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Contaminants in Land-Applied Biosolids: Characterization and Modeling of Fate and Transport During Rainfall Events, and Determination of Effects of Triclocarban on a Freshwater Mudsnail

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By

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Abstract

Studies are described in which the fate and transport of contaminants in land-applied biosolids was characterized via direct measurements and then modeled successfully. Additionally, the effects of one such contaminant, triclocarban (TCC), were investigated in a freshwater mudsnail.

Rainfall simulations were conducted on soil plots amended with biosolids. Surface runoff and leachate was collected and analyzed for the endocrine disrupting chemicals (EDCs) bisphenol A, 17α-ethynylestradiol, triclocarban, triclosan, octylphenol, and nonylphenol; sixteen metals; and estrogenic activity via the ER-CALUX bioassay. Triclosan, nickel, and copper were detected at levels that might pose risk to aquatic life, though levels of metals in the biosolids were well below regulatory limits. ER-CALUX results were mostly explained by background bisphenol A contamination and octylphenol, though unknown contributors and/or matrix effects were also found.

An existing model, Groundwater Loading Effects of Agricultural Management Systems (GLEAMS), was modified to include addition of a biosolids phase with labile organic carbon (distinct from soil organic carbon), and was used to predict the fate and transport of trace organic contaminants from land-applied biosolids. The model was calibrated using existing data from literature studies, including experiments described in above, and showed good agreement for acetaminophen, ibuprofen, triclosan, triclocarban, and estrone with reasonable input parameters. It was then applied to various theoretical scenarios using chemicals of varied properties to examine the effects

of K_{OC} and half-life, application date, and application method (surface spreading vs. incorporation) on long-term chemical losses.

The effects of TCC were studied in the freshwater mudsnail *Potamopyrgus antipodarum*. After 4 weeks exposure, environmentally relevant TCC concentrations of 1.6 to 10.5 μg/L resulted in statistically significant increases in the number of unshelled embryos, while 0.2, 1.6, and 10.5 μg/L exposures significantly increased numbers of shelled embryos. The lowest observed effect concentration (LOEC) was 0.2 μg/L, the no observed effect concentration (NOEC) was 0.05 μg/L, and the median effective concentration (EC50) for unshelled effects was 2.5 μg/L. Results indicate that TCC may be causing reproductive effects in the environment. Furthermore, environmental risk from a new class of EDCs is both qualitatively and quantitatively similar to risk from existing classes of EDCs.

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With these, the last sentences I write as part of my seven year journey at the University of California, Davis, comes an outpouring of relief and gratitude for the assistance, support, and encouragement of those who have made it possible.

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Overview

Contaminants in Land-Applied Biosolids: Fate, Transport, and Effects

Introduction

Municipal biosolids are commonly applied to land as soil amendment or fertilizer as a form of beneficial reuse of what could otherwise be viewed as waste. Balanced against this benefit are potential risks to groundwater and surface water quality from constituents that may be mobilized during storm events. In this dissertation, a series of three studies are described that:

1) characterize mobilization of selected constituents from land-applied biosolids during simulated rainfall events;

2) modify an existing model to predict the fate and transport of trace organic constituents from land-applied biosolids; and

3) investigate the effects of one such chemical, triclocarban, on embryo production in a freshwater mudsnail.

In chapter 1, the mobilization of selected endocrine disrupting compounds (EDCs), heavy metals, and total estrogenic activity in rainfall runoff from land-applied biosolids is characterized. Rainfall simulations were conducted on soil plots amended with biosolids. Surface runoff and leachate was collected and analyzed for several EDCs; a suite of sixteen metals; and estrogenic activity via the ER-CALUX bioassay [1].

In chapter 2, an existing model, Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) is modified to predict the fate and transport of trace

organic contaminants from land-applied biosolids. Modifications include addition of the biosolids phase (to already present soil and water phases), and degradation of organic carbon in the biosolids phase. The model is calibrated using existing data from literature studies, including experiments described in chapter 1, then applied to various theoretical scenarios to investigate management options for land application of biosolids.

In chapter 3, the effects of the antimicrobial chemical triclocarban (TCC) on embryo production were studied in the freshwater mudsnail *Potamopyrgus antipodarum*. TCC is commonly found in biosolids at among the highest concentrations of any trace organic contaminant, is highly persistent, and was examined in chapters 1 and 2. Specimens were exposed to environmentally relevant concentrations in laboratory enclosures, were removed and dissected, and embryos contained within the brood pouch were counted and classified as shelled or unshelled after 2 and 4 weeks of exposure [2].

References

1. Giudice BD, Young TM. 2011. Mobilization of endocrine-disrupting chemicals and estrogenic activity in simulated rainfall runoff from land-applied biosolids. *Environmental Toxicology and Chemistry* 30:2220-2228.
2. Giudice BD, Young TM. 2010. The antimicrobial triclocarban stimulates embryo production in the freshwater mudsnail *Potamopyrgus antipodarum*. *Environmental Toxicology and Chemistry* 29:966-970.

Chapter 1

Mobilization of Endocrine Disrupting Chemicals and Estrogenic Activity in Simulated Rainfall Runoff from Land-Applied Biosolids

Abstract

Municipal biosolids are commonly applied to land as soil amendment or fertilizer as a form of beneficial reuse of what could otherwise be viewed as waste. Balanced against this benefit are potential risks to groundwater and surface water quality from constituents that may be mobilized during storm events. The objective of the present study was to characterize the mobilization of selected endocrine disrupting compounds (EDCs), heavy metals, and total estrogenic activity in rainfall runoff from land-applied biosolids. Rainfall simulations were conducted on soil plots amended with biosolids. Surface runoff and leachate was collected and analyzed for the EDCs bisphenol A, 17α-ethynylestradiol, triclocarban, triclosan, octylphenol, and nonylphenol; a suite of sixteen metals; and estrogenic activity via the ER-CALUX bioassay. Triclocarban (2.3-17.3 ng/L), triclosan (<51-309 ng/L), and octylphenol (<4.9-203 ng/L) were commonly detected. Chromium (2.0-22 µg/L), cobalt (2.5-10 µg/L), nickel (28-235 µg/L), copper (14-110 µg/L), arsenic (1.2-2.7 µg/L), and selenium (0.29-12 µg/L) were quantifiable over background levels. Triclosan, nickel, and copper were detected at levels that might pose some risk to aquatic life, though levels of metals in the biosolids were well below maximum allowable regulatory limits. ER-CALUX results were mostly explained by background bisphenol A contamination and octylphenol in runoff, though unknown contributors and/or matrix effects were also found.

1 Introduction

In the United States 3.4 million dry tons of biosolids are used as soil amendment or fertilizer each year, 61% of the biosolids that are generated during municipal wastewater treatment [1]. Biosolids contain high levels of nutrients and organic matter that can be a valuable resource to agricultural sites and sites in need of remediation, and state and federal agencies have long promoted the practice of land application [2]. The beneficial reuse of biosolids must be evaluated relative to potential risks, which include, among other concerns, impacts to water quality due to mobilization of nutrients, heavy metals, pathogens, and organic compounds. Disposal and reuse of biosolids in the United States is subject to 40 CFR 503, which includes regulations governing acceptable land and climate characteristics, maximum application rates, and limits on the heavy metal and pathogen content of the biosolids (http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr503_main_02.tpl).

Heavy metals in biosolids have historically been the primary concern related to their beneficial reuse. Several studies that have investigated the movement of metals from land-applied biosolids have described higher than expected mobility of metals in column studies and have linked this phenomenon to transport associated with dissolved organic carbon (DOC) [3-5]. More recently, concerns have arisen over levels of several groups of organic chemicals in biosolids, including endocrine disrupting chemicals and pharmaceuticals and personal care products (PPCPs). While it is uncertain what effects these chemicals may have in the environment, the potential risks have led to a few studies that have examined their mobilization from land-applied biosolids. These

studies included analysis of PPCPs in tile drainage [6, 7] and surface runoff [8, 9] following application of liquid municipal biosolids and dewatered municipal biosolids. In general, concentrations found in tile drainage and surface runoff were far lower than those found in treated wastewater effluent, and with few exceptions, are well below the lowest known environmental endpoints. Cell-based bioassays have been used to characterize endocrine disrupting potential in municipal biosolids [10, 11], but have not, to our knowledge, been used previously to characterize the movement of this potential in runoff from land-applied biosolids. In the present study, the Chemical Activated LUciferase gene eXpression (ER-CALUX) assay, which uses light to measure binding to the estrogen receptor, is applied to rainfall runoff and leachate.

The objective of the present study is to characterize the mobilization of selected endocrine disrupting chemicals, heavy metals, and estrogenic activity in runoff from land-applied biosolids during controlled rainfall simulations. The study examines whether the rate of loss of these constituents in surface runoff changes throughout the storm and whether overall levels could pose a threat to receiving waters. Furthermore, results of the present study will be used to determine whether current regulations governing maximum allowable levels of metals in biosolids are sufficiently protective to address concerns related to PPCPs.

2 Methods and Materials

2.1 Chemicals

Acetonitrile, ethyl acetate, methanol, acetone, hydrochloric, nitric, and acetic acid were all obtained from Fisher Scientific (USA) and were the highest purity available. Bisphenol A (BPA), 17α-ethynylestradiol (EE2), 4-*t*-octylphenol (OP), *n*-

nonylphenol (NP), triclosan (TCS), triclocarban (TCC), and zearalanone (ZAN) were obtained from Sigma-Aldrich (USA). Deuterated triclocarban (TCC-d7), and ^{13}C labeled triclocarban ($^{13}C_6$-TCC), ^{13}C labeled bisphenol A ($^{13}C_{12}$-BPA), and ^{13}C labeled nonylphenol ($^{13}C_6$-NP) were obtained from Cambridge Isotope laboratories (USA). 17β-estradiol (E2), and phenol red-free Dulbecco's modified Eagle medium (DMEM) were purchased from Sigma; cell culture reagents and media were obtained from Gibco/BRL®. Properties of chemicals analyzed are shown in **Table 1-1**.

Table 1-1. Chemical properties

Constituent	Chemical Abstracts Service (CAS) #	Molecular Weight g/mol	pK_a	Solubility [a] mg/l	Log K_{OW} [a]	EEF CALUX [b]
Bisphenol A (BPA)	80-05-7	228.3	9.6 [25]	85 [25]	3.32-4.16 [25]	7.80×10^{-6} [26]
17-α Ethynylestradiol (EE2)	57-63-6	296.4	10.4 [27]	4.8 [28]	3.67-4.15 [28]	1.2 [26]
Triclocarban (TCC)	101-20-2	315.6	12.7 [29]	0.02-1.55 [7]	4.9 [29]	0 [30]
4-t-Octylphenol (OP)	140-66-9	206.3	10.2 [21]	12.6 [21]	4.12 [21]	1.40×10^{-6} [26]
4-Nonylphenol (NP)	104-40-5	220.4	10.7 [21]	5.43 [21]	4.48 [21]	2.30×10^{-5} [26]
Triclosan (TCS)	3380-34-5	289.54	8.1 [19]	1.97-10 [7]	4.8 [29]	0 [30]

[a] Measured or estimated at 20 °C, pH 7.
[b] Molar based estradiol equivalent factor (EEF), the estrogenic potency relative to estradiol, using the Estrogen Receptor Chemical Activated LUciferase gene eXpression (ER-CALUX) bioassay.

2.2 Batch desorption experiments

Batch desorption experiments were performed to examine how the water extractable biosolids concentrations compared with the solvent extractable concentrations, to compare against concentrations found in runoff from the rainfall simulations (described below), and to compare the water extractable concentrations of analytes in the biosolids sample used in the rainfall simulations to other biosolids samples from publicly owned treatment works (POTWs) in California. Six different biosolids samples from six POTWs in California (including the biosolids used in the rainfall simulations) and one commercial biosolids fertilizer product were analyzed.

Descriptions of the facilities from which these biosolids samples originated can be found in (Ogunyoku and Young, University of California Davis, Davis, CA, USA, unpublished data). Approximately 1 to 2 g of biosolids and approximately 40 ml of Milli-Q water were added to centrifuge tubes. The biosolids were then broken up by means of crushing and stirring with mini-spatulae, and the tubes were tumbled at room temperature in the dark for 1 h to approximately simulate rainfall simulation contact times. The tubes were centrifuged for 30 min at 3660 rpm, then decanted into new vials. Five ml was removed and acidified for metals analysis as described below, and the remaining water was extracted via solid phase extraction and analyzed via liquid chromatography/mass spectrometry (LC/MS) for organics as described below. Results were corrected by recovery of surrogates, which were spiked into the solution immediately prior to extraction, and method blank concentrations were subtracted.

2.3 Rainfall simulator and test-plot design and operation

Two identical rainfall simulators were placed side-by-side over each of three test-plots. Rainfall simulators were constructed largely according to [12]. Briefly, each simulator consisted of a 1 m x 1 m acrylic reservoir with 900 hypodermic syringe needles (23 gauge) as drop formers suspended 1.7 m above plot surfaces by aluminum and polyvinyl chloride (PVC) tubing. Plastic sheeting was attached to each simulator to prevent wind from blowing drops off course. Water was supplied to each from a polyethylene tank filled with well-water by a centrifugal pump. A needle valve on each simulator provided flow adjustment control. As water in the tank was used, a valve opened and the tank was refilled by fresh well-water filtered through a sediment filter. Water in the tanks was continuously cycled through a carbon filter attached to each tank

by the pumps to remove chlorine and other contaminants from the water. Prior to each simulated rain event, steel pans were inserted between the simulator and the plot to allow rainfall rate to reach steady state. Rainfall was directed off these pans into a graduated cylinder and timed to calibrate rainfall rate, which was always adjusted to 60 mm/h. This intensity was meant to approximate the maximum 30-min duration rainfall recorded at most rainfall stations in the Sacramento River Basin. Storm simulation duration was variable because the simulations were stopped when 24 liters of runoff had been collected. It is estimated that drops were 2.5 mm in diameter, and in falling 1.7 meters, reached approximately 65% of their terminal velocity at the time of impact on the soil surface [12, 13].

Three replicate plots were constructed. The plots consisted of a 2 m long x 1 m wide x 0.38 m deep box constructed out of 1.6 cm (5/8") plywood. Each box was lined with 0.15 mm (6 mil) plastic sheeting adhered by silicone and fastened across the top edge by duct tape. A makeshift PVC tile-drain consisting of 1.3 cm (½") PVC with 0.64 cm (¼") holes drilled at 2.5 cm (1") intervals was placed on the bottom of the box with a drain exiting at the down-slope end of the box. The bottom layer of fill was 6.4 cm (2.5") of all-purpose pea-gravel pre-rinsed to remove clay particles. The middle layer of fill was an agricultural soil 19 cm (7.5") thick, compacted with a 15 cm x 15 cm (6" x 6") soil tamper approximately every 5 cm (2"). The top layer was 7.6 cm (3") of loose soil. The soil was a sandy loam with the following properties: pH 8.0, EC 0.60 dS/m, cation exchange capacity 20.7 meq/100 g, organic matter 0.64%, organic carbon 0.37%, $CaCO_3$ 1.3%, 60:26:14 sand:silt:clay. This soil is a typical agricultural soil in the region, although contained more sand and less clay than soils that have previously

been analyzed in nearby agricultural fields. Care was taken to ensure minimal leakage out of the plastic sheeting so that all water not running off would exit through the tile-drain. One end of each plot was elevated using a pallet-jack to achieve a 3.5 to 4 degree slope. Although most fields in California's Central Valley are very flat, this slope is within typical ranges found in agricultural fields. A collection flume and cover to block rainfall from directly entering the flume similar to that used in [12] were attached to the downslope end of the plot. The flume directed water through teflon tubing into collection bottles.

