummingbirds can be exposed through nectar collection in fields before they are sprayed. The probability of exposure will depend on how often imadacloprid is residual in soils and plants, and how often hummingbirds forage for nectar in blueberry fields. Bumble bees collected during bloom did not have detectable imadacloprid, suggesting that the probability of exposure during the blueberry bloom is variable. The imidacloprid concentration we found in blueberry flowers from one farm was consistent with that from *Apis* bees in other studies (Blacquière et al. 2012). It was also consistent with the concentration in pollen from bumble bees in our study; some of the pollen was collected after blueberry bloom and so contained pollen from non-blueberry sources. Imidicloprid in blueberry flowers is also consistent with the known persistence of imidacloprid in treated soils of 157 to 973 d (Pest Management Regulatory Agency, Government of Canada 2016a), and the presence of imidacloprid in nectar of citrus fruit trees at 232 d post soil application (Byrne et al. 2014) and in nectar of flowers from fruit crops (<10 ng/mL; Byrne et al. 2014), and in wildflowers (up to 12.29 ng/g) collected from the edges of sprayed cereal fields (Botias et al. 2015).

The cloacal fluid results also demonstrate consistent exposure of hummingbirds to neonicotinoid pesticides in both years of the study in the Fraser River Valley. Our analysis of sugar-water samples and our reference site findings from Vancouver Island address 2 important aspects of the pesticide exposure scenario for hummingbirds. Although the hummingbirds are sipping tap water plus retail white sugar at feeders at all of our trapping sites, the nondetectable results in our sugar-water test samples and from cloacal fluid from Vancouver island birds indicate that hummingbirds are not being exposed to pesticides via sugar or water in bird feeders. In 2016, when Vancouver Island samples solely comprised the reference samples, neonicotinoids were not detected from the sites far from agricultural fields. However, in 2015 when the Vancouver Island and Fraser Valley reference site samples were pooled, imidacloprid was detected. This suggested that hummingbirds, even in more remote areas of the Fraser Valley, may be exposed to neonicotinoids. In our many observations in British Columbia, investigation of recaptured birds from banding sites indicated that hummingbirds typically move to forage at a distance of up to 1 km but occasionally 2 to 8 km between banding stations, suggesting that these birds may be exposed to pesticide sources >1 km away from our sample sites in the Fraser Valley.

In many of the same birds that contained neonicotinoid insecticides in cloacal fluid, we did not detect these compounds in fecal pellets, which may be due to detection limits that were 2 orders of magnitude higher in the biosolid methods relative to the cloacal fluid analytical methods. Also, the tendency of neonicotinoids to be excreted in urine as unchanged compounds due to their high water solubility (Ford and Casida 2006a, 2006b) may mean that fecal pellets did not contain detectable levels of these pesticides. However, the fecal pellets may be equally useful in revealing other types of chemical exposure. Piperonyl butoxide (detected in fecal pellets) is an organic compound added to pesticide formulations to increase potency, primarily of pyrethroids, because it suppresses the insect cytochrome P-450 mixed-function oxidase system (Brooks and Harrison 1964; Wilkinson et al. 1984). Synthetic pyrethroids are registered for use on many crops and for domestic use in the Fraser Valley (British Columbia Ministry of Agriculture 2009, 2012, 2017, 2018; Pest Management Regulatory Agency, Government of Canada 2017), suggesting that exposure sources could be widely available to hummingbirds.

To understand pesticide sources for the hummingbirds, we must also consider our sampling locations and timing of sampling. The spatial variation in neonicotinoid concentrations in cloacal fluid reflected proximity to blueberry fields. Imidacloprid was detected in blueberry flowers on one farm, indicating that nectar sources can be contaminated in the fields. However, our pooled fluid and fecal samples were collected from April to June when blueberries are in bloom for just 3 wk during that period. The cloacal fluid may therefore represent a composition of feeding sites. Hummingbirds may also be exposed via wildflowers growing near treated agricultural fields (Botias et al. 2015) and/or garden plants grown for the nursery trade or other crops such as the raspberries grown in the region. A variety of crop types in the Fraser Valley use neonicotinoids, pyrethroids, and organophosphate pesticides as well as carbamates and fungicides (British Columbia Ministry of Agriculture 2009, 2012, 2017, 2018), and hummingbirds may come into contact with these crops when they are in bloom or may come into contact with insects that have fed on the crops.

Our findings from the bumble bees and their pollen and from blueberry samples further suggest that the source(s) and timing of pesticide exposure may be more landscape

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driven than simple exposure from within conventionally sprayed blueberry crops. *Bombus* sp. commonly forage over distances from 0.5 to 1.5 km, as determined by a variety of food sources within the landscape composition and configuration (Osborne et al. 2008; Redhead et al. 2016). This factor suggests that exposure could occur within field margins of blueberry fields and/or other crop types including organic blueberry fields, which, in our study area, were located next to conventionally sprayed fields. Although bumble bees collected during bloom did not contain detectable neonicotinoids, these compounds were found in pollen from bees in pooled samples (collected both during and after bloom) on both conventional and organic farms. The concentration of imidacloprid in bumble bee pollen from the Fraser Valley were within the same range ($\leq$25.55 ng/g) detected in honey bee (*Apis mellifera*) pollen from cereal crop field margins (Botias et al. 2015). Observations that bumble bees in the Fraser Valley prefer feeding off- blueberry crop (Bobiwash et al. 2018) and that pollen was collected for our analysis both during and after the 3-wk blueberry bloom suggest that other types of pollen could also be sources of pesticide exposure. Our detection of diazinon in pollen is further evidence that other crops were probably visited by the bumble bees that were subsequently sampled from within blueberry fields. Diazinon was phased out in Canada in 2012 from use in airblast sprayers such as the type used in blueberry fields in the Fraser Valley; however, diazinon use on raspberries was not phased out until the end of 2016 (Pest Management Regulatory Agency, Government of Canada 2013). Raspberries are also commonly grown in the Fraser Valley in close proximity to some of our blueberry study fields (for locations of raspberry farms in the Fraser Valley, see Raspberry Industry Development Council 2017).

SUMMARY AND CONCLUSIONS

In this first examination of pesticide exposure in hummingbirds, the results revealed wide-ranging chemical exposure. By examining vertebrate and invertebrate pollinators, we applied an approach that enhanced our understanding of the complexity of pesticide exposure in a pollinator guild within the Fraser Valley. This approach may be valuable wherever mosaics of agricultural and suburban landscapes occur.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4174.

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Data availability—Associated data will be available through this journal's data repository and linked to the final published article.

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