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A multi-year field study to evaluate the environmental fate and agronomic effects of insecticide mixtures

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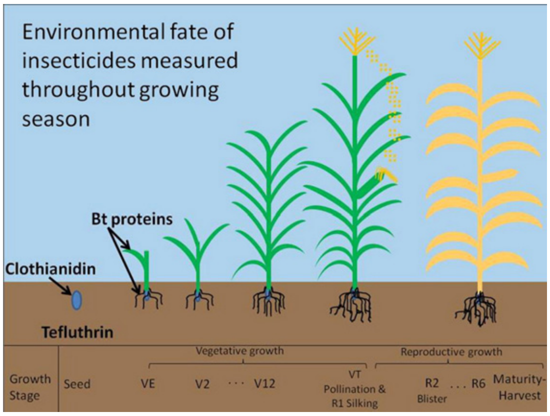
HIGHLIGHTS

- Cry1Ab proteins were found in soil and runoff water, but dissipated quickly.
- Clothianidin was found in all environmental matrices.
- Tefluthrin was measured at high levels in soil, runoff water, and runoff sediment.
- The addition of tefluthrin to Bt corn had no impact on grain yield or pest damage.

GRAPHICAL ABSTRACT



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ABSTRACT

A mixture of insecticides used in corn production was monitored over a three-year period in a field study to determine how long each persists in the environment, where each insecticide travels within the corn field, and the efficacy of using soil-applied insecticides with genetically modified corn. The genetically modified corn contained the insecticidal Cry1Ab and Cry3Bb1 proteins (Bt corn) and the Cry1Ab protein was found to persist only during the corn growing season in soil, runoff water, and runoff sediment with highest concentrations measured during pollination. Very low concentrations of Cry1Ab proteins were measured in soil collected in the non-Bt corn field, and no Cry1Ab proteins were detected in shallow groundwater or soil pore water. Clothianidin, a neonicotinoid insecticide used as a seed coating, was detected in all matrices and remained persistent throughout the year in soil pore water. Tefluthrin, a pyrethroid insecticide applied at planting to control corn rootworm larvae (*Diabrotica* spp., Coleoptera: Chrysomelidae) populations, was consistently detected in soil, runoff water, and runoff sediment during the corn growing season, but was not detected in groundwater or soil pore water. Tefluthrin did not have an effect on root damage from corn rootworm larvae feeding to Bt corn, but did prevent damage to non-Bt corn. A slight reduction in grain yield was observed in the non-Bt, no tefluthrin treatment when compared to all other treatments, but no significant difference in grain yield was observed among Bt

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corn treatments regardless of soil insecticide application. In the current study, the use of tefluthrin on Bt corn did not significantly affect crop damage or yield, and tefluthrin may travel off-site in runoff water and sediment.

1. Introduction

The corn rootworm complex, *Diabrotica* spp. (Coleoptera: Chrysomelidae), has been a major pest of field corn (*Zea mays*) in the United States for decades causing substantial crop damage and; therefore, reductions in corn grain yield (Dun et al., 2010). Transgenic or genetically modified (GM) corn was introduced in the United States in 1996 as a new method to control corn pests with a specific mode of action so that non-target and beneficial insects were not directly affected by its use (Roush, 1998). Currently registered insect-protected GM corn contains genetic code from the bacterium *Bacillus thuringiensis* Berliner (Bt) and the corn produces insecticidal crystal protein endotoxins (Cry proteins) that are lethal to target pest species when ingested (Roush, 1998).

Since the first Bt crop was released in 1996 and the first trait for rootworm control was released in 2003, adoption of this technology has increased exponentially in the United States from 8% of total corn acreage grown in the United States in 1997 to 90% in 2013, while some conventional insecticide use has decreased (USDA, 2013). However, recent studies found that some rootworm populations have developed evolutionary resistance to the GM corn trait Bt-Cry3Bb1 (Gassmann et al., 2011, 2012). A recent pest management strategy has been the addition of seed coatings with neonicotinoid insecticides (example: clothianidin). Neonicotinoid insecticides, like clothianidin, are the most widely used insecticides in the world (Goulson, 2013), and almost all seed corn purchased in the United States is coated with a neonicotinoid insecticide (Mullin et al., 2005). Conventional soil insecticide application of the pyrethroid, tefluthrin, at planting is another strategy to control rootworm genetic resistance to Bt crops, although the benefits of this method have not been researched in depth (Petzold-Maxwell et al., 2013).

The current study fills important gaps in knowledge about the environmental fate and agronomic efficacy of the combination of conventional insecticides with Bt corn production. Concentrations of each insecticide were measured in runoff water and sediment, groundwater, soil pore water, and soil before, during, and after the corn growing season throughout three years at a research site in central Illinois using a continuous corn, no-till agricultural system. Corn grain yields and root damage from pests were also measured in order to determine agronomic efficiency of using this corn production system. The goal of the study was to determine how long these insecticides persist in the environment, where the insecticides are found in the environment after application, and if the combination of insecticides affects grain yields and pest damage.

2. Methods

2.1. Solvents and chemicals

Purified Cry1Ab protein was purchased from Abraxis, LLC (Warminster, PA, USA). Neonicotinoids including clothianidin and acetamiprid were 99.9% pure (Sigma-Aldrich, St. Louis, MO, USA) and imidacloprid (ChemService, West Chester, PA, USA) was 99.5% pure. Surrogates for the clothianidin analysis were imidacloprid and acetamiprid. The tefluthrin stock used for quantification was 96.4% pure (Fluka Analytical, Sigma-Aldrich). The surrogate used for the tefluthrin analysis was decachlorobiphenyl (DCBP, 200 μg mL^{-1} in acetone, Supelco Analytical, Sigma-Aldrich).