An initial control storm (CS) and three successive treatment storm events (TS1, TS2, and TS3) following biosolids application were simulated on each of the three replicate plots between March 23 and April 24, 2009. The control storm was simulated 5 d prior to application of biosolids. Biosolids were applied to plots (day 0) at a rate equivalent to about 10 tons/acre (2.25 kg/m^2, dry wt) and incorporated into the top 3-6" (7-15 cm) of soil less than 24 h later (day 1). The application rate is the maximum typically applied to corn, and a moderate rate for soybeans. Treatment storm events Treatment storms TS1, TS2, and TS3 were conducted at day 3, 9, and 24, respectively. Soil was undisturbed and allowed to dry between the treatment storms. It should be noted that the top 3 to 6" (7-15 cm) of soil were loose and disturbed for CS and TS1, but compacted by the storm events for the start of TS2 and TS3. Approximately 16 mm (0.6") of natural rain fell on the plots between treatment storms 2 and 3 (days 11-14); however, the plots were not inclined and the relatively light intensity of the rainfall meant that no runoff and very little leaching occurred.

Biosolids were obtained from a POTW in the Central Valley of California. The POTW, which provides sewerage to residential, commercial, and industrial users, as well as state correctional facilities, is a conventional activated sludge facility, and sludge undergoes anaerobic digestion and is dewatered on a belt filter press. Biosolids were collected directly after the belt press and had an initial solids content of 15%. Typically, the facility allows biosolids to dry in the sun for several months before being used or disposed, and solids contents at that time are at least 70%. To simulate these conditions, biosolids were pre-dried in a large outdoor oven at 85 °C for 18 h to achieve a solids content of 30%. After spreading, high winds and low humidity further dried biosolids, and solids content was approximately 60% at the time of incorporation into the soil.

2.4 Analysis of biosolids

Biosolids samples were prepared and analyzed for TCC and TCS according to methods in (Ogunyoku and Young, unpublished data). This method had been previously developed for TCC and TCS only, so no attempt was made to measure levels of BPA, EE2, NP, or OP in biosolids. Biosolids samples (1 g) were dried in an oven at 70°C for 24 h and homogenized. Samples were spiked with the surrogate standard (TCC – d7) and allowed to dry, extracted with 15 ml of 1:1 acetone/methanol on a shaker table for 24 h at 55°C, centrifuged for 30 min at 3660 rpm, and the supernatant filtered to 0.2 microns using polytetrafluoroethylene syringe filters. A 300 µl aliquot of the sample was transferred to a vial and diluted with 250 µl of pure methanol and 50 µl of 2 µg/ml $^{13}C_6$-TCC (internal standard) in methanol, and analyzed via LC/MS. A Phenomenex C18 Prodigy™ (5µm, 100 Å pore size; 2.0 x 100 mm) with a guard

column (2.0 x 4.0 mm) was used at 40°C with an injection volume of 10µl. A gradient method consisting of 90:10 MilliQ water/acetonitrile with 10 mM acetic acid and 50:50 methanol/acetonitrile with 35 mM acetic acid at a constant flow rate of 0.500 ml/min was used for the analysis of the sample. Detection was achieved using an Agilent (USA) 1100 series LC/MS ion trap with electrospray ionization in negative ion mode and multiple reaction monitoring. The criteria used for positive identification of TCC were the retention time (RT ± 0.1 min), the parent ion (*m/z* 313), and transition ion (*m/z* 160). Triclosan identification criteria were RT ± 0.1 min, the parent ion (m/z 287), and spectra matching. Triclocarban product ion and TCS parent ion were used for quantification.

Metals were extracted using a modified version of U.S. Environmental Protection Agency (U.S. EPA) method 3050 [14]. Briefly, 5 ml of trace metals grade nitric acid was added to approximately 500 mg of pre-dried biosolids in test tubes. The tubes were capped and left for 24 h, then sonicated for 1 h at 50°C. Slowly, 5 ml of 1:1 30% hydrogen peroxide was added and the tubes allowed to sit for 1 h. After another 1 h of sonication, the solution was diluted to 50 ml with Milli-Q water. Samples were analyzed for metals using an Agilent (USA) 7500i inductively coupled plasma mass spectrometer (ICP/MS). Dilute nitric acid in ultra-pure water rinses were analyzed every 20 to 25 samples to quantify machine drift. Counts-per-second of 10 elements were detected for each sample. Elements measured were chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), silver (Ag), cadmium (Cd), and lead (Pb). Instrument detection limits (three standard deviations, U.S. EPA 6020) were approximately 0.01 ng/ml. Sample concentrations always exceeded method

detection limits, which varied by metal, but were all less than or equal to 0.2 µg/L (0.02 mg/kg). Concentrations for each element were adjusted to a baseline zero point by subtracting the average background levels determined in all analyzed rinses throughout the sample run.

2.5 Analysis of runoff

Six runoff samples from each storm simulation were collected in 4 liter amber bottles. Leachate samples were collected in amber bottles and periodically dumped into a large glass reservoir. A single 2.5 L sample of leachate was taken from the composite reservoir at the end of each simulation. Total suspended solids concentration was measured in all samples using Standard Method 2540D [15]. Subsamples of 50 ml were centrifuged, 0.45 µm filtered, and analyzed for dissolved organic carbon (DOC, method detection limit = 0.5 mg/L) for one replicate set of samples from each storm simulation. Approximately 425 ml of each sample was centrifuged for 45 min at 3660 rpm in polypropylene centrifuge bottles. From each sample, 5 ml was removed and acidified with 50 µl of nitric acid for metals analysis via inductively coupled plasma mass spectrometry (ICP/MS). The remaining supernatant was acidified to pH 2 with hydrochloric acid and extracted via solid phase extraction (SPE) within 24 h. The extraction was carried out on Waters (USA) OASIS HLB 6cc disposable cartridges. Each cartridge was conditioned with 5 ml 75:25 ethyl acetate/acetone mixture followed by 5 ml methanol and then 5 ml acidified (pH 2 with HCl) Milli-Q water. Samples of approximately 400 ml were loaded at a rate of 2 ml/min and then dried for 10 min. Cartridges were eluted with 8 ml of 75:25 ethyl acetate/acetone. Eluates were evaporated to dryness under a gentle stream of nitrogen at 65°C. Finally, extracts were

redissolved in 150 µl of dimethylsulfoxide (DMSO) for analysis via the ER-CALUX bioassay and LC/MS. For TS1, separate samples were prepared for LC/MS and ER-CALUX, and surrogate compounds $^{13}C_{12}$-BPA, TCC d7, and $^{13}C_6$-NP were spiked into samples that were to be run on the LC/MS prior to extraction to calculate average recovery.

Extracts were analyzed for organics via liquid chromatography with tandem mass spectrometry. Injection volume was 50 µl, and separation was achieved on an Ascentis® C18 25 cm x 4.6 mm, 5 µm (Supelco, USA) column at 30°C. The binary mobile phase consisted of 0.9 ml/min of A: Milli-Q and B: acetonitrile, each with 0.2% acetic acid. The gradient was as follows: 40% B from 0 to 5 min, linear gradient to 75% B at 19 min, linear gradient to 95% B at 21 min, linear gradient to 100% B at 25 min, 100% B until 27 min, linear gradient to 40% B at 33 min. Detection was achieved using an Agilent 1100 series LC/MSD ion trap with electrospray ionization in negative ion mode and multiple reaction monitoring. From 20 to 33 min, post-column injection of 0.1 ml/min of 50 mM ammonium hydroxide was utilized to amplify signal of OP and NP.

The requirements for detection were presence of the fragment ion above the instrument detection limit and elution within expected the retention time window (± 0.1 min). All sample analyte responses for all three storm simulations were corrected by average recovery of their respective surrogates from TS1 samples. Calibration was via external standards, and solvent blanks and a mid-level standard were included to ensure no carryover, degradation, or significant change in instrument response. Method detection limits were determined via extraction and analysis of 7 replicate, low level

Milli-Q water samples spiked with target compounds, and subsequent multiplication of the standard deviation of the response by the associated student's *t* value (per U.S. EPA guidance), and were as follows: BPA 5.2 ng/L; EE2 10 ng/L; TCC 1.0 ng/L; TCS 51 ng/L; OP 4.9 ng/L; and NP 8.8 ng/L. All data were analyzed using Bruker Daltonik DataAnalysis Version 2.1 software (Bremen, Germany).

Recombinant human ovarian cancer cells (BG1Luc4E$_2$, ER-α–positive) were grown and maintained as described in Rogers and Denison [16]. These cells contain a stably integrated, ER-responsive firefly luciferase reporter plasmid, pGudLuc7ERE. Cells were maintained in estrogen-stripped media for 5 d before they were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells/well and allowed to attach for 24 h. Cells were then incubated with carrier solvent (DMSO: 1% final solvent concentration), E2 (1 nM), and runoff sample extracts for 24 h at 37°C. For luciferase measurement, sample wells were washed twice with phosphate-buffered saline, followed by addition of cell lysis buffer (Promega); the plates were then shaken for 20 min at room temperature to allow cell lysis. Luciferase activity in each well was measured with an Orion microplate luminometer (Berthold) with automatic injection of Promega stabilized luciferase reagent. Luciferase activity in each well is expressed relative to that maximally induced by 1nM E2.

Following runoff sample preparation steps discussed above, metals were analyzed identically to metals in biosolids extracts described above.

3 Results

3.1 Batch desorption experiments

Results for the biosolids that were also used in the rainfall simulations are shown in **Table 1-2**. Results for all 7 of the biosolids tested are shown in **Table 1-3**. The water extractable concentrations of metals in the rainfall simulator biosolids were generally the highest among the POTW samples. The commercial fertilizer product Milorganite had the highest levels of leachable metals among all of the samples. The water extractable concentrations of OP, TCS, and TCC of these biosolids were toward the low, middle, and high end of the range of results found for the 7 biosolids samples analyzed (Table 1-3). Estrogenic activity as measured by ER-CALUX was lowest for the rainfall simulator biosolids among the 7 samples.

Table 1-2. Levels of constituents measured in biosolids, runoff, and leachate [a, b]

	Biosolids Concentrations			Runoff and Leachate Event Mean Concentrations								
Constituent	Total extractable[c] (mg/kg)	Water extractable (mg/kg)	Ceiling concentrations [d] (mg/kg)	Control storm (CS)		Treatment storm 1 (TS1)		Treatment storm 2 (TS2)		Treatment storm 3 (TS3)		Units
				Runoff	Leachate	Runoff	Leachate	Runoff	Leachate	Runoff	Leachate	
Cr	53.6	0.13	-	2.8 (A)	6.7	3.4 (A)	22	3.3 (A)	2.0	0.07 (B)	9.0	µg/L
Co	5.1	0.16	-	0.69 (A)	0.46	2.5 (B)	10	8.8 (C)	4.1	2.8 (B)	8.6	µg/L
Ni	57.6	1.66	420	6.4 (A)	6.8	39.6 (B)	235	90.9 (C)	46	28.1 (B)	51	µg/L
Cu	362.8	0.84	4300	0.25 (A)	2.2	19 (BC)	110	26.8 (B)	14	11.4 (C)	27	µg/L
Zn	538.0	0.98	7500	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	µg/L
As	4.8	0.15	75	0.5 (A)	1.6	2.7 (B)	2.5	2.8 (B)	2.1	1.7 (C)	1.2	µg/L
Se	6.1	0.10	100	0.09 (A)	1.8	0.94 (B)	12	1.6 (C)	0.73	0.29 (A)	2.3	µg/L
Ag	3.4	0.00027	-	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	µg/L
Cd	1.6	0.0015	85	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	µg/L
Pb	7.8	0.012	840	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	µg/L
TCC	17.6	0.11	-	ND	ND	13.1 (AB)	2.7	6.3 (A)	2.3	17.3 (B)	5.2	ng/L
TCS	15.9	0.12	-	ND	ND	ND	ND	282.1 (A)	ND	309.6 (A)	ND	ng/L
OP	NM	0.012	-	ND	ND	41.5 (A)	ND	82.7 (A)	ND	203.2 (B)	38	ng/L
NP	NM	ND	-	ND	ND	**20** [e]	ND	**38** [f]	ND	ND	ND	ng/L
EE2	NM	0.047	-	ND	ND	ND	ND	ND	ND	ND	ND	ng/L
BPA	NM	NQ	-	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	ng/L
ER-CALUX	NM	**37.5**	-	17 (A)	30	43 (B)	15	55 (B)	42	50 (B)	19	% 1 nM E2
TSS [g]	-	-	-	4094 (A)	2170	4543 (A)	2258	1579 (B)	2734	1199 (B)	85	mg/L
DOC [g]	-	-	-	1.0	3.4	24	207	83	42	18	34	mg/L

[a] NM = not measured, ND = not detectable (i.e., below method detection limits), NQ = not quantifiable (i.e., not significantly different from field/laboratory blank results). Organic chemical and ER-CALUX abbreviations shown in Table 1-1. Method detection limits as follows: BPA 5.2 ng/L; EE2 10 ng/L; TCC 1.0 ng/L; TCS 51 ng/L; OP 4.9 ng/L; and NP 8.8 ng/L

[b] (A), (B), and (C) represent runoff event mean concentration (EMC) results that are significantly different (p<0.05) between storms.

[c] Acid digestion for metals, solvent extraction for organics.

[d] Code of Federal Regulations Title 40 part 503.13 Table 1 Land Application Ceiling Concentrations.

[e] n = 2, Non-detect (ND) omitted.

[f] n = 3, Non-detect (ND) omitted.

Table 1-3. Batch experiment one-hour leachable concentrations of metals, organics, and ER-CALUX activity in biosolids samples from various POTWs and a commercial biosolids product (µg/kg, except ER-CALUX in % 1 nM E2). [a]

Constituent	POTW Rainfall Simulators	POTW 1	POTW 2	POTW 3	POTW 4	POTW 5	Milorganite
Cr	127	38.0	5.69	9.66	19.6	10.9	471
Co	157	44.0	14.2	17.8	19.5	20.3	466
Ni	1660	464	66.1	113	409	144	2377
Cu	841	469	48.5	162	86.1	40.4	14012
Zn	977	584	217	355	120	177	4081
As	147	58.5	43.8	42.8	67.0	44.3	421
Se	104	13.4	1.66	2.87	6.34	6.06	158
Ag	0.27	ND	ND	ND	11.1	ND	40.36
Cd	1.49	ND	ND	ND	ND	ND	6.19
Pb	12	8.21	ND	ND	ND	ND	13.35
TCC	105	93.5	5.79	12.2	24.2	7.38	9.77
TCS	120	ND	127	182	ND	ND	ND
OP	11.9	ND	2.38	58.1	153	37.7	ND
NP	ND	ND	24.5	36.5	13.8	ND	ND
EE2	47.2	ND	20.9	ND	25.1	ND	ND
BPA	ND	ND	ND	124	ND	ND	ND
ER-CALUX	22.2	41.9	43.9	48.9	42.3	44.4	29.5

[a] Organic chemical and ER-CALUX abbreviations shown in Table 1-1.