Solvents were purchased from Fisher Scientific (Waltham, MA, USA) and included pesticide grade hexanes, acetone, and dichloromethane (DCM), Optima grade acetonitrile (ACN), and HPLC grade submicron filtered water. Trifluoroacetic acid (≥98%) was purchased from Sigma-Aldrich. The solid phase extraction (SPE) cartridges used to clean sample extracts were 6 mL dual layer Supelclean™ ENVI™-Carb II/primary secondary amine (PSA) 300/600 mg (Supelco Analytical).

2.2. Field site

Research was conducted on a 36 ha farm in Christian County, Illinois, USA. Three replicates of six different treatments were planted in 2011, 2012, and 2013, with the treatments planted in the same locations in the field in each of the three years. The treatments included: (1) Bt corn MON88017 × MON810 with the insertion of Cry3Bb1 and Cry1Ab genes, respectively, with no tefluthrin; (2) Bt corn MON88017 × MON810 with half the normal rate of tefluthrin applied which was 0.06 kg ha^{-1}; (3) Bt corn MON88017 × MON810 with the full rate of tefluthrin 0.11 kg ha^{-1}; (4) a near isoline of non-Bt corn with no tefluthrin applied; (5) non-Bt corn with half the normal rate of tefluthrin applied; and (6) non-Bt corn with the full rate of tefluthrin applied. Only the Cry1Ab protein (which targets Lepidopteran pests) was quantified in environmental matrices due to lack of commercial availability of standards of the Cry3Bb1 protein. There are differences in protein expression within the plant (e.g. Cry3Bb1 found at high levels in roots, and Cry1Ab found at high levels in ear tissue — see Table 5 in USEPA, 2010), but based on a literature review and the capabilities in our laboratory, it was determined that Cry1Ab could be examined in the environment and compared to other Bt Cry proteins. Corn seed from all treatments was uniformly coated with 0.25 mg of the active ingredient clothianidin per seed. In order to replicate typical field conditions of an average grower's practice in Central Illinois, all seed was treated with a seed treatment, therefore, there was no field control for clothianidin. There were no foliar applications of fungicides to any treatment, however some fungicides were used as a component of the seed coating. Fungicides in the seed treatment varied between years, but were always uniformly applied. Seed was planted at a rate of 86,488 seeds per ha in 2011 and 2012 and at a rate of 84,017 seeds per ha in 2013. Supplemental fertilizer including nitrogen, phosphorus, and potassium were applied to each treatment every year as per standard local corn production practices. All treatments had the same rate of herbicides isoxaflutole, thiencarbazone-methyl, and glyphosate applied within a week after planting each year to provide essentially a weed-free experiment.

2.3. Field sample collection

Groundwater was collected from seven - 5.08 mm diameter, 4 m deep polyvinylchloride (PVC) wells spaced so that each treatment was represented at least once. Wells were purged prior to sample collection with a submersible pump (Water Spout II Super Purge Pump, Forestry Suppliers, Inc., Jackson, MS, USA) (USEPA, 2004). Soil pore water was collected from lysimeters buried 1 m in the ground (Large volume "Ultra" sampler, Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Based on Farm Service Agency aerial images, slopes on the research field ranged from 0 to 2% and from 2 to 5% slopes in other areas of the field (Surety software, Agridata, Inc., Grand Forks, ND, USA). Most runoff samples from the current study were collected from the section with 2 to 5% slopes. Runoff water and sediment were collected after rain events (at least 1.27 cm of rain accumulation) by overland samplers (troughs) consisting of a 3.05 m segment of 0.3 m diameter PVC pipe cut in half with caps on the ends installed level with the soil surface. Troughs were cleaned before the rain event and the sample,

Table 1
Environmental characteristics of water samples collected in the field.

Sample	Total organic carbon (mg/L)	Total suspended solids (mg/L)	Conductivity (μS/cm)	Dissolved oxygen (mg/L)	pH
Soil pore water	2.6	4.0	351.0	8.4	7.4
Groundwater	1.1	ND	579.0	7.8	7.8
Runoff water	45.7	25.0	60.9	5.8	6.7

ND = non-detect.

including water and sediment, was taken directly from the trough using an aluminum scoop within 24 h of the rain event. Precipitation was monitored from an onsite hydrological weather station (Campbell Scientific, Inc., Logan, UT, USA). Runoff samples were only collected during the growing season. Each runoff sampler and lysimeter was placed in the middle of the treatment with three replicates of each of the six treatments for a total of 18 lysimeters and 18 runoff samplers. All water samples were collected in acetone-rinsed clean glass jars, stored at 4 °C until processed, and extracted for all compounds within 14 days of collection to prevent degradation (Slanina et al., 1979). Water characteristics are listed in Table 1. Conductivity was measured using a YSI 30 salinity, conductivity and temperature meter (YSI Inc., Yellow Springs, OH, USA), dissolved oxygen (DO) was measured with a YSI 55 dissolved oxygen probe, and pH was measured with an Orion 4 Star pH meter (Thermo Scientific, Chelmsford, MA, USA). Total suspended solids (TSS) and total organic carbon (TOC) were measured in water by Midwest Laboratories (Omaha, NE, USA) using standard methods 2540D and 5310B, respectively.

Soil samples were collected using a stainless steel hand trowel that was cleaned with a cloth between each sample leaving no residual soil on the trowel between samples. Two soil samples were taken from the top 3 cm of soil with the hand trowel between the rows of corn and in the corn row near the roots. The two samples were stored in the same container and later homogenized with a 2 mm sieve with all visible plant material removed before analysis (Ahmad et al., 2005). Runoff sediment that settled from water samples was separated and frozen until chemical analysis. Soil samples were on average 27% clay, 70% silt, and 3% sand, had a total cation exchange capacity of 30 meq 100 g^{-1}, had a pH of 6.6, and had a total organic carbon amount of 2.2%. Field samples for insecticide quantification were collected before planting, four times during the corn growing season, and after harvest. Analytical methods for extracting the insecticides from environmental samples are provided in the Supplemental information (SI).