3.2 Rainfall simulations

Duration of storms ranged from 29.5 (TS3) to 83 min (CS), while volume of water leached during the simulation ranged from 0.5 (TS3) to 17.5 L (CS). The average duration across replicates for CS, TS1, TS2, and TS3 were 67.1 (*standard deviation (SD) = 16.7*), 55.3 (*SD = 4.9*), 32.6 (*SD = 0.8*), and 33.3 (*SD = 3.3*) min, respectively. Surface runoff flow rate generally increased throughout each storm and approached a steady state value of between 0.8 and 1.45 L/min near the end of each storm. It is important to recognize that the top 3 inches (7.6 cm) of soil was loose (uncompacted) prior to CS and TS1, but had compacted and settled due to previous storms prior to TS2

and TS3. This accounts for some of the disparity in hydraulics between CS/TS1 and TS2/TS3. Runoff timing and TSS concentrations are shown in **Figure 1-1**.

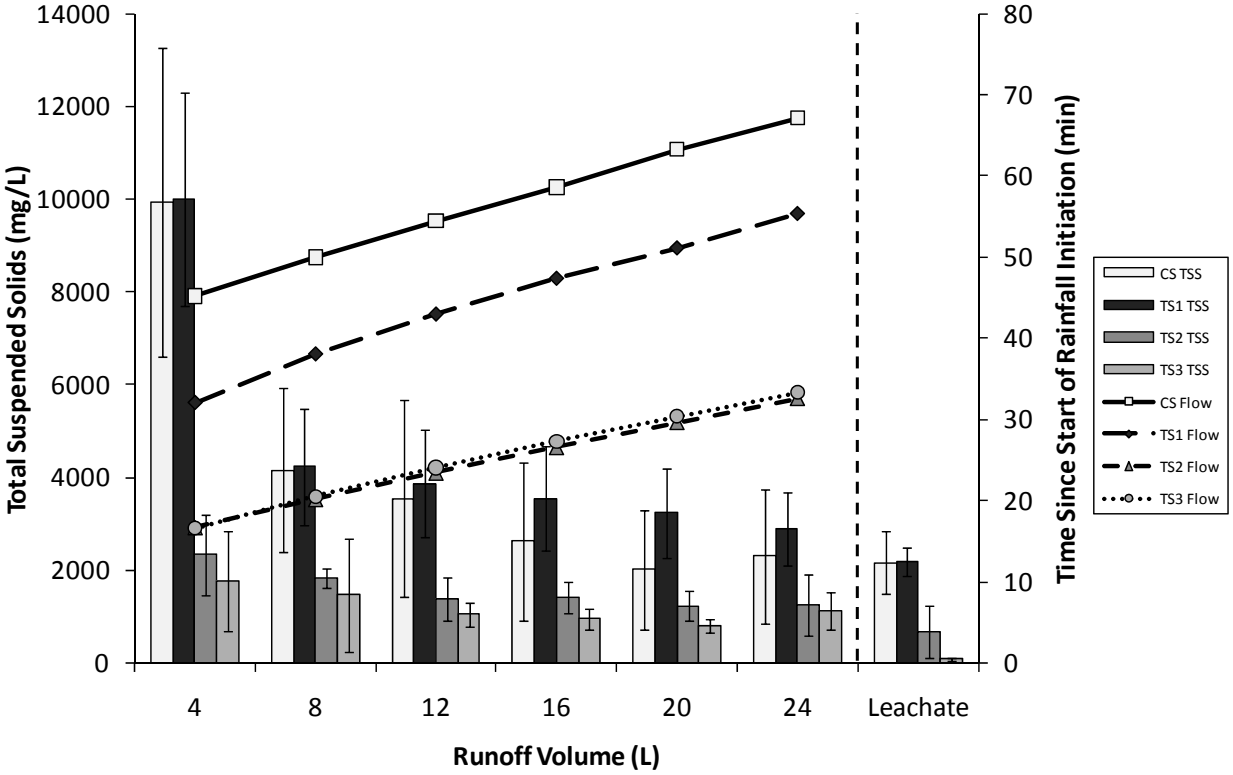

Figure 1-1. Total suspended solids (TSS) and timing of runoff fractions vs. runoff volume. Error bars for TSS measurements represent ± 1 standard deviation.

Dissolved organic carbon averaged 1 mg/L (*standard error of the mean* (*SEM) = 0.19*) in all fractions of CS runoff, and was 3.4 mg/L in CS leachate. In the one replicate of TS1 runoff fractions analyzed, DOC decreased from 33.5 to 15.3 mg/L throughout the storm and averaged 23.9 mg/L (*SEM = 3.1*), but was 206.9 mg/L in leachate. There was an increase in TS2, in which DOC in the first runoff fraction was 128 mg/L and decreased throughout the storm to 46.6 mg/L, but was 41.8 mg/L in leachate. Levels in TS3 were similar to those in TS1, decreasing from 29.4 mg/L to 10.2 mg/L at the end of the storm, and was 33.9 mg/L in leachate. Total suspended

solids (TSS) varied from an average of 1.1 g/L (*SEM = 0.23*) in the final fraction of TS3 to 10.0 g/L (*SEM = 1.3*) in the initial fraction of TS1.

3.2.1 Organics

The biosolids used in the rainfall simulations contained 17.6 mg/kg TCC and 15.9 mg/kg TCS. Median levels in biosolids from U.S. POTWs are 22 mg/kg for TCC and 4 mg/kg for TCS [17]. The total contents of other organic chemicals were not measured in biosolids.

17α-Ethynylestradiol was never detected in any of the samples. Nonylphenol was detected in less than 10% of samples and averaged 27.5 ng/L (*SEM = 7.5*) when detected. Bisphenol A in runoff was always detectable, but was unable to be quantified due to large amounts in the field blanks. Octylphenol and TCC were both detected in every surface runoff sample except one (OP) from every treatment storm. Triclosan was also detected in every surface runoff sample from TS2 and TS3, but was not able to be detected in TS1 due to high matrix interference. Triclosan was never detected in any leachate sample. Octylphenol was not detectable in leachate for TS1 and TS2, but averaged 37.8 ng/L (*SEM = 8.0*) in TS3, approximately 18% of the surface runoff concentration. Triclocarban was generally detected in leachate, gradually increasing from TS1 to TS2 to TS3, and averaged 3.2 ng/L (*SEM = 0.47*).

For TCC, TCS, and OP, total mass lost in runoff appeared to be linearly correlated with runoff volume (**Figure 1-2**). The magnitude of runoff losses showed an interesting trend with subsequent storms. For TCC, event mean concentrations (EMCs; total mass lost divided by total runoff volume) in runoff from least to greatest were TS2, TS1, then TS3. However, EMCs for TS2 and TS1 and for TS2 and TS3 were not

significantly different (p<0.05). For OP, the EMC trend from least to greatest was in chronological order: TS1, TS2, then TS3, and all were significantly different from each other. Triclosan EMCs were not significantly different.

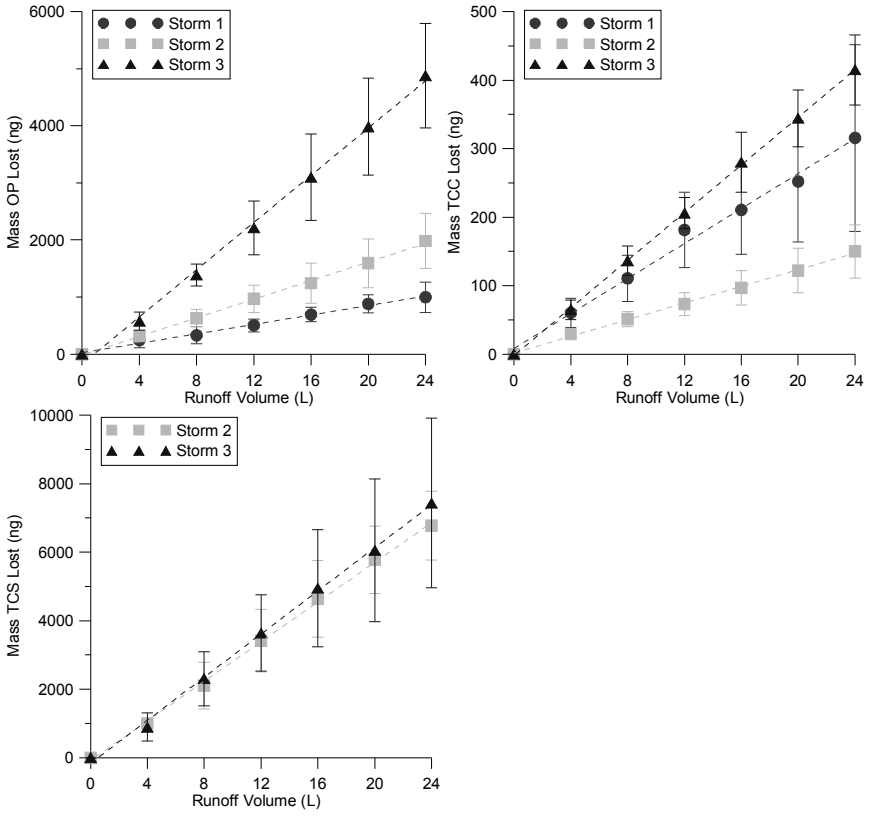

Figure 1-2. Loss of 4-t-octyphenol (OP), triclocarban (TCC), and triclosan (TCS) with increasing runoff fraction. TCS was not quantifiable during Storm 1 due to high matrix interference. Error bars represent ± 1 standard deviation.

3.2.2 ER-Calux

ER-Calux response showed a significant increase from CS to the treatment storms. Control storm response averaged 17% in runoff fractions and 29% in leachate, while averages across treatment storms varied from 43 to 55% in runoff fractions and 13 to 43% in leachate (Table 1-2). Results of treatment storms did not differ significantly from each other, except the leachate fraction from TS2 exhibited significantly greater response than TS1 and TS3. Time varying runoff samples within

storms did not significantly differ from each other; therefore no time-dependent trends were detected in response.

3.2.3 Metals

Three metals, Pb, Ag, and Cd were negligible in all runoff samples. Zinc could not be quantified due to large amounts in field blanks. The only metals that showed significantly elevated runoff concentrations were Cr, Co, Ni, Cu, As, and Se; results are shown in Table 1-2. Co, Ni, Cu, and Se showed the same trend: concentrations increased from TS1 to TS2, then decreased to below TS1 levels in TS3. Chromium and As concentrations remained approximately the same in TS1 and TS2, then decreased in TS3. All reported concentrations are corrected by subtracting average field blank concentrations.

4 Discussion

Water extractable concentrations of TCC and TCS were similar, and bore a similar relationship to their solvent extractable concentrations as well. The OP had an order of magnitude lower water extractable concentrations, though in rainfall runoff its concentration was much higher than TCC. 17α-Ethynylestradiol ~~EE2~~ 17α-Ethynylestradiol was detected at low levels in the water extractable portion, though it was never detectable in runoff samples.

Runoff and leachate flow behaved as expected, with runoff being lower and infiltration being higher when soil was loose and uncompacted, and runoff becoming higher and infiltration lower after a storm had compacted the soil. Total suspended solids levels also behaved as expected, with values being highest when soil was loose and early in storms. The DOC showed a dramatic increase in TS1 when compared to

CS due to the addition of the biosolids, which contain high amounts of organic carbon. The increase in DOC from TS1 to TS2 in rainfall runoff can be attributed to one of two factors: as microbial activity increased in the biosolids after TS1, organic carbon was liberated from the biosolids matrix and could be more easily mobilized in runoff (which has less contact time with the biosolids than does leachate) in TS2, or the compacted nature of the soil in TS2 limited infiltration flow, and thus a greater portion of the mobilized DOC was transported in the runoff. The total mass of DOC lost from the plots in runoff and leachate was greatest for TS1, followed by TS2, then TS3. Following TS2, the combination of microbial use of organic carbon and loss to runoff/infiltration are likely to have caused the decrease in TS3.

Concentrations of TCS found in runoff in the present study are within the range of concentrations found in similar studies (**Table 1-4**), but were slightly higher than most other reported values. This is likely due to a combination of factors, including lower soil organic carbon content, a higher concentration in the biosolids, and a higher soil pH. Concentrations of TCC in runoff were five times greater than the next highest concentration found in similar studies [9]. Again, this can be attributed to the lower soil organic carbon content and the significantly greater concentration of TCC in the biosolids than in previous runoff studies, although the levels here were typical of those found in U.S. biosolids [17]. The difference in concentrations of TCC and TCS in runoff in the present study is likely due to the difference in their pK_a values. While the reported Log K_{OW}s for the two compounds are similar (Table 1-1), the pK_a of TCC is 12.7 [18], while TCS is 8.1 [19]. The soil pH was 8.0, and runoff pH varied from 7.8 to 8.0. This indicates that nearly half of the TCS present in the biosolids was ionized, and

is thus much more likely to partition into the runoff than the neutral TCC. The ratio of TCS/TCC in runoff in the present study varied from 18 to 45, which is similar to the ratios of approximately 32 to 40 found by others [9].

Table 1-4. Comparison of present study TCS and TCC results to other studies.[a]

Study	Soil Organic Carbon (%)	Soil pH	Type of Biosolids[b]	Type of Sample	Rainfall Rate (mm/hr)	TCS Biosolids (mg/kg)	TCS Water (ng/l)	TCC Biosolids (mg/kg)	TCC Water (ng/l)
Lapen et al.	0.5-2.1	6.6-6.8	LMB	Tile Drainage	Varied	3.8	3680 [c]	NA	NA
Edwards et al.	0.4-2.0	7.0-7.5	DMB	Tile Drainage	Varied	14	230 [c]	8.0	< 5
Topp et al.	1.97	7.5	LMB	Surface Runoff	210	NR [d]	258 [e]	NA	NA
Sabourin et al.	1.97	7.5	DMB	Surface Runoff	186	7.0	110 [c]	8.2	3.4
Present Study	0.37	8	DMB	Surface Runoff	60	16	310 [e]	18	17.3

[a] Organic chemical abbreviations shown in Table 1-1.
[b] LMB = liquid municipal biosolids, DMB = dewatered municipal biosolids.
[c] Maximum detected concentration in a grab sample.
[d] NR = not reported.
[e] Maximum event mean concentration (EMC).

The reason for the difference between losses of TCC and OP with subsequent storms is not clear. An increasing trend from TS2 to TS3 was shown for both, however. By observation, it was clear that the biosolids were breaking up into smaller pieces as they were impacted by raindrops and as they dried out after simulated storms. The increased specific surface area exposed to runoff would likely lead to this common trend between TCC and OP. Additionally, as organic carbon was degraded by microorganisms or lost through runoff in previous storms, less was available for TCC and OP to sorb to, and thus, mobilization may have increased for this reason. It is also possible that octylphenol ethoxylates present in the biosolids were being transformed

into OP over time, and thus the amount of OP available to mobilization increased with each additional storm.

Concentrations of dissolved organic compounds showed no relationship to measured DOC, which indicates that organics lost from the plots and measured were either truly dissolved or bound to suspended solids that were extracted with the dissolved fraction. Samples were not filtered, so the maximum particle size remaining in suspension after centrifugation was calculated to be approximately 3.6 microns (for reference, an estimated 50% of 2.5 micron particles and 25% of 1.9 micron particles were removed via centrifugation). Dissolved organic compounds also showed no relationship to TSS within the samples. A limitation of this study was that organics bound to suspended solids were not analyzed. Because organics measured in this study strongly bind to particulates, it is likely that total mass of compounds lost from the site would correlate to TSS measurements, though this should be confirmed by future studies.