2.4. Yield and crop damage methods

Corn roots were harvested in the middle of the corn growing season when rootworm pressure was predicted to be highest on one occasion per year. To evaluate root damage, three plants with intact roots were harvested in three different locations within each of the different replicate treatments of tefluthrin and Bt corn for a total of 27 plants evaluated per treatment. After roots were washed with water, corn rootworm larval injury to the roots was evaluated based on the node-injury scale developed by Oleson et al. (2005), which scores a plant from zero to three. A score of zero indicates no root damage and a score of three is the highest possible score and indicates that the first three nodes of roots were completely destroyed by rootworm feeding (Oleson et al., 2005). A plant given a score of three is likely to have a severe reduction in yield and could be completely lodged, which means that it cannot stand straight and is bent. A full description of the root node-injury scale can be found at http://www.ent.iastate.edu/pest/rootworm/nodeinjury/nodeinjury.html. When corn reached physiological maturity, it was harvested by a 12-row combine and grain yields were determined by the on-board SMS™ software (Ag Leader Technology, Ames, IA, USA) (Jaynes, 2013).

2.5. Statistical analysis

Water insecticide concentrations are reported in ng L^{-1}. The Cry1Ab protein concentrations in soil and sediment are reported in ng g^{-1} wet weight and tefluthrin and clothianidin in soil and sediment are reported in ng g^{-1} dry weight. Means and standard deviations were calculated for each compound in each matrix at each time point. Outliers were removed from statistical analyses if data points exceeded the mean ± 2.5 times the standard deviation. A maximum of 6% of data was removed. The method detection limit (MDL) was measured for each insecticide in water and soil matrices by taking the standard deviation of seven replicate samples and multiplying it by the Student's t value at 99% and six degrees of freedom of 3.14 (USEPA, 2012). Spiking levels for the MDL were near the final MDL value (Table S1). A reporting limit was established for each insecticide in each matrix at three times the MDL. Samples that had values below the reporting limit were substituted with half the value of the reporting limit for statistical analyses. Reporting limits for all compounds are listed in Table S1. Half-lives for tefluthrin and clothianidin were determined in soil by calculating the exponential decay curve using each year's data and solving for half the value of the time zero concentration. There were not enough water samples collected to measure half-lives of the insecticides in water, and half-lives were not calculated for the Cry1Ab protein in soil due to the lack of samples containing detectable levels of the protein.

2.6. Quality assurance and quality control

For every 20 samples processed, a laboratory blank sample (known blank soil, water, or laboratory sand), a matrix spike (MS), and a matrix spike duplicate (MSD), which consisted of a sample from the batch spiked with a known amount of insecticide standard, were processed. A batch passed the quality assurance test if the relative percent difference (RPD) between the MS and MSD was below 20% and the surrogate recovery was 80 to 120%. During the gas chromatography with an electron capture

Table 2
Number of samples collected in 2011, 2012, and 2013, respectively.

Sample type	Sampling event					
	Pre-planting	During season (VE)	During season (V7)	During season (VT/R1)	During season (R6)	Post-harvest
Soil	18, 18,18	18, 18,18	18, 18,18	18, 18,18	18, 18,18	18, 18,18
Runoff sediment	0, 0, 0	18, 18, 18	0, 18, 18	0, 18, 18	0, 18, 18	0, 0, 0
Runoff water	0, 0, 0	18, 18, 18	0, 18, 18	0, 18, 18	0, 18, 18	0, 0, 0
Lysimeter and groundwater	23, 23, 23	23, 23, 23	22, 23, 22	5, 23, 23	9, 23, 14	0, 14, 0

VE = emergence vegetative stage of corn; V7 = middle vegetative stage of corn; VT = tassels fully developed in vegetative corn stage and R1 = first reproductive stage of corn development; and, R6 = physiological maturity at end of corn growing season.

detector and high performance liquid chromatorgraphy with a diode array detector quantification, a blank and a known standard were injected every nine samples with required recovery of the standard at 80 to 120%.

3. Results

The number of samples collected for each matrix at each time point during the project is reported in Table 2. In 2011, only one runoff event was collected after planting because it did not rain more than 1.27 cm in one 24 h period for the remainder of the growing season. Fig. 1 is a conceptual model based on measured values that describes the fate and transport of each of the insecticides examined in the current study summarized over the three growing seasons.

3.1. Cry1Ab protein

3.1.1. Water

The Cry1Ab protein was not detected in any groundwater or soil pore water samples (Fig. 1). Conversely, runoff water from both the Bt and non-Bt fields often contained the Cry1Ab protein. The highest recorded concentration of Cry1Ab in runoff water was 129 ng L^{-1} from a sample collected in September 2013. Several runoff water samples collected from the non-Bt field contained the Bt protein (69 and 89% detection frequency in 2012, 2013, respectively) indicating movement and dispersal of the Cry1Ab protein between fields.

3.1.2. Soil and runoff sediment

Even though the Cry1Ab protein was mobile throughout both fields in runoff water, the same result was not observed in soil or runoff sediment samples. In fact, only 4% of non-Bt soil samples processed during the three-year project contained detectable concentrations of the Cry1Ab protein. The Cry1Ab protein was detected in soils collected from the Bt field, but at consistently low concentrations (Fig. 2). The highest average Cry1Ab protein concentration in a monthly sample was 9 ng g^{-1} in July 2012, which was during pollination. The Cry1Ab protein was also detected in runoff sediment samples, but only after pollination (Fig. 1). The Cry1Ab protein was measured in runoff water and sediment, and in soil samples indicating some potential for off-site travel, but depended on the growth stage of corn (Fig. 1).

3.2. Clothianidin

3.2.1. Water

Soil pore water had the highest recorded clothianidin concentrations when compared to the other water matrices tested (Fig. 3). This is likely due to the close proximity of the coated seed to the soil pore water and the relatively high water solubility of clothianidin (340 mg L^{-1} at 20 °C). Clothianidin was present in the soil pore water during all sampling events regardless of the presence of corn (Fig. 3). Some clothianidin leached into the groundwater table (Fig. 3). In 2012, a steady increase in clothianidin concentrations was observed in groundwater over the growing season; however, in 2013, clothianidin concentrations in groundwater decreased throughout the growing season (Fig. 3). Groundwater collected from this field could receive inputs from neighboring farms and; therefore, is not only a representation of the environmental fate from the research field, but also a representative of the location. Runoff water also contained measureable concentrations of clothianidin, but usually at lower concentrations than soil pore water and concentrations decreased throughout the growing season (Figs. 1 and 3).

3.2.2. Soil and runoff sediment

Residual clothianidin concentrations were measured in soil samples collected before the start of the next growing season in each of the three years. In all three field seasons, the highest soil clothianidin concentrations were measured immediately after planting, but then decreased throughout the season (Figs. 1 and 2). Unlike the water samples collected, clothianidin quickly dissipated in soil with half-lives calculated at 28 days for both 2011 and 2012 and at 20 days for 2013. Runoff sediment often contained clothianidin during the middle of the growing season, but at lower concentrations than what was measured in soil. Clothianidin was measured in every matrix examined in the current study indicating high potential for off-site travel.

3.3. Tefluthrin

Data presented in this section are only from the full rate of tefluthrin treatments due to very little tefluthrin cross contamination between the full rate treatments into the half rate and no tefluthrin treatments. Also, the same temporal trends were observed in the half-rate tefluthrin as well as the full-rate tefluthrin treatments. Since there was no carryover of tefluthrin between treatments and similar trends were observed, the data below represent the maximum potential level of application.

3.3.1. Water

Throughout the three years of the current study, no groundwater samples and only 1.5% of soil pore water samples contained tefluthrin above the reporting limit. Conversely, runoff water frequently contained tefluthrin (Fig. 1). In 2012, 19 days after application, the maximum tefluthrin concentration in runoff water was 393 ng L^{-1} and the maximum concentration 130 days after application (right before harvest) was 186 ng L^{-1}. In 2013, the maximum tefluthrin concentration 10 days after application was 169 ng L^{-1}, and the maximum concentration before harvest was 24 ng L^{-1}. Overall, tefluthrin concentrations in runoff water decreased throughout the corn growing season.

3.3.2. Soil and runoff sediment

Tefluthrin is applied in granular form to soil at planting; therefore, soil samples consistently contained detectable levels of this insecticide (Figs. 1 and 2). Tefluthrin concentrations in soil decreased throughout the growing season with half-lives calculated at 46, 30, and 28 days, in 2011, 2012, and 2013, respectively. It is important to note that tefluthrin soil concentrations measured in the current study were a dilution of the most concentrated soil, because the samples were a combination of soil collected next to the plant and soil from in between corn rows.

Tefluthrin was present in runoff sediment samples processed throughout the project (Fig. 1). Therefore, there is potential for tefluthrin to be carried off-site in runoff sediment and runoff water, especially immediately following application. Soil and sediment were the primary matrices containing tefluthrin, but some was present in the runoff water, further indicating a potential for off-site travel.

3.4. Crop damage and yield

During the three years of the project, the application of tefluthrin to Bt corn had no significant impact on corn yield or root damage (Fig. 4). Although, the Bt corn roots were damaged less by corn rootworm larvae than non-Bt roots, and the application of tefluthrin reduced damage to non-Bt corn roots, a similar effect on yield was not observed (Fig. 4). In fact, yields in the non-Bt field were at least equivalent to yields in the Bt field in two of the three years of the study.

4. Discussion

4.1. Cry1Ab protein

This is the first study of its kind to examine the environmental fate of Cry1Ab proteins in both water and soil matrices collected in a corn field. Panel A of Fig. 1 describes the fate of the Cry1Ab protein over the corn growing season in the three matrices in which it was measured. At the beginning of the growing season during corn vegetative emergence (VE corn growth stage), the Cry1Ab protein was measured in runoff water, but at low concentrations in soil and runoff sediment. One source of the Cry1Ab protein in runoff water could be from residual plant material left from the previous year's harvest, since the current study was conducted using a no-till management system. In the middle of the season at the vegetative 7 stage (V7), a similar trend was observed as the VE stage with the Cry1Ab protein measured at medium level concentrations in runoff water, and measured at low concentrations in soil and runoff sediment. Some of the detected Cry1Ab proteins in soil and runoff water could come from root exudates and residual plant material (Saxena and Stotzky, 2001). During pollination, which is the vegetative tasseling stage (VT) and the reproductive 1 stage (VT/R1), soil and runoff sediment contained the highest mean concentrations of the Cry1Ab protein, and runoff water also had medium level concentrations of the Cry1Ab protein. At physiological maturity of the corn (R6), concentrations of the Cry1Ab protein decreased in soil and runoff sediment, but increased in runoff water (Fig. 1).

The Cry1Ab protein was not detected in soil pore water or shallow groundwater indicating little movement of the protein below the surface of the soil. Tank et al. (2010) was the first study to track the fate of a Cry protein in stream water, and the authors provided several hypotheses on how the protein traveled into the stream. The current study's lack of Cry1Ab in groundwater provides evidence against one