Concentrations of TCC, TCS, and OP in runoff were below or toward the low range of typical levels in treated wastewater effluent (100-6000 ng/L for TCC [20], 27-2700 ng/L for TCS [20], and 20-1700 ng/L for OP [21]). Concentrations of TCC and OP were below their most sensitive known environmental endpoints (60 ng/L no-observed-effect concentration [NOEC] for TCC, *Mysidopsis bahia* reproduction [20]; 1 µg/L NOEC for OP, *Oncorhynchus mykiss* vitellogenin synthesis [22]), while TCS was slightly above its most sensitive value found in the literature (200 ng/L NOEC, *Selenastrum capricornutum* growth [20]).

The ER-Calux response in CS of approximately 17% can largely be attributed to background BPA. Literature dose-response values for BPA indicate a response of 33% for 1000 nM BPA (228 μg/L) [23], and our own dose-response tests indicate a typical response of 28% for 3400 μg/L. Some of the increase in response during treatment storms is attributable to detected concentrations of OP. The literature indicates a response of 15% to 1000 nM OP (206 μg/L) [24], and our own dose-response tests indicate typical responses of 18% to the same concentration. Taking this into account, there still exists between 5 and 15% of response unaccounted for by chemical measurements. This could be due to matrix enhancement effects or chemicals not analyzed, or possibly a combination of the two. For example, it is known that steroid hormones such as estradiol and estrone can be present in biosolids and elicit an estrogenic response [11], but these were not analyzed in the present study. As with organics, discussed above, ER-Calux response showed no relationship to DOC, suggesting that measured responses were largely associated with freely dissolved organics. Finally, it is important to note that no significant decrease of ER-Calux activity was apparent in the runoff from three storms over the three week study period. As discussed above, concentrations of OP increased throughout this period, although the specific mechanism for this increase is unknown. The conclusion that can be drawn from this result is that mobilization of estrogenic activity from land-applied biosolids is not limited to the first storm following biosolid application, and may not even reach its maximum value in that storm. This complex process clearly requires further study.

Nickel concentrations were highly linearly correlated with DOC ($r = 0.986$). Copper and other metals were not as tightly correlated, but showed strong relationships

to DOC nonetheless. Results for nickel, copper, and arsenic are shown in **Figure 1-3**. These results confirm findings from previous studies on the mobility of metals from biosolids being correlated with DOC [3, 5].

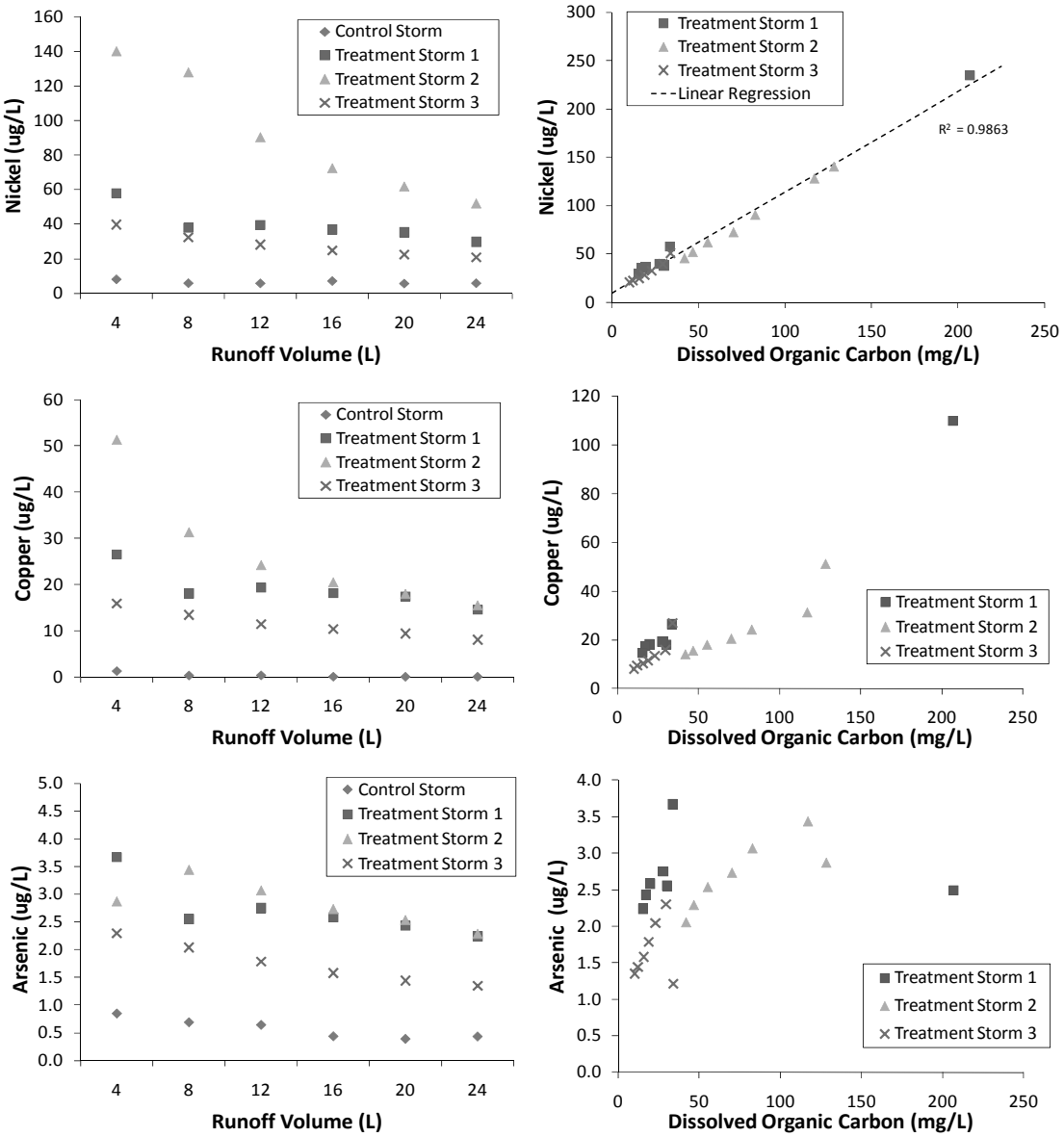

Figure 1-3. Mean nickel, copper, and arsenic concentrations in runoff fractions vs. runoff volume, and in runoff and leachate fractions vs. dissolved organic carbon.

Concentrations of metals were thus highest in the leachate from TS1 and the runoff from TS2, due to the higher levels of DOC measured in those samples. As

mentioned above, the increase in DOC in TS2 runoff may have been due to either increased liberation from the biosolid matrix following TS1, or due to reduced infiltration (relative to TS1) in the compacted soil of TS2. In either case, since biosolids are a source of organic carbon in the settings in which they are applied, and infiltration will generally be highest in the first storm following incorporation of the biosolids into the soil, these results have implications for when maximum concentrations of metals would be expected in leachate and runoff. In many settings, metals transport in leachate will be highest in the first storm following biosolids application and incorporation, while maximum concentrations in runoff may be seen in subsequent storms, eventually diminishing as DOC concentrations decrease.

Concentrations of nickel in runoff were below the California Toxics Rule (CTR; http://www.epa.gov/region9/water/ctr/) maximum freshwater criterion of 591 μg/L (based on the study water hardness of 131 mg/L), while copper concentrations were above the CTR maximum freshwater criterion of 18 μg/L for TS1 and TS2, but below for TS3. Had rainwater been used for the simulations, hardness would have likely been much lower, which would have lowered the criteria and resulted in more exceedances.

Although levels of metals in biosolids were generally far below maximum allowable limits, possible environmental risk in rainfall runoff was demonstrated for copper, nickel, and TCS. In a field setting, setbacks and erosion control (i.e., berms) can mitigate this risk, but the findings of this study nonetheless indicate that current limits on metals concentrations in biosolids may not be sufficiently protective with respect to either metals or TCS in runoff.

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Chapter 2

Modification of GLEAMS for Prediction of Fate and Transport of Trace Organic Contaminants from Land-Applied Biosolids

Abstract

Municipal biosolids are commonly applied to agricultural lands as fertilizer, but this also poses potential risks to groundwater and surface water quality from constituents that may be mobilized during storm events. In the present study, an existing model, Groundwater Loading Effects of Agricultural Management Systems (GLEAMS), is modified to predict the fate and transport of trace organic contaminants from land-applied biosolids. The primary modification is the addition of a labile biosolids organic carbon phase distinct from soil organic carbon. The model is calibrated using existing data from literature studies, including experiments described in chapter 1. It is then applied to various scenarios using chemicals of varied properties to examine the effects of K_{OC} and half-life, to examine the effect of application date for a perennial application scenario in the arid west, and to examine differences in chemical loss under different application methods (e.g., surface spreading vs. incorporation) on long-term chemical losses. The calibrated model showed good agreement with field runoff data for acetaminophen, ibuprofen, triclosan, triclocarban, and estrone, but substantially under predicted measured concentrations for carbamazepine, androstenedione, and progesterone with reasonable input parameters. In applying the model, as expected, chemicals with long half-lives and low K_{OC}s exhibited the highest overall losses, while chemicals with short half-lives and high K_{OC}s exhibited the lowest overall losses. For short half-life chemicals, perennial application at the beginning of the California dry season resulted in the lowest overall losses. However, for long half-

life chemicals with high K_{OC}, perennial application during the rainy season resulted in the lowest losses, because application of the biosolids caused organic carbon to be high during the period of highest runoff. As expected, surface application of biosolids resulted in greater losses than incorporation for all chemicals tested. Results from this study can help predict environmental risk from land-application of municipal biosolids, highlight gaps in our knowledge about how chemicals are mobilized and transported from biosolids, and can help identify management practices that result in minimal impacts to water quality.

1 Introduction

In the United States, 61% of the biosolids that are generated during municipal wastewater treatment are used as soil amendment or fertilizer [1]. Biosolids contain high levels of nutrients and organic matter that can be a valuable resource to agricultural sites, but the beneficial reuse of biosolids must be evaluated relative to potential risks, which include, among other concerns, impacts to water quality due to mobilization of trace organic compounds (TrOCs).

Several studies have measured the mobilization of TrOCs from land-applied biosolids. These studies included analysis of PPCPs in tile drainage [1, 2] and surface runoff [3-5], and of hormones in surface runoff [6] following application of liquid municipal biosolids or dewatered municipal biosolids. Concentrations found in tile drainage and surface runoff were generally of the same magnitude or lower than those found in treated wastewater effluent, but some were still comparable or above the lowest known environmental endpoints. These endpoints are not typically related to mortality, but are generally sublethal developmental or reproductive effects of chronic exposure to low levels.

Some researchers have developed or applied models to examine losses due to subsurface transport from liquid municipal biosolids [7, 8]. Models have also been developed for transport of nutrients from land-applied manure [9, 10]. A fugacity-based model has been developed for land-applied biosolids that does not include transport via rainfall runoff [11]. To our knowledge, no models have been developed that allow for prediction of chemical loss via both surface runoff and subsurface transport, and that also allow the user to examine effects of agricultural management practices (such as

crop rotation, erosion control measures, tillage management, etc.) on chemical fate and transport from land-applied biosolids.

The Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) model has been used previously to model pesticide losses from agricultural fields [12, 13], and losses of nutrients from land-applied manure [10, 14]. The model contains a pesticide fate and transport module that predicts movement of agriculturally applied pesticides in surface runoff and the subsurface. With the recognition that mobilization of trace organic constituents from biosolids and biosolids amended soil is mechanistically similar to pesticides from soil, in this study, GLEAMS is modified to allow the user to input biosolids application parameters, and then predicts the fate and transport of target chemicals from the field.

In addition to GLEAMS, the other currently used model for predicting pesticide fate and transport is the Pesticide Root Zone Model (PRZM). Many studies have compared the accuracy and deficiencies of GLEAMS and PRZM using field data for runoff volume, pesticide concentrations in leachate and runoff, and pesticide concentrations in the soil profile. Although many of the studies have used older versions of the models, and the focus of the majority of the studies was on subsurface pesticide leaching and concentrations in the soil profile (as opposed to concentrations in runoff), some patterns have emerged in terms of their relative benefits for different uses. Multiple studies have shown that GLEAMS produces better results specifically for runoff volumes and concentrations [15-20], others have shown that PRZM is preferable for leachate concentrations and soil concentrations with depth in the soil profile [20-22], and others have shown that both models produce acceptable results [23, 24].

The specific objectives of this study were to: (1) modify an existing chemical fate and transport model that incorporates agricultural management practices, and calibrate this model using existing studies in the literature, to enable simulation of chemical losses from land-applied municipal biosolids, and (2) apply the model to various hypothetical scenarios to investigate and make general observations about the effects of different factors and management decisions on chemical losses from land-applied biosolids. Results from this study can help predict environmental risk from land-application of municipal biosolids, highlight gaps in our knowledge about how chemicals are mobilized and transported from biosolids, and can help identify management practices that result in minimal impacts to water quality.

2 Model Description

GLEAMS was developed in the 1980s based on the Chemicals, Runoff, and Erosion in Agricultural Management Systems (CREAMS) model, which was developed in the late 1970s. GLEAMS contains hydrology, erosion, pesticide, and nutrient submodels. The model was developed to evaluate complex interactions among soils, pesticide chemistry, climate, and management practices that affect chemical movement in and through the root-zone [25, 26]. The model can be used for plot or field sized units, in which soil, management, and areal precipitation are uniform. Soil properties vary with depth, and therefore, parameter values are required for each horizon. Computational layers are used to track and route water and chemicals. The surface layer is assumed to be a fixed thickness of 1 cm, even though it is known that factors such as tillage, time since last tillage, rainfall, soil texture, soil water content, and soil

cover, among other things, affect infiltration control and interaction of runoff and chemical extraction [25].

The model runs on a daily time-step, and daily climactic data are used to calculate the water balance. Runoff and infiltration due to precipitation are determined using the curve-number method. A storage-routing technique is used to simulate distribution of water and percolation in the subsurface. Evapotranspiration is estimated using a modified Penman equation or the Priestly-Taylor method. The erosion submodel uses a modified Universal Soil Loss Equation to simulate storm-by-storm rill and interrill erosion. Sediment in runoff is affected by particle size, and thus sediment enrichment ratios are used in simulating adsorbed pesticide transport. In essence, coarser soil material is deposited or left in place, so the transported sediment has a higher per unit mass adsorptive capacity and adsorbed chemical concentration than that of the whole soil. The enrichment ratio is calculated in the erosion submodel based on the specific surface area of the sediment leaving the field and the specific surface area of the whole soil matrix [25, 27].

The pesticide submodel tracks pesticide movement in runoff and sediment, as well as in the subsurface. Degradation, extraction into runoff, and movement of pesticide in the subsurface are described in more detail in the following section, which describes model modifications. Pesticide applications can be by surface application, incorporation, injection, or chemigation (i.e., application of pesticides dissolved in irrigation water). Complete descriptions of the model can be found in the model and related documentation [25, 27, 28].