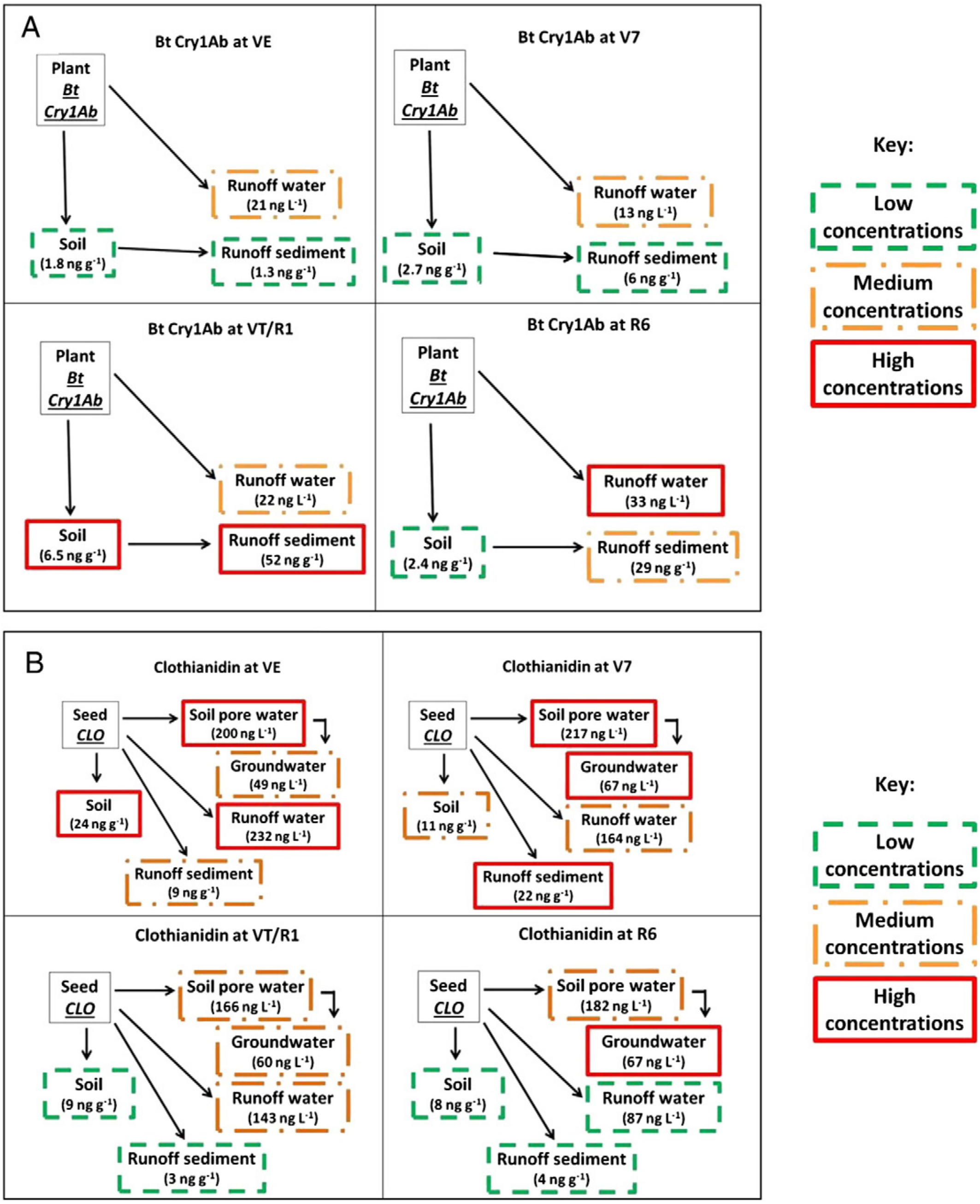

Fig. 1. Summary of the fate of the insecticides over the growing season examined in the current study at four time points: VE = emergence vegetative stage of corn; V7 = middle vegetative stage of corn; VT = tassels fully developed in vegetative corn stage and R1 = first reproductive stage of corn development; and, R6 = physiological maturity at end of corn growing season. A) the Bt protein, Cry1Ab; B) clothianidin; and, C) tefluthrin. Values in parentheses indicate means for each matrix at individual time points over the three-year study. "High" concentrations indicate the highest mean concentration measured in the current study for each insecticide over time, "medium" concentrations indicate mean concentrations that were between the maximum and minimum mean concentrations over the season, and "low" concentrations indicate means that were near reporting limits. High, medium, and low designations are relative to the current study and may not indicate ecological relevance. Very few soil pore water and groundwater samples contained the Bt Cry1Ab protein or tefluthrin which is why they were not presented in the figure.

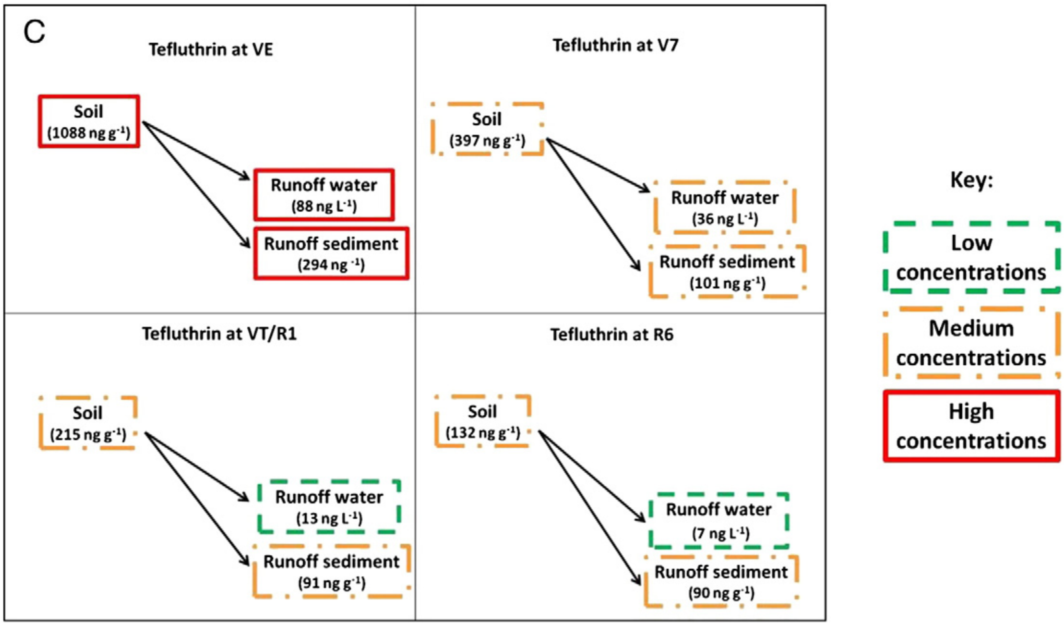

Fig. 1 (*continued*).

hypothesis given by Tank et al. (2010) that the presence of the Cry1Ab protein in streams adjacent to corn fields could be from groundwater sources. The insecticidal protein is likely traveling to nearby streams primarily by overland surface runoff and from wind dispersal of pollen and plant material. In the current study, the protein was found in abundance in runoff water samples collected from the Bt and non-Bt fields.