3 Model Modifications

Two modifications to GLEAMS were made: addition of a biosolids phase (to soil and water phases already present), and degradation of organic carbon in the biosolids phase. Instead of exclusively simulating pesticide fate and transport, the modified model simulates the fate and transport of any organic constituent present in the biosolids whose sorption can be described using linear partitioning to organic matter and whose degradation follows first order decay. Applications of the chemical are now no longer strictly chemical only applications, but applications of the chemical can occur as part of the application of the biosolids that contain the chemical. Application methods include surface application, incorporation, and injection.

In the model, equilibrium between the three phases is established in each computational layer every time rainfall occurs. This means that there are times when the three phases are not at equilibrium (i.e., when conditions affecting equilibrium have changed but rainfall has not yet occurred). The most important factor in the modified model that causes this is the organic carbon degradation in the biosolids. There are therefore parameters, such as soil and biosolids concentrations of chemical, which the user can view as output on a daily time-step, but which may not be at equilibrium. However, since the primary objective of the model is to estimate losses due to rainfall, establishment of equilibrium only on days when rainfall occurs is considered an appropriate simplification.

3.1 Addition of Biosolids Phase

3.1.1 Degradation of chemical

Degradation of chemical in soil and biosolids is assumed to follow a first-order relationship, and is defined in terms of the empirical half-life,

$$C_{s(t+\Delta t)} = C_{s(t)} \cdot \exp\left(-0.693 \cdot \frac{\Delta t}{S_{1/2\,s}}\right) \tag{1}$$

$$C_{b(t+\Delta t)} = C_{b(t)} \cdot \exp\left(-0.693 \cdot \frac{\Delta t}{S_{1/2\,b}}\right) \tag{2}$$

where $C_{s(t+\Delta t)}$=concentration in soil at time t+Δt (mg/kg); $C_{s(t)}$=concentration in soil at time t (mg/kg); Δt=time interval between computation (d); $S_{1/2s}$=half-life in soil phase (d); $S_{1/2b}$=half-life in biosolids phase (d); $C_{b(t+\Delta t)}$=concentration in biosolids at time t+Δt (mg/kg); and $C_{b(t)}$=concentration in biosolids at time t (mg/kg).

3.1.2 Basic system description

Chemical distribution between the solution phase and the soil phase, and between the solution phase and the biosolids phase, is described as a simple linear sorption isotherm,

$$K_{ds} = \frac{C_s}{C_w} \tag{3}$$

$$K_{db} = \frac{C_b}{C_w} \tag{4}$$

where, at equilibrium, K_{ds}=soil-water distribution coefficient (L/kg); C_s=concentration in soil (mg/kg); C_w=concentration in water (mg/L); K_{db}=biosolids-water distribution coefficient (L/kg); and C_b=concentration in biosolids (mg/kg). Because the distribution

coefficients are dependent on the organic carbon content of the soil or biosolids at a given time, they are defined in terms of the organic carbon normalized distribution coefficients,

$$K_{ds} = K_{OCs} \cdot \frac{OC_s}{100} \tag{5}$$

$$K_{db} = K_{OCb} \cdot \frac{OC_b}{100} \tag{6}$$

where K_{OCs}=organic carbon normalized soil-water partitioning coefficient (L/kg); OC_s=% organic carbon in soil; K_{OCb}= organic carbon normalized biosolids-water partitioning coefficient (L/kg); and OC_b=% organic carbon in biosolids. Studies have shown that K_{OCs} and K_{OCb} are not always equal to each other—either because soil or biosolids components other than organic carbon participate significantly in the sorption process or because the different composition of the two phases cause variation in their sorptive efficiency [29-31]. The model allows for users to input different values for the K_{OCs} and K_{OCb}.

At saturation, the volume of water per unit volume of the soil-biosolids matrix is

$$V_{fw} = POR \tag{7}$$

where V_{fw}=volume of water per unit volume of saturated soil-biosolids mixture (L). Next we define the density of soils and biosolids as:

$$\rho_s = \frac{M_s}{V_s} \tag{8}$$

$$\rho_b = \frac{M_b}{V_b} \tag{9}$$

where ρ_b=density of biosolids (kg/L). We can then define the volumes of soil and biosolids as:

$$V_{fs} = 1 - POR - \frac{M_b}{\rho_b} \tag{10}$$

$$V_{fb} = 1 - POR - \frac{M_s}{\rho_s} \tag{11}$$

where V_{fs}=volume of soil per unit volume of saturated soil-biosolids mixture (L); and V_{fb}=volume of biosolids per unit volume of saturated soil-biosolids mixture (L). The mass of biosolids in each layer is determined according to the application rate and method of application (surface spreading, incorporation, or injection). The mass of soil in each layer is determined according to the bulk density of the soil. The approach described above assumes that porosity is unaffected by biosolids addition.

3.1.3 Pesticide losses and movement during rainfall

In the following section, three processes simulated by the model are described. First, loss of chemical from the surface layer of soil-biosolids mixture due to infiltration into the layer beneath is determined. Second, loss of chemical due to extraction from the remaining mass of chemical in the surface layer into overland flow is determined. Last, routing of chemical through subsurface layers is described.

3.1.3.1 Loss of chemical in surface layer due to infiltration

At saturation, the chemical mass in the surface layer of the soil-biosolids mixture is

$$z = C_w \cdot V_{fw} + C_s \cdot V_{fs} \cdot \rho_s + C_b \cdot V_{fb} \cdot \rho_b \tag{12}$$

where z=mass of chemical in the surface layer per unit volume of soil-biosolids mixture in the surface layer (mg). Now, combining equation 12 with equations 3, 4, and 7,

$$z = C_w \cdot POR + K_{ds} \cdot C_w \cdot V_{fs} \cdot \rho_s + K_{db} \cdot C_w \cdot V_{fb} \cdot \rho_b \tag{13}$$

The rate of change of chemical mass in the surface layer due to infiltration during a storm is

$$-dz = C_w \cdot f \cdot dT \tag{14}$$

where f=water flux (L/h); and T=time (storm duration) (h). Rearranging equation 13 gives

$$C_w = \frac{z}{POR + K_{ds} \cdot V_{fs} \cdot \rho_s + K_{db} \cdot V_{fb} \cdot \rho_b} \tag{15}$$

Combining equations 14 and 15, we then integrate from z_o to z and from T_0 to T:

$$\int_{z_0}^{z} -dz = \int_{T_0}^{T} \frac{z \cdot f \cdot dT}{POR + K_{ds} \cdot V_{fs} \cdot \rho_s + K_{db} \cdot V_{fb} \cdot \rho_b} \tag{16}$$

where z_0=concentration of chemical in surface layer at the beginning of the storm per unit volume of soil-biosolids mixture in the surface layer (mg). This yields:

$$z = z_0 \cdot \exp\left(\frac{-f \cdot T}{POR + K_{ds} \cdot V_{fs} \cdot \rho_s + K_{db} \cdot V_{fs} \cdot \rho_b}\right) \tag{17}$$

The infiltration flux through the top layer of soil is

$$f = \frac{P - Q - AWS}{T} \tag{18}$$

where P=rainfall depth (cm); Q=surface runoff depth (cm); and AWS=soil water storage capacity to saturation (initial abstraction) (cm). It can be shown that the volume of 1 cm water depth equals the unit volume of soil-biosolids matrix chosen, since the depth of the surface layer is assumed to be 1 cm. Thus, although P, Q, and AWS are given/calculated in GLEAMS in units of cm, on a per unit volume basis (in this case the unit volume being 1 L), the numeric values are the same in units of L (and, therefore, f (cm/h) = f (L/h)). Thus, we can combine equations 17 and 18 to give:

$$z = z_0 \cdot \exp\left(\frac{-(P - Q - AWS)}{POR + K_{ds} \cdot V_{fs} \cdot \rho_s + K_{db} \cdot V_{fb} \cdot \rho_b}\right) \tag{19}$$

The parameter z_0, the mass of chemical in the surface layer at the beginning of the storm per unit volume of soil-biosolids mixture in the surface layer, is calculated as the weighted average concentration of soil and biosolids times the weighted average bulk density of the mixture,

$$z_0 = \frac{C_s(t) \cdot M_s + C_b(t) \cdot M_b}{M_s + M_b} \cdot \left(V_{fs} \cdot \rho_s + V_{fb} \cdot \rho_b\right) \tag{20}$$

Thus, the total mass of chemical lost via infiltration from the surface layer is:

$$PERCM_1 = z - z_0 \tag{21}$$

where $PERCM_1$=mass of chemical lost via infiltration from the surface layer (mg).

3.1.3.2 Extraction and movement of chemical in overland flow

In the model, at the time runoff occurs, the surface layer of soil contains the chemical mass determined after losses due to infiltration (vertical translocation), which are described above. The concentration of chemical available to runoff in this model is defined as the mass of chemical in the surface layer per unit volume of soil-biosolids mixture in the surface layer divided by the weighted average bulk density of the mixture,

$$C_{av(mix)} = \frac{z}{\left(V_{fs} \cdot \rho_s + V_{fb} \cdot \rho_b\right)} \tag{22}$$

where $C_{av(mix)}$= concentration of chemical in surface layer of soil-biosolids mixture available to runoff (mg/kg). However, at the interface between the soil/biosolids matrix and the overland flow, only some portion of the soil-biosolids mixture is effective in supplying chemical to the flow. Thus, we introduce the term $B_{av(mix)}$, the soil-biosolids

mixture mass available to supplying chemical to the overland flow per unit volume of overland flow (extraction coefficient; kg). The mass of chemical in the soil-biosolids mixture available to the overland flow is assumed to equilibrate instantly between the soil/biosolids mixture and the overland flow, so

$$C_{av(mix)} \cdot B_{av(mix)} = C_w \cdot V + C_s \cdot B_s + C_b \cdot B_b \tag{23}$$

where V=volume of water per unit volume of runoff interface (L), and B_s and B_b are the soil and biosolids (respectively) masses available to supplying chemical to the overland flow per unit volume of overland flow (kg). B_s and B_b represent a portion of the total mass of soil and biosolids, respectively, and thus must meet the following requirements:

$$B_s \leq M_s \tag{24}$$

$$B_b \leq M_b \tag{25}$$

In equation 23, we can disregard the volume of the runoff interface occupied by soil compared to the much larger volume of water, so that V = 1. Combining equations 3 and 4 with equation 23, we derive the expressions for the equilibrium concentrations in the overland flow, soil, and biosolids:

$$C_w = \frac{B_{av(mix)} \cdot C_{av(mix)}}{1 + B_s \cdot K_{ds} + B_b \cdot K_{db}} \tag{26}$$

$$C_s = \frac{B_{av(mix)} \cdot C_{av(mix)}}{\frac{1 + K_{db} \cdot B_b}{K_{ds}} + B_s} \tag{27}$$

$$C_b = \frac{B_{av(mix)} \cdot C_{av(mix)}}{\frac{1 + K_{ds} \cdot B_s}{K_{db}} + B_b} \tag{28}$$

The total mass of aqueous chemical lost via runoff from the surface layer is:

$$QM = Q \cdot C_w \tag{29}$$

where QM=mass of aqueous chemical lost via runoff from the surface layer (mg); and Q=surface runoff (L). The total mass of chemical sorbed to sediment lost via runoff from the surface layer is:

$$SM = S \cdot E \cdot C_s \tag{30}$$

Where SM=mass of chemical sorbed to sediment lost via runoff from the surface layer (mg); S=sediment loss (kg, calculated in the erosion component); and E=enrichment ratio (calculated in the erosion component, as described previously).

As noted in [25], a functional relationship developed in the original GLEAMS relates B_s to K_d as follows:

$$B_s = \begin{cases} 0.5, & K_{ds} \leq 1.0 \\ 0.7 - 0.2 * K_{ds}, & 1.0 < K_{ds} \leq 3.0 \\ 0.1, & K_{ds} > 3.0 \end{cases} \tag{31}$$

Little is presented in [25] regarding the development of these rules. In the present work, the rules for B_s were modified based on the calibration performed, which is discussed in a subsequent section. Although no previous work has been done to determine the value of B_b, it will be shown later that acceptable results are obtained by equating the value of B_b to the entire mass of biosolids per unit volume of overland flow (i.e., all of the biosolids phase present in the 1 cm surface layer is available to supplying chemical to the overland flow). Thus,

$$B_b = M_b \tag{32}$$

The value of $B_{av(mix)}$ is calculated as the average of B_s and B_b, weighted by the K_d of each compartment,

$$B_{av(mix)} = \frac{K_{ds} \cdot B_s + K_{db} \cdot B_b}{K_{ds} + K_{db}} \tag{33}$$

3.1.3.3 Vertical movement of chemical in the subsurface

The mass of chemical that infiltrates from the surface layer into the layer below was defined in equation 19. The mass of chemical that infiltrates from a given subsurface layer into the layer below it is:

$$PERCM_i = PERC_i \cdot C_{wi} \tag{34}$$

where $PERCM_i$= mass of chemical in percolate from subsurface layer i (mg); $PERC_i$= volume of water percolated from subsurface layer i (L); and C_{wi}= concentration of chemical in water in subsurface layer i (mg/L). This mass is added to any existing mass in the layer below, and the total mass of chemical in each layer is computed in this fashion. The total mass of chemical in any subsurface layer, i, is divided between the three phases:

$$PMS_i = C_{bi} \cdot M_{bi} + C_{si} \cdot M_{si} + C_{wi} \cdot V_{wi} \tag{35}$$

where PMS_i= mass of chemical in subsurface layer i (mg); C_{bi}= concentration of chemical in biosolids in subsurface layer i (mg/kg); M_{bi}= mass of biosolids in subsurface layer i (kg); C_{si}= concentration of chemical in soil in subsurface layer i (mg/kg); M_{si}= mass of soil in subsurface layer i (kg); C_{wi}= concentration of chemical in water in subsurface layer i (mg/L); and V_{wi}= volume of water in subsurface layer i (L). Assuming equilibrium, substituting equations 4 and 5 into the above equation gives

$$PMS_i = \frac{K_{db}}{K_{ds}} \cdot C_{si} \cdot M_{bi} + C_{si} \cdot M_{si} + \frac{C_{si}}{K_{ds}} \cdot V_w \tag{36}$$

$$C_{si} = \frac{PMS_i}{\frac{K_{db}}{K_{ds}} \cdot M_{bi} + M_{si} + \frac{V_{wi}}{K_{ds}}} \tag{37}$$

And, again, at equilibrium,

$$C_{bi} = \frac{K_{db}}{K_{ds}} \cdot C_{si} \tag{38}$$

$$C_{wi} = \frac{C_{si}}{K_{ds}} \tag{39}$$

3.2 Degradation of Organic Carbon in Biosolids Phase

Mineralization of organic carbon in biosolids is treated in the model as a first order process acting on two compartments: fast-degrading organic carbon and slow-degrading organic carbon. A third compartment, recalcitrant organic carbon, does not degrade. The biosolids-borne organic carbon remaining at time t is thus the sum of these three components, so,

$$MOC_b(t) = MOC_{b,f}(t) + MOC_{b,s}(t) + MOC_{b,r}(t) \tag{40}$$

$$1 = fOC_{b,f}(t) + fOC_{b,s}(t) + fOC_{b,r}(t) \tag{41}$$

where $MOC_b(t)$=mass of organic carbon in biosolids at time t (kg); $MOC_{b,f}(t)$=mass of fast-degrading organic carbon in biosolids at time t (kg); $MOC_{b,s}(t)$=mass of slow-degrading organic carbon in biosolids at time t (kg); $MOC_{b,r}(t)$=mass of recalcitrant organic carbon in biosolids at time t (kg); $fOC_{b,f}(t)$=fraction of organic carbon that is fast-degrading at time t; $fOC_{b,s}(t)$=fraction of organic carbon that is slow-degrading at time t; and $fOC_{b,r}(t)$=fraction of organic carbon that is recalcitrant at time t. The equations for calculating mass in each fraction at any time are,

$$MOC_{b,f}(t + \Delta t) = MOC_{b,f}(t) \cdot \exp\left(-k_{b,f} \cdot \Delta t\right) \tag{42}$$

$$MOC_{b,s}(t + \Delta t) = MOC_{b,s}(t) \cdot \exp(-k_{b,s} \cdot \Delta t) \tag{43}$$

$$MOC_{b,r}(t) = MOC_{b,r}(0) \tag{44}$$

where $k_{b,f}$=rate constant for degradation of fast-degrading organic carbon (d^{-1}); and $k_{b,s}$=rate constant for degradation of slow-degrading organic carbon (d^{-1}). The initial masses of organic carbon in each fraction are found by,

$$MOC_{b,f}(0) = fOC_{b,f}(0) \cdot MOC_b(0) \tag{45}$$

$$MOC_{b,s}(0) = fOC_{b,s}(0) \cdot MOC_b(0) \tag{46}$$

$$MOC_{b,r}(0) = fOC_{b,r}(0) \cdot MOC_b(0) \tag{47}$$

While the total initial mass of organic carbon is found by

$$MOC_b(0) = M_b \cdot \frac{OC_b(0)}{100} \tag{48}$$

3.3 Assumptions Used for Perennial Biosolids Application

Since biosolids are tracked as a distinct phase, to keep the mass of biosolids in each layer from growing unrealistically large in perennial application scenarios, some simplifying assumptions were employed. Specifically, immediately prior to the second and subsequent perennial biosolids applications:

1. The mass of biosolids is set to 0.
2. The masses of organic carbon and of all chemicals in the remaining biosolids phase are added to the soil phase.