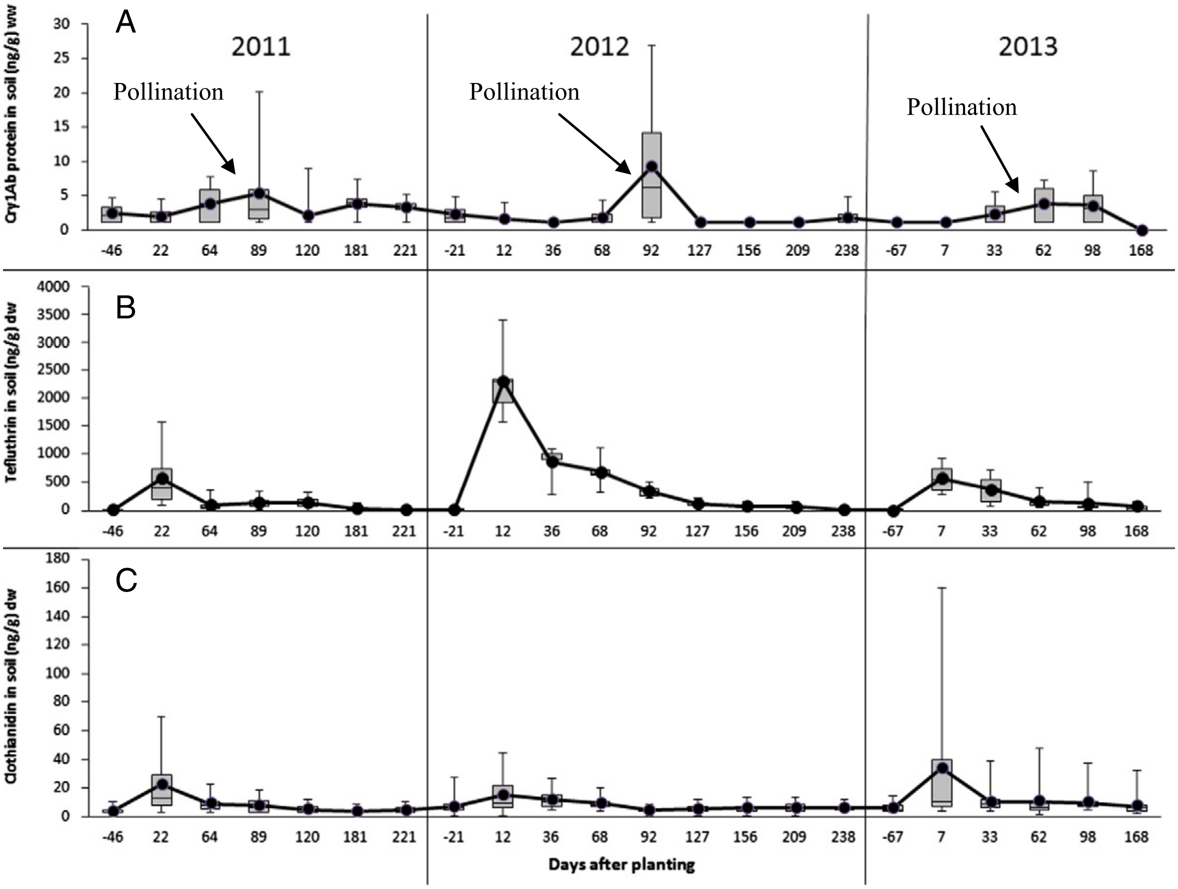

Fig. 2. Soil concentrations of each insecticide over the three year study. Negative values on the x-axis indicate days prior to planting in each year. Box and whisker plots represent the maximum and minimum concentration with the error bars, and the third and first quartiles with the outline of the box, and the median of the data with the line in the middle of the box. Mean values for each time point are represented by the circles. A) The Cry1Ab protein, B) tefluthrin, and C) clothianidin.

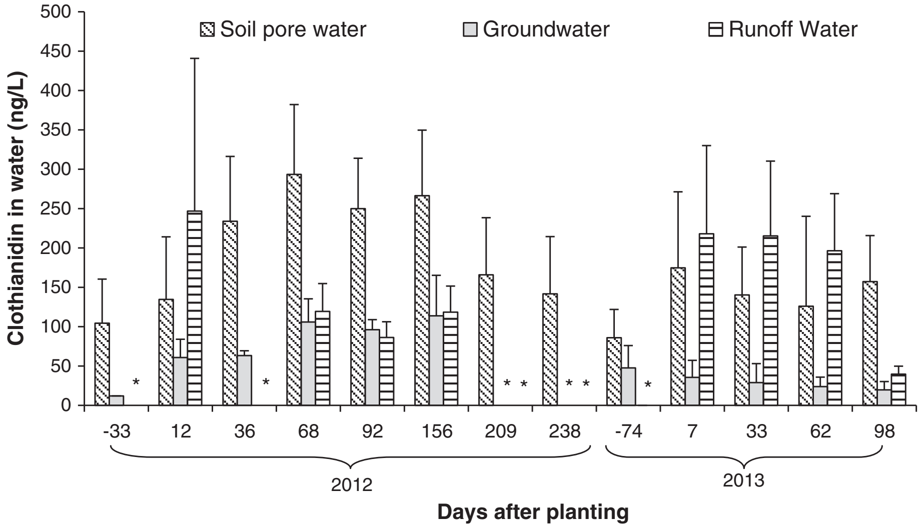

Fig. 3. Clothianidin concentrations measured in soil pore water and groundwater over two seasons. Negative values on the x-axis indicate days prior to planting in each year. Values indicate the mean ± standard deviation. An asterisk indicates no samples were collected at this time point. Due to drought conditions in 2011, very few water samples were collected; therefore, they are not reported in this figure.

Concentrations of the Cry1Ab protein in runoff water ranged from non-detect to 48 ng L^{-1} in all samples collected in 2012 and from non-detect to 129 ng L^{-1} in all samples collected in 2013 (Fig. 1). A study conducted to examine environmental fate of Cry1Ab protein expressed in rice yielded similar results, but with slightly lower concentrations, which ranged from non-detect to 31 ng L^{-1} in water samples adjacent to the rice plants (Wang et al., 2013).

The Cry1Ab protein was detected in field soils and runoff sediments collected from the Bt treatment in the current study (Figs. 1 and 2), and in a few samples from the non-Bt treatment. This is the first study to measure Cry1Ab proteins in runoff sediment and we found an increase in concentrations over the growing season, which correlated to pollination (Fig. 1). Higher concentrations of the Cry1Ab protein were measured in runoff sediment than soil with the highest measured Cry1Ab protein concentration in runoff sediment at 143 ng g^{-1}. When compared to other studies conducted on the environmental fate of Bt Cry proteins in soil in a field setting, we measured similar, but with slightly higher means, especially our samples that were taken during pollination. For example, Dubelman et al. (2005) attempted to measure Cry1Ab protein concentrations in soil and found only one sample above 30 ng g^{-1}. The Cry3Bb1 protein was measured at concentrations from 3.4 to 6.9 ng g^{-1} in field-collected soil samples (Ahmad et al., 2005). A field study on the Cry1F protein in soil also yielded similar results with no samples above 4.5 ng g^{-1} (Shan et al., 2008). One of the challenges with examining Bt Cry proteins in the environment and trying to compare results to other studies is that extraction methods are widely varied and many are not fully validated. Also, each type of protein can exhibit different behaviors in the environment from the varied levels of expression within the plant. Therefore, caution must be used during comparisons; however, the conclusion that very low concentrations of Cry proteins are measured in the environment is consistent among almost all other studies.

4.2. Clothianidin

Clothianidin was found primarily in water samples and was present in soil and sediment samples, but at low concentrations (Figs. 1, 2,

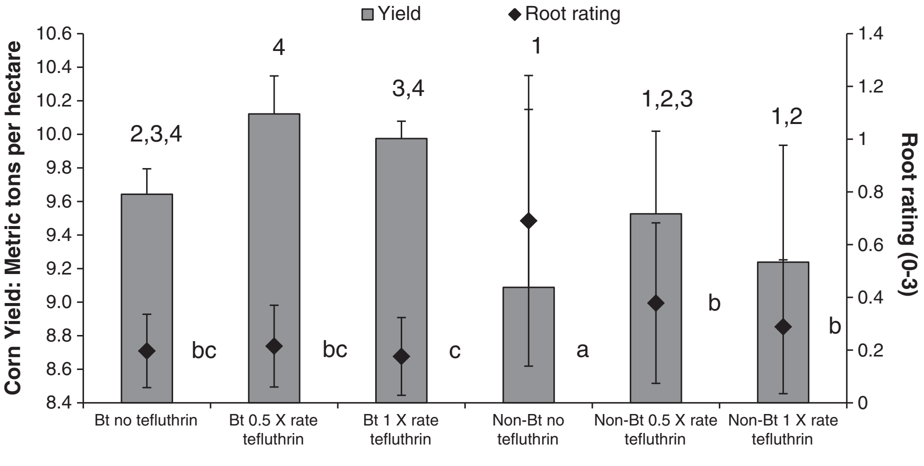

Fig. 4. Mean values with vertical error bars indicate ± standard deviation. The root ratings range from 0 to 3 with 0 indicating no damage to roots from corn rootworm larvae, and 3 indicating three nodes of corn roots completely destroyed from rootworm larvae feeding which would lead to complete failure of the plant (Oleson et al., 2005). Letters to the side of bars indicate significantly different groups based on a Tukey post-hoc from a repeated measures ANOVA on the root rating data. Numbers above bars indicate significantly different groups based on a Tukey post-hoc from a repeated measures ANOVA on the yield data.

and 3). Changes in clothianidin concentrations in environmental matrices overtime are featured in panel B of Fig. 1. At the beginning of the corn growing season, when clothianidin is first released into the environment from the seed coating, it was measured in all matrices tested. During the subsequent sampling events, clothianidin soil concentrations decreased and runoff sediment clothianidin concentrations decreased after the second sampling event of the corn growing season. In the sampling events around pollination (VT/R1) and physiological maturity (R6), clothianidin was primarily measured only in the environmental water matrices. Soil pore water and groundwater maintained a constant concentration of clothianidin; however, runoff water concentrations of clothianidin decreased over the corn growing season (Figs. 1 and 3).

This is one of the first studies to examine the fate of clothianidin through a field study in soil pore water, groundwater, and runoff water. Two government documents reported a high potential for clothianidin to leach into groundwater based on models (Rexrode et al., 2003; DeCant and Barrett, 2010), but neither of these had actual field measurements. Despite predictions for clothianidin to leach into groundwater, the current study provided evidence against clothianidin traveling to the groundwater table in high concentrations. Clothianidin may bind to the soil organic matter and this could prevent some leaching from the soil pore water to the groundwater table. Rexrode et al. (2003) made a prediction based on computer models that the highest expected groundwater clothianidin concentration would be 910 ng L^{-1}; but the highest measured groundwater sample from current study was much lower at 224 ng L^{-1}. Risk to non-target species residing in the soil pore water matrix environment needs to be evaluated, since elevated concentrations of clothianidin in this matrix were measured throughout the year. Rexrode et al. (2003) predicted from laboratory tests that clothianidin has a high potential to be transported via surface water runoff, and this result was confirmed based on the current study's findings that clothianidin is prevalent in runoff water.