These assumptions are meant to approximate the mineralization of biosolids that occurs over time, in which biosolids as a unique phase separate from the soil cease to be recognizable due to natural phenomena. Over both short-term and long-term simulations with a single or with perennial applications, such as those employed in the present study, these assumptions are reasonable approximations. For scenarios that

involve multiple applications per year, more sophisticated approaches may be more appropriate.

4 Methods

4.1 Calibration

Three case studies were selected to use for calibrating the model [3, 5, 6]. All three studies applied dewatered municipal biosolids to small (2-6 m^2) plots and used rainfall simulators to simulate intense (>60 mm/hr) rainfall events periodically over the course of 24-36 days following biosolids application. From the chemicals analyzed in these studies, a subset of chemicals were selected to use for calibrating the model. The chemicals selected met three criteria: 1) they were quantified in biosolids and runoff from rainfall simulations, 2) their sorption could be described by linear isotherms to organic carbon, and 3) data was available in the literature regarding their expected half-life and K_{OC}. **Table 2-1** summarizes chemicals analyzed in the studies, whether they were included in the present study for calibration, and the reasons for exclusion, if any. **Tables 2-2 through 2-4** summarize important parameters used in the initial model runs to replicate the scenarios described in these studies. Additional climate parameters and precipitation input files are shown in Appendix A. For parameters that were not measured or reported in the studies, estimates or assumptions were made, as necessary, or other data sources were consulted.

There were two phases to the calibration process. The first phase was to develop a set of relationships for B_s that would result in generally acceptable agreement between predicted and measured runoff concentrations for as many chemicals as

possible. The second phase was to modify chemical and scenario-specific parameters to obtain better agreement between model results and experimental data. Changing certain parameters resulted in chemical-specific changes, while other parameters affected all chemicals simultaneously. Also, changing certain parameters affected results for all storm simulations, while others primarily affected later storms. **Table 2-5** summarizes the calibration parameters that were altered to investigate effects on the modeling results and to obtain better agreement between the model and experimental results.

Runoff volume and erosion mass were not calibrated for the simulations. Previous work has shown that GLEAMS produces adequate predictions of long-term runoff and erosion, though short-term (< 1 year, or by storm) estimates may be quite variable [32]. It was for this reason that runoff concentrations, which were relatively insensitive to runoff volume, were used for calibration, and not mass of chemical. However, application of the model on a long-term basis and evaluation of chemical mass losses should still be valid, based on this and previous work. Sediment concentrations were not calibrated, as insufficient data was available in the literature to determine accurate runoff sediment concentrations. Concentration in rainfall that infiltrated (percolate) were also not able to be calibrated. These were only measured in [3], and only detected for a single chemical. Moreover, measurements in [3] may have been affected by edge effects and soil cracking which cannot be accounted for in the model.

Table 2-1. Chemicals Analyzed in Previous Studies and Status of Inclusion/Exclusion in the Present Study.

Sabourin et al., 2009		Yang et al., 2012	
atenolol	DNQ	11-ketotestosterone	ND
acetaminophen	**Used**	17-alpha estradiol	ND
caffeine	[a]	17-beta estradiol	ND
carbamazepine	**Used**	**androstenedione**	**Used**
cotinine	DNQ	cholesterol	DNQ
gemfibrozil	ND	cis-androsterone	[c]
ibuprofen	**Used**	coprostanol	DNQ
naproxen	ND	diethylstilbesterol	ND
sulfamethoxazole	[b]	dihydrotestosterone	[c]
triclocarban	**Used**	epitestosterone	ND
triclosan	**Used**	equilenin	ND
Giudice and Young, 2011		equilin	DNQ
bisphenol a	ND	estriol	ND
ethynylestradiol	ND	**estrone**	**Used**
nonylphenol	ND	ethinylestradiol	ND
octylphenol	NM	mestranol	ND
triclocarban	**Used**	norethindrone	ND
triclosan	**Used**	**progesterone**	**Used**
		testosterone	ND

DNQ = did not quantify
Used = used for calibration in the present study
ND = not detected in biosolids and/or runoff, or not detected in runoff above concentrations measured during control (no biosolids) storms
NM = not measured in biosolids
[a] – Caffeine is ionic at normal pH. Very few literature K_{OC} values could be found, and the ones that were varied by several orders of magnitude, prohibiting the ability to accurately and objectively calibrate.
[b] – Sorption of sulfamethoxazole is known not to be governed by a linear isotherm to organic carbon.
[c] – No literature values of K_{OC} or half-life could be found.

Table 2-2. Input Hydrologic, Erosion, and General Chemical and Biosolids Parameters for the Three Scenarios Used for Calibrating the Model.

Parameter	Description	Units	Sabourin et al., 2009 [5]	Giudice and Young, 2011 [3]	Yang et al., 2012 [6]	Source [a]
Hydro						
HBDATE	Beginning year and julian day of simulation		2006219	2009081	2008113	-
DAREA	Total drainage area of field	ha	0.0002	0.0002	0.0006	Measured
RC	Effective saturated conductivity of soil below root zone	cm/hr	0.13	1.3	0.13	Estimated
BST	Fraction of plant-available water at start of simulation		0	0	0	Assumed [b]
CONA	Soil evaporation parameter	$m/d^{0.5}$	4.5	3.5	3.3	Lookup
CN2	SCS curve number for mosture condition II		75	86	75	Lookup
CHS	Hydraulic slope of the field	m/m	0.07	0.06555	0.0524	Measured
RD	Effective rooting depth	cm	90	33	90	Estimated
Erosion						
NPTSO	Number of points for overland flow profile slope		1	1	1	-
DAOVR	Drainage area represented by overland flow profile	ha	0.0002	0.0002	0.0006	Measured
XOVC()	Distance from top of profile to slope point	m	1	1	1.5	Measured
SLOV()	Slope of profile at XOV()	m/m	0.07	0.065	0.0524	Measured
XSOIL()	Rel. horizontal distance from top slope to bot. segment		1	1	1	-
KSOIL()	Soil erodibility factor for slope segment above XSOIL		0.4563	0.322	0.198	Lookup
XFACT()	Rel. horizontal distance from top slope to bot. segment		1	1	1	-
CFACT()	Soil loss ratio for overland flow profile segment		0.06	1	0.2	Lookup
PFACT()	Contouring factor for overland flow profile segment		0.5	0.5	0.5	Lookup
NFACT()	Mannings "n" for overland flow profile segment		0.04	0.01	0.1	Lookup
Pesticides						
PDATE	Year in rot. cycle and application date		1220	1087	1119	-
IPST	Number of pesticides (i.e., chemicals) applied on PDATE		5	2	3	-
APRATE	Rate of application of chemical	kg/ha	Not Used [c]	Not Used [c]	Not Used [c]	-
DEPINC	Depth of incorporation	cm	15	11.4	15	Measured
Biosolids						
RATEB	Biosolids application rate	kg/ha	8000	22500	3500	Measured
DENSB	Density of biosolids	kg/m^3	1000	1000	1000	Assumed
OCBA	Organic carbon in applied biosolids	%	29	29	29	Assumed [d]
OCBFA	Fast degrading organic carbon in applied biosolids	%	20	20 (19)[e]	20	Assumed [d]
OCBSA	Slow degrading organic carbon in applied biosolids	%	7	7 (5)[e]	7	Assumed [d]
HLOCF	Half-life of fast degrading organic carbon	days	5	5 (30)[e]	5	Assumed [d]
HLOCS	Half-life of slow degrading organic carbon	days	230	230	230	Assumed [d]

[a] – Measured: values reported in cited reference; estimated: values selected based on scientific judgment and for which runoff concentrations were insensitive; lookup: values from lookup tables in [28] based on soil texture or other measured parameters.

[b] – Soil moisture was assumed to be at the wilting point (i.e., BST = 0) to begin each simulation. On short time scales such as used for calibration, runoff and therefore mass losses are sensitive to this parameter, but concentrations (on which calibration was based) are not.

[c] – For biosolids simulations, the application rate of chemicals is based on RATEB and CONCB instead of APRATE.

[d] – Based on typical values found in [33].

[e] – Values in parentheses are final calibrated values.

Table 2-3. Input Soil Parameters for the Three Scenarios Used for Calibrating the Model.

			Sabourin et al., 2009 [5]			Giudice and Young, 2011 [3]			Yang et al., 2012 [6]		
Parameter	**Description**	**Units**	*Layer 1*	*Layer 2*	*Layer 3*	*Layer 1*	*Layer 2*	*Layer 3*	*Layer 1*	*Layer 2*	*Layer 3*
BOTHOR()	Depth to bottom of each soil horizon	cm	15	30	90	11.4	26.6	33	15	30	90
POR()	Porosity of each soil horizon [a]	cc/cc	0.43	0.47	0.47	0.4	0.4	0.4	0.4	0.4	0.4
FC()	Field capacity of each soil horizon [a]	cm/cm	0.32	0.36	0.36	0.22	0.22	0.11	0.19	0.19	0.19
BR15()	Wilting point of each soil horizon [a]	cm/cm	0.12	0.2	0.2	0.08	0.08	0.03	0.05	0.05	0.05
SATK()	Saturated conductivity in each soil horizon [a]	cm/hr	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57
OM()	Organic matter content of each soil horizon [b]	%	4	3	1	0.64	0.64	0.01	0.3	0.3	0.3
CLAY()	Percent of soil mass in each soil horizon that is clay [b]	%	15	15	15	14	14	0	5	5	5
SILT()	Percent of soil mass in each soil horizon that is silt [b]	%	67	67	67	26	26	0	10	10	10

[a] – Values from lookup tables in [28] based on soil texture or other measured parameters.
[b] – Values reported in cited reference.

Table 2-4. Input Chemical Parameters for the Three Scenarios Used for Calibrating the Model.

Parameter	Description	Units	Sabourin et al., 2009 [5]					Giudice and Young, 2011 [3]		Yang et al., 2012 [6]		
NOPEST	Pesticide (i.e., chemical) ID number	-	1	2	3	4	5	1	2	1	2	3
PSTNAM	Pesticide (i.e., chemical) name [a]	-	ACT	CBZ	IBU	TCC	TCS	TCC	TCS	AD	E1	PR
H2OSOL	Water solubility [b]	mg/L	14000	17.7	21	0.0237	10	0.0237	10	57.8	1.3	8.81
KOC	OC normalized partition coeff. in soil [c]	L/kg	263 (75)	180	66 (338)	71687	7946 (3000)	71687	7946 (5000)	5248	1585	1995
SOLLIF	Soil half-life [d]	days	50	495	32 (50)	1000	187	1000	187	2	2.7 (20)	0.21
CONCB	Conc. of chemical in applied biosolids [e]	mg/kg	0.0286	0.0067	0.657	8.194	7.066	17.6	15.1	0.0217	0.07871	0.01498
KOCB	OC normalized partition coeff. in biosolids [f]	L/kg	263 (75)	180	66 (338)	71687	7946 (3000)	71687	7946 (5000)	5248	1585	1995
HFLIFB	Biosolids half-life [f]	days	50	495	32 (50)	1000	187	1000	187	2	2.7 (20)	0.21

Values in parentheses are final calibrated values.

[a] – ACT = acetaminophen, CBZ = carbamazepine, IBU = ibuprofen, TCC = triclocarban, TCS = triclosan, AD = androstenedione, E1 = estrone, PR = progesterone.

[b] – Water solubility of ACT, CBZ, IBU, TCC, TCS as cited in [5]; AD, E1, PR as cited in [6].

[c] – OC normalized partition coefficient in soil of ACT, IBU taken from [34]; CBZ taken from [35]; TCC, TCS taken from [30]; AD, E1 taken from [36]; PR value was assumed based on K_{OC}s for other hormones and K_{OW} of progesterone relative to K_{OW}s for other hormones.

[d] – Soil half-life of ACT was assumed value (no reliable reference could be found); CBZ, TCC, TCS taken from [37]; IBU taken from [38]; AD value was assumed based on half-lives for other hormones with similar structure (no reliable reference could be found); E1 taken from [39]; PR taken from [40].

[e] – Values reported in cited reference.

[f] – Taken from the values for these parameters in soil for purposes of calibration.

Table 2-5. Parameters Used for Calibrating the Model.

	All Storms	**Latter Storms (i.e., not 1st Storm)**
Chemical Specific	K_{oc} B_s, B_b	Chemical Half-life
Chemical Generic	Biosolids OC % B_s, B_b Porosity	Biosolids OC Half-lives

4.2 Application

Several theoretical scenarios were modeled to examine the effects of K_{OC} and half-life, to examine the effect of application date for an annual application scenario in the arid west, and to examine differences in chemical loss under surface spreading vs. incorporation application methods on long-term chemical losses. Twelve generic chemicals, each with a different combination of half-life (10, 100, and 1000 days) and Log K_{OC} (2, 3, 4, and 5) were simulated. Their names in this report are denoted by HL[*half-life, in days*]LKOC[*Log KOC*], where [*half-life*] and [*Log KOC*] are the generic chemical's half-life and Log K_{OC}. All scenarios used the soil and erosion parameters that were used in the Giudice and Young, 2011 calibration model runs (Tables 2-2 and 2-3). Precipitation data was obtained from the California Irrigation Management Information System database (CIMIS, [41]) for the 25-year period January 1, 1987 through December 31, 2011. Annual biosolids application was either on January 2, May 1, or September 1. All chemicals were at equivalent concentrations of 10 mg/kg in the applied biosolids and were assumed to have essentially unlimited solubility (1000 mg/L). Solubility is an input to the model only insofar as it will limit aqueous concentrations to the solubility should they have exceeded it otherwise.

Biosolids were applied at a rate of 10,000 kg/ha, and either incorporated to a depth of 11.4 cm, or surface-applied.

5 Results and Discussion

5.1 Calibration

As mentioned above, the first phase of calibration was to develop relationships for the parameter B_s that would result in generally acceptable agreement with runoff concentration data for as many chemicals as possible. It is known that transfer of chemical from the soil/soil-pore water in the upper layer of soil to overland flow is dependent on chemical properties such as K_d and molecular diffusivity, and rainfall/runoff characteristics such as rainfall intensity and bottom shear stress, both of which affect turbulent mixing [42-44]. However, the original version of GLEAMS employs a functional simplification of this process. In the present study, the same approach was taken, but a new relationship was developed for cases in which $K_{ds} > 3.0$ in order to obtain acceptable agreement with the experimental results. **Figure 2-1** shows the equations used to estimate B_s based on the value of K_{ds} in the calibrated model. For the two instances in which $1.0 \leq K_{ds} \leq 3.0$, the relationship from the original GLEAMS was maintained. For the instances in which $K_{ds} > 3.0$, a power law relationship was developed that resulted in acceptable agreement with experimental results. Thus, the value of B_s in the model is calculated as follows:

$$B_s = \begin{cases} 0.5, & K_{ds} \leq 1.0 \\ 0.7 - 0.2 * K_{ds}, & 1.0 < K_{ds} \leq 3.0 \\ 0.0914 * {K_{ds}}^{-0.274}, & K_{ds} > 3.0 \end{cases} \quad (49)$$

The value of B_b was always set equal to the entire mass of biosolids per unit volume of overland flow (i.e., all of the biosolids phase is available to supplying chemical to the overland flow). Further work is necessary to determine whether this assumption always provides acceptable results, but in the present study, no lesser value of B_b provided better agreement with experimental values.

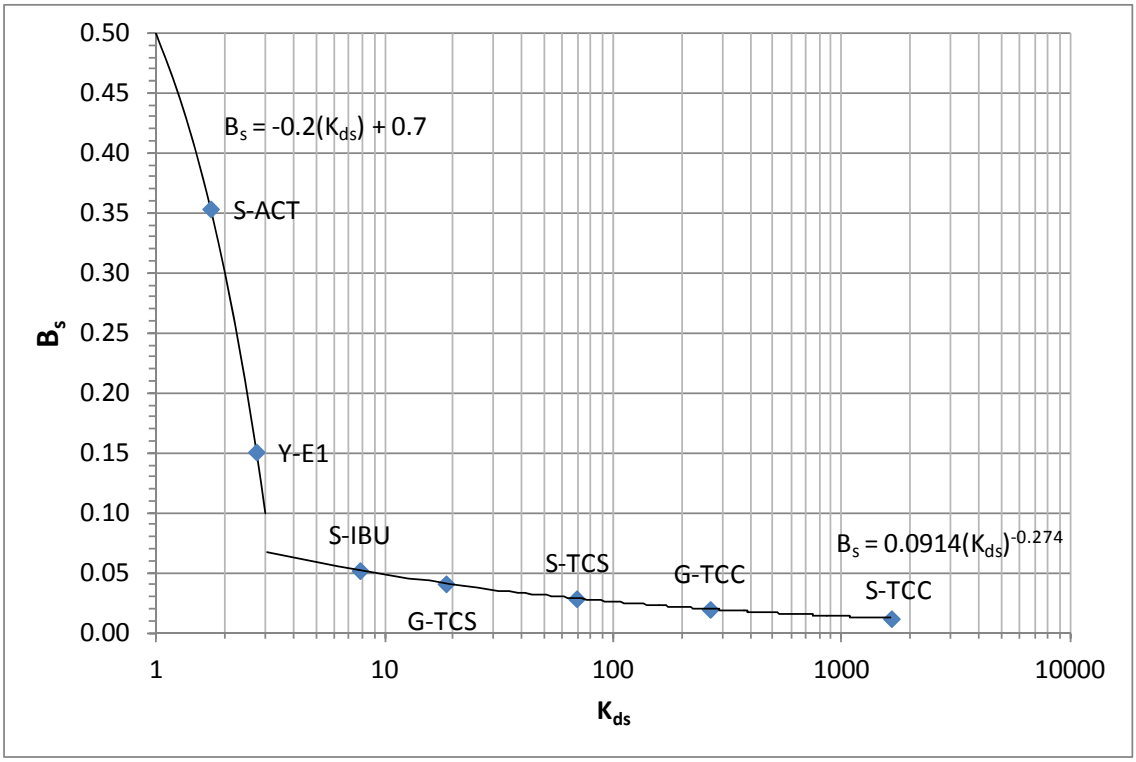

Figure 2-1. Relationship developed between B_s and K_{ds}. Data labels denote the calibration scenario (S=Sabourin et al., 2009; G=Giudice and Young, 2011; Y=Yang et al., 2012) and the chemical (abbreviations shown in footnote to Table 2-4).

Results of the calibration for all scenarios are shown in **Figures 2-2 and 2-3**. After calibration, seven of the ten chemicals showed acceptable agreement with experimental runoff concentrations (Figure 2-2), and the remaining three did not (Figure 2-3).

Results of the initial simulation for the Sabourin et al., 2009 [5] experiments showed generally acceptable agreement with experimental results for triclocarban. Adjusting the ibuprofen half-life from 32 days to 50 days and the K_{OC} from 66 to 338

(as in [45]) improved agreement for ibuprofen. Adjusting the acetaminophen K_{OC} from 263 to 75 improved agreement for acetaminophen. The improved agreement due to the change in K_{OC} for acetaminophen was due to 2 factors: the direct impact on K_{ds} and K_{db} of a lower K_{OC} in equations 5 and 6, and thus on concentrations in equations 26-28; and the impact of a decrease in K_{ds} to less than 3.0 in the surface layer, which increased B_s substantially. For triclosan, the K_{OC} in soil and biosolids had to be adjusted down from its initial estimate of 7946 to 3000, and the half-life from 187 to 15 days, to obtain acceptable agreement. For carbamazepine, results of the initial simulation were far below experimental values, and no reasonable adjustments to model parameters (including B_s) resulted in acceptable agreement.

The primary reason for the discrepancy for carbamazepine is related to the mass of carbamazepine available to runoff in the model. In [5], it was reported that over the course of the 5 rainfall simulations, approximately 20% of the carbamazepine applied was lost via runoff. Since biosolids were incorporated to a depth of 15 cm, and the surface runoff zone of the model is only the top 1 cm of the soil/biosolids, a maximum of 6.7% of the applied mass of carbamazepine is available for runoff in the model. Therefore, it would be impossible for the model to approach the concentrations in [5] without either increasing the depth of the computational layer of overland flow or increasing the initial mass of carbamazepine in the applied biosolids. Carbamazepine may also experience colloid facilitated transport [46] that limits its retardation in a field setting, resulting in higher concentrations than the model would predict.

Results of the initial simulation for the Giudice and Young, 2011 experiments [3] showed generally acceptable agreement with experimental runoff concentrations for

triclocarban and triclosan. In order to achieve better agreement, the triclosan K_{OC} in soil and biosolids was lowered from 7946 to 5000. Additionally, fractions of fast degrading and slow degrading organic carbon in the biosolids were adjusted, and the half-life of fast-degrading organic carbon increased, and these changes improved agreement for both triclocarban and triclosan. A reduction in the literature derived K_{OC} for triclosan in both the Giudice and Young, 2011, and the Sabourin et al., 2009 scenarios is justified, since soil and runoff pH in the former was 8.0 to 8.1 (approximately the pK_a of triclosan) and biosolids pH in the latter was 8.0, while literature K_{OC}s have been derived under more acidic conditions. Wu et al. found that the K_d of the anionic form of triclosan was 0.5 to 0.66 the K_d of the neutral form in soils amended with biosolids, making the overall K_d at pH 8 between 0.7 and 0.8 the K_d at pH 5 or 6, for which the K_{OC} for triclosan was derived [30]. Concentrations of both triclocarban and triclosan were < 0.1 ng/L in the percolate for all storm events. Triclosan was not detected in any of the experimental events, and triclocarban was 2.7 to 5.2 ng/L. As mentioned above, percolate concentrations were not able to be calibrated for the modified model.

Results of the base simulation for the Yang et al., 2012 experiments [6] showed acceptable agreement between modeled results and experimental results for estrone, but modeled concentrations for androstenedione and progesterone were far below measured values. Increasing the half-life for estrone from 2.7 to 20 days improved agreement for the latter storms. No reasonable adjustments of model parameters (including B_s) resulted in acceptable agreement for androstenedione and progesterone. There are several potential reasons for the lack of agreement. First, hormone concentrations in the

soil and biosolids may change rapidly on a day-to-day basis as hormones are conjugated or deconjugated, or due to interconversion of hormones [6]. For example, studies have shown increases in androstenedione and progesterone in manure and manure-soil mixtures as they aged [47, 48]. Colloid facilitated transport and leaching via preferential subsurface pathways have also been shown to be major transport pathways for hormones [49, 50]. Taken together, it is clear that the assumptions contained within the modified GLEAMS model described herein make simulation of the fate and transport of some hormones inappropriate, and more sophisticated approaches are necessary for these compounds.

Although model results were only moderately sensitive to soil porosity (and then only for very mobile chemicals) in the original GLEAMS [27], the developments described above make porosity a more sensitive parameter. This is because, in addition to the direct dependence on porosity in equations 10, 11, and 19, the mass of soil used in equations 10 and 11 is dependent on the bulk density, which depends on the soil porosity. In short, porosity now directly affects the ratio of the mass of three phases to each other, as opposed to only two previously. Because changing the porosity affected concentrations of all chemicals simultaneously, and some chemicals had acceptable agreement initially, this parameter was not adjusted for calibration purposes.

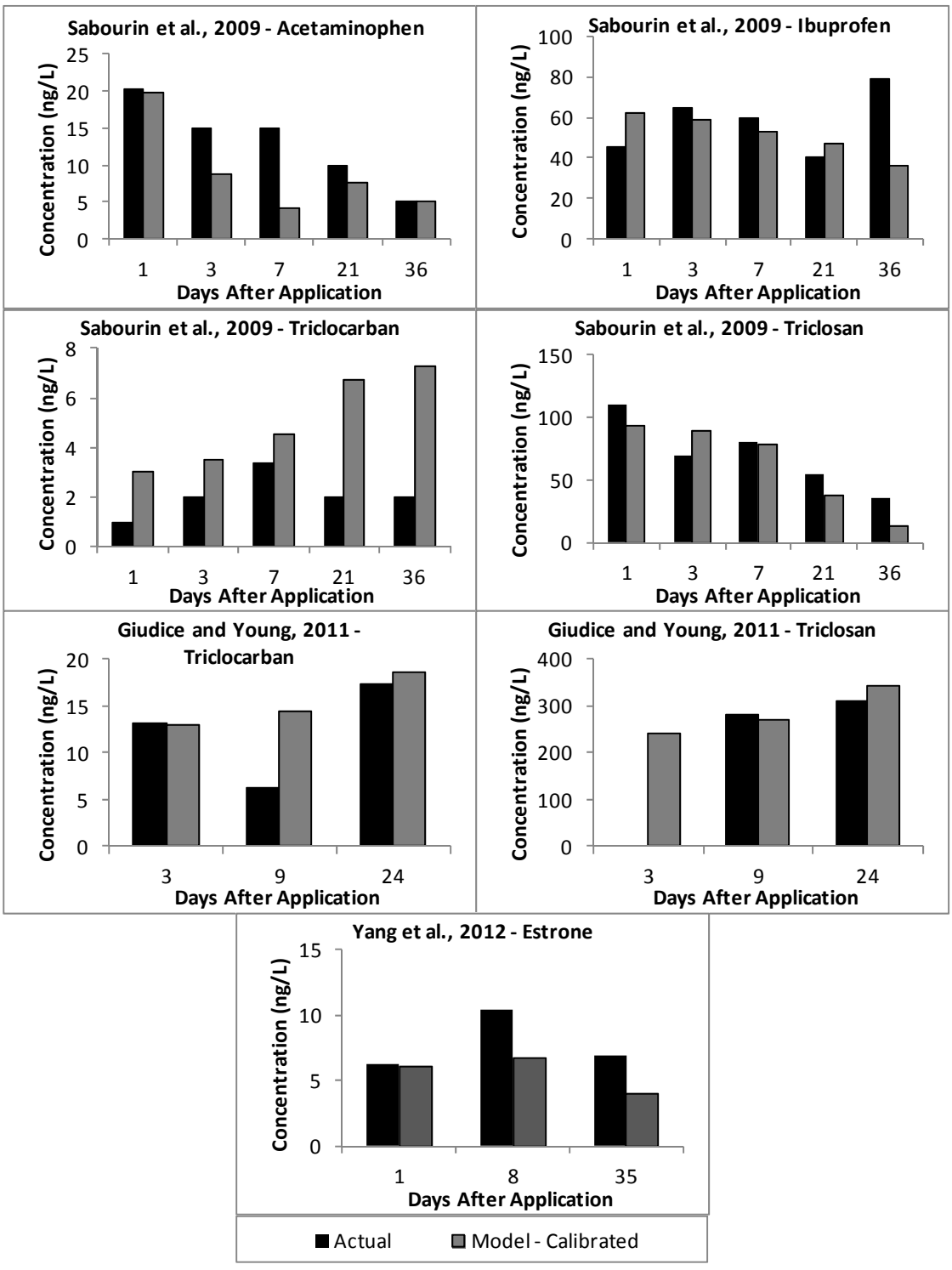

Figure 2-2. Results of experiments and calibrated simulations for chemicals for which acceptable agreement was obtained: Sabourin et al., 2009 (acetaminophen, ibuprofen, triclocarban, and triclosan) Giudice and Young, 2011 (triclocarban and triclosan), and Yang et al., 2012 (estrone).

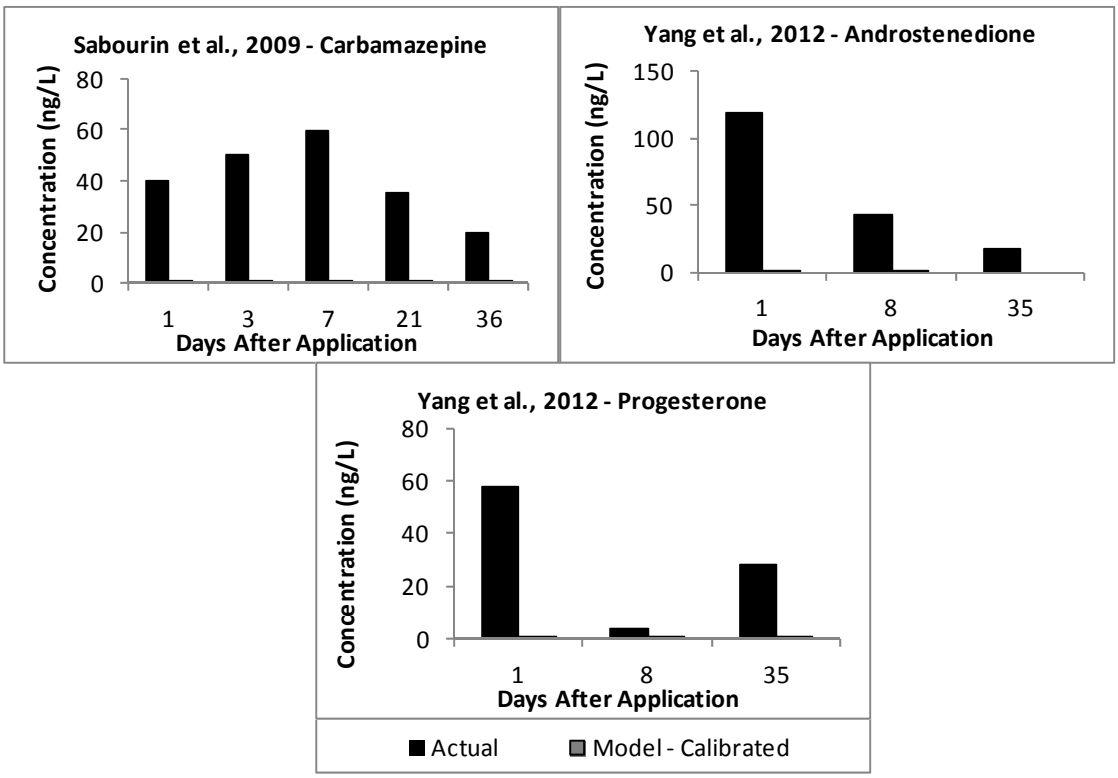

Figure 2-3. Results of experiments and calibrated simulations for chemicals for which no acceptable agreement could be obtained: Sabourin et al., 2009 (carbamazepine), and Yang et al., 2012 (androstenedione, progesterone).

5.2 Application

Total annual chemical loss in runoff is plotted as a function of the recurrence interval for biosolids applied annually on January 2 in **Figure 2-4**. As expected, the chemical with the highest K_{OC} and shortest half-life (HL10LKOC5) exhibited the smallest losses, while the chemical with the lowest K_{OC} and longest half-life (HL1000LKOC2) exhibited the greatest losses. For a given recurrence interval (or probability of occurrence), the maximum annual chemical loss was 4 to 5 orders of magnitude greater than the minimum loss. For each factor of 10 K_{OC} decrease (e.g., Log K_{OC} 5 to Log K_{OC} 4) and half-life increase (e.g., 10 days to 100 days), total annual chemical loss at a given recurrence interval increased by approximately a factor of 10. For the chemical with the highest losses, HL1000LKOC2, over the course of the

simulation, in many years the total amount of chemical lost was on the same order of the amount applied (Figure 2-4).

Figure 2-5 shows the relative contributions of sorbed and aqueous phases in runoff and of percolated rainfall of the 12 generic chemicals to their average annual mass loss. The runoff aqueous phase makes up the majority of the mass loss for chemicals with Log $K_{OC} < 4$. For chemicals with Log $K_{OC} = 4$, loss via runoff is in almost equal parts aqueous and sorbed phases. For chemicals with Log $K_{OC} > 4$, the sorbed phase in runoff makes up the majority of the loss. Loss via percolate is correlated with loss in the aqueous phase of runoff, though is always less, and is essentially zero for chemicals with Log $K_{OC} > 4$. It should be noted that the modifications to the model were not calibrated for the sorbed phase. The mass of triclosan and triclocarban lost via the sorbed phase was investigated in [5], and was found to be generally 10-20% of the mass lost in the aqueous phase, though it was only slightly less than lost in the aqueous phase for TCC at 36 days post application. Likewise, the mass of hormones lost via the sorbed phase was investigated in [6]. Approximately 30-40% of the total mass of estrone lost was via the sorbed phase, 10-30% of the mass of androstenedione, and less than 5% of the progesterone. Concentrations associated with the sorbed phase were not reported in either work. Because total mass loss via the sorbed phase is dependent on the total mass of solids lost, which also was not reported in sufficient detail to be of use, there is limited ability to use the results for meaningful calibration. Further work is necessary to examine the impact of biosolids application on erosion and particle characteristics in runoff.

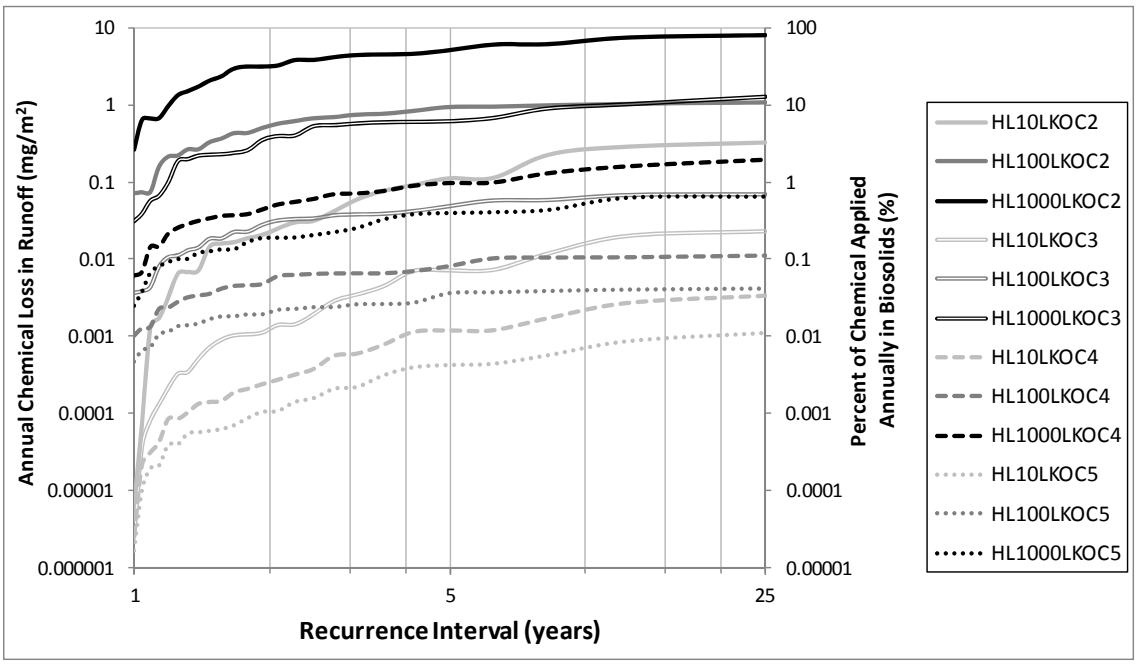

Figure 2-4. Annual mass loss of generic chemicals in runoff (aqueous plus sorbed) vs. recurrence interval given annual application of biosolids on January 2.

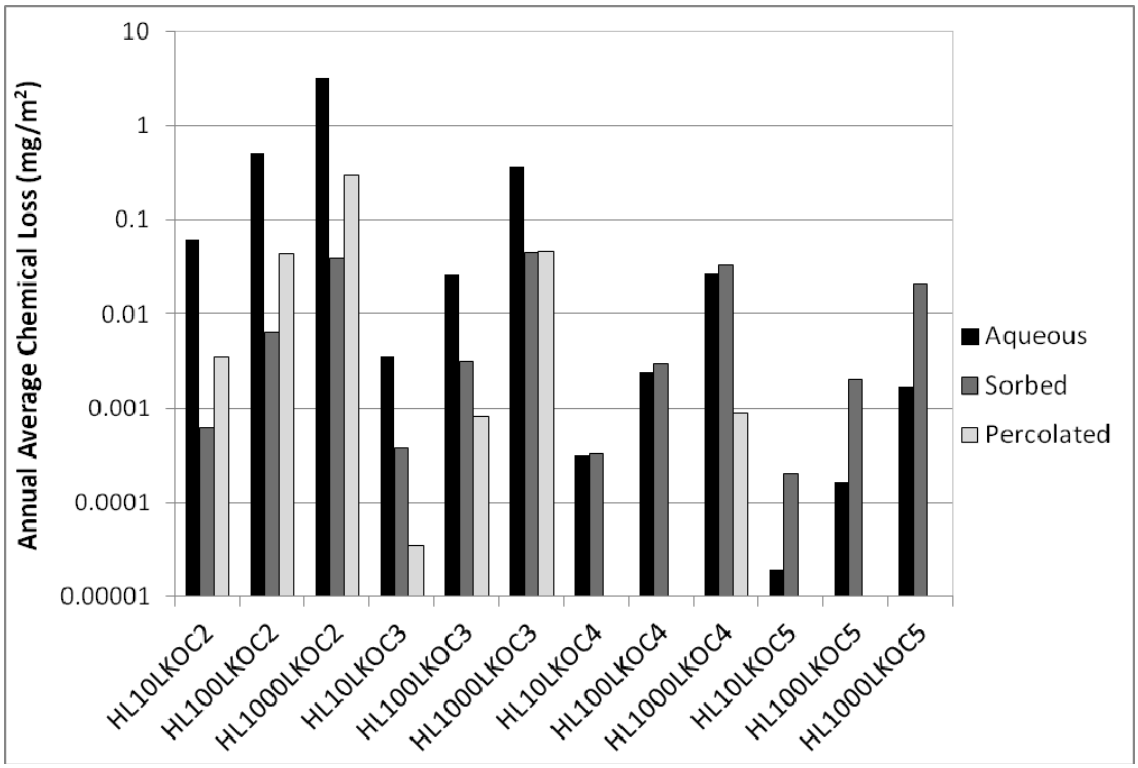

Figure 2-5. Average annual mass loss of generic chemicals for aqueous and sorbed phases in runoff and in percolate given annual application of biosolids on January 2. Annual application rate of each chemical was 10 mg/m^2

The effect of the annual application day is shown in **Figure 2-6**. For chemicals with short half-lives (i.e., half-lives of 10 and 100 days; bottom two panels of Figure 2-6), annual application on May 1 results in the least chemical mobilized. This is because virtually all of the precipitation at this site occurs between November and April. Therefore, after annual application on May 1, in almost all years there is ample time for chemicals to degrade prior to any significant (i.e., runoff inducing) rainfall. However, for chemicals with long half-lives, on the order of 1 year or longer (i.e., the 1000 day chemicals; top panel of Figure 2-6), and relatively high K_{OC}, annual application on January 2 results in the least chemical mobilized. This is because for these chemicals, organic carbon added to the plots as part of the biosolids matrix degrades much more rapidly than the chemicals themselves. The chemicals accumulate in the soil over a period of years, and the primary driver for temporal variation in mobility is not chemical concentration, but soil/biosolids organic carbon content. The highest organic carbon content in the biosolids phase occurs in the weeks and months immediately following application, and for a January 2 application date, this coincides with the majority of the rainfall.

Figure 2-6 also shows the effect of application type. In all cases, surface spreading results in greater, and many times considerably greater, median annual losses in runoff than when incorporated. This is because in the case of surface spreading, all of the mass of chemical that is applied is done so into the top 1 cm of soil/biosolids, which is the layer that is available to surface runoff. In this case, since incorporation is to a depth of 11.4 cm, incorporation results in only about 9% of the chemical applied into the top layer (i.e., 1 cm/11.4 cm).

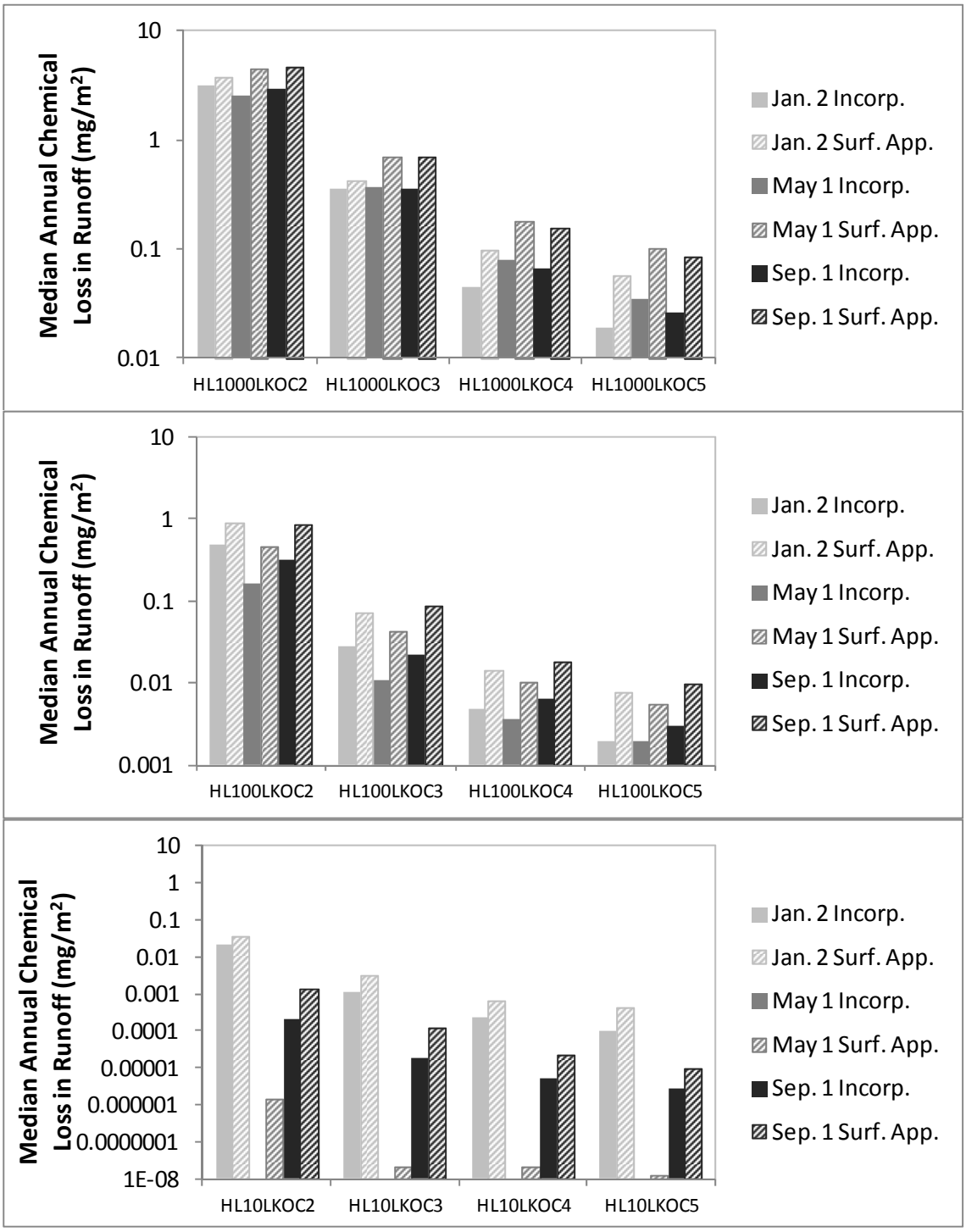

Figure 2-6. Median annual mass loss of generic chemicals in runoff (aqueous plus sorbed) given annual biosolids applications of January 2, May 1, and September 1 for surface application and incorporation into the top 11.4 cm of soil.

6 Summary and Conclusions

The model presented herein simulated the fate and transport in runoff of trace organic chemicals in land-applied biosolids. Seven out of ten chemical-scenarios for which experimental data was available in the literature (across three independent studies) showed good agreement between model predictions and experimental concentrations. Based on the chemicals for which the model was successful and unsuccessful, the model is useful for predicting the concentration in runoff of target chemicals for which concentrations are unlikely to increase due to degradation/interconversion of parent/related chemicals, and whose transport in soil and biosolids can be modeled via linear partitioning