The current study fills an important gap in knowledge requested by Goulson (2013) on the environmental fate of neonicotinoids in soil with actual levels of neonicotinoids measured on systems where repeated application is common. Drastically different clothianidin soil half-lives have been reported in the literature. In the USEPA's risk assessment of the use of clothianidin as a seed coating, clothianidin half-lives in soil from regulatory studies were reported from 148 to 6932 days in 10 different soils in a laboratory study and from 277 to 1386 days in four field studies conducted in North America in which clothianidin was applied as a broadcast spray (Rexrode et al., 2003). However, our findings (half-lives calculated at 28 and 20 days depending on the year) were consistent with Li et al. (2012) who measured clothianidin half-lives in soil from 6 to 12 days under field conditions. In the current study, the highest clothianidin soil concentrations were measured within weeks of planting and decreased by the end of the growing season, but low, detectable levels remained in soil throughout the winter and into the spring of the following year (Fig. 2). While the current study did not measure clothianidin concentrations in the corn tissue, we can conclude that it remains in the environment in water for extended periods of time well beyond the corn growing season.

4.3. Tefluthrin

Throughout the corn growing season, tefluthrin was only measured in soil, runoff water, and runoff sediment (Fig. 1C). Concentrations in each of these three matrices decreased over time, but remained at detectable levels over the growing season. Tefluthrin was not measured in groundwater or soil pore water, but it was transported by and commonly detected in runoff water (Fig. 1). Schreiber et al. (1993) is the only other study describing environmental fate of tefluthrin. They also found no detectable levels of tefluthrin in shallow groundwater (<2.5 m) at any time during the season. Maximum concentrations of tefluthrin in runoff water one week after planting were 100 and 640 ng L^{-1} depending on the year, and decreased to 50 and 20 ng L^{-1} in samples taken before harvest depending on the year (Schreiber et al., 1993). The tefluthrin concentrations in runoff water from Schreiber et al. (1993) were comparable to measurements in the current study.

Soil and runoff sediment were the primary matrices containing tefluthrin in the current study (Fig. 1). The lethal concentration at which 50% of the population was effected (LC50) for corn rootworm larvae exposed to tefluthrin in soil was 300 ng g^{-1} (McDonald et al., 1986). The first sampling event in 2011; the first, second, and third soil sampling events in 2012; and the first and second sampling events after application in 2013, of the current study exceeded this benchmark. Therefore, in 2012 and 2013 soil tefluthrin concentrations were compliant with desirability of a soil insecticide to last 6 to 10 weeks to cause a pest insect population to decrease in size. The half-life of tefluthrin in soil in the current study averaged 35 days and at six months post-application an average of 7% of tefluthrin remained in the soil. The amount of tefluthrin before planting in the subsequent year was 3% and 0.2% from the original concentration, in 2012 and 2013, respectively.

The current study measured tefluthrin at much higher concentrations in runoff sediment than Schreiber et al. (1993) who found maximum concentrations of tefluthrin at 60 ng g^{-1} and 70 ng g^{-1} one week after application and maximum concentrations at 3 and 4 ng g^{-1} before harvest. The current study found maximum tefluthrin concentrations in runoff sediment of 661 and 953 ng g^{-1} after application and 251 and 81 ng g^{-1} before harvest in 2012 and 2013, respectively. Tefluthrin was applied to the field at a slightly lower concentration in Schreiber et al. (1993) at 0.11 kg ha^{-1} and in the current study was applied at 0.15 kg ha^{-1}, which could help explain why the current study had more tefluthrin in the runoff sediment. From this information, it can be concluded that tefluthrin can be transported off-site in runoff water and sediment following application, with decreasing concentrations throughout the growing season.

4.4. Crop damage and yield

Sutter et al. (1989) found that root protection from corn rootworm larvae achieved by a soil-applied insecticide was extremely variable and that soil moisture, planting date, and several other factors have a significant influence on rootworm larvae population success and control.

Mortality achieved by soil applied insecticides may be too low to have a meaningful effect on evolutionary resistance among rootworm populations (Petzold-Maxwell et al., 2013), but using a pyramiding resistance management plan (Bt + soil insecticide) may delay the resistance process (Roush, 1998). In the current study, tefluthrin did not have an effect on root damage in GM corn, but did prevent damage to non-Bt corn, which is in agreement with the findings of Petzold-Maxwell et al. (2013). A slight reduction in yield was observed in the non-Bt, no tefluthrin treatment when compared to all other treatments, but no difference in yield was observed among Bt corn treatments regardless of the soil insecticide application (Fig. 4).

5. Conclusions

This is the first study to examine the environmental fate of a Bt corn insecticidal protein, a seed coating insecticide, and a soil insecticide in the same field over multiple years. Fig. 1 highlights the fate of the insecticides examined in the current study over the corn growing season and pinpoints the environmental matrices that are most likely to retain each insecticide. Use of two of the insecticides (Bt crops and neonicotinoids) in the current study is being intensely debated in public and scientific communities. Several countries in Europe have banned the use of both Bt crops (Waltz, 2009) and neonicotinoid seed coatings (EFSA, 2013; USEPA, 2013). Results from the current study indicate that the Bt corn proteins rapidly dissipate in the environment; therefore, risk to non-target species from this exposure is most likely limited. The neonicotinoid seed coating, clothianidin, remained in environmental water matrices for extended periods of time; however, it dissipated within a few months in soil matrices. Further investigation into potential non-target aquatic species toxicity to clothianidin should be conducted to determine if this finding is of ecological concern. Tefluthrin, which is currently used in abundance in the Midwestern United States, was measured in soil, runoff water, and runoff sediment at elevated concentrations during the corn growing season; therefore, potential transport of tefluthrin off-site could be an exposure route for non-target species. As expected, tefluthrin applied with non-Bt corn helped prevent damage from pest feeding. A slight reduction in grain yield was observed in the non-Bt, no tefluthrin treatment when compared to all other treatments, but no significant difference in grain yield was observed among Bt corn treatments regardless of soil insecticide application. Also, since the application of tefluthrin to Bt corn did not prevent damage to roots from corn rootworm larvae feeding, this prophylactic use warrants further investigation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.07.115.

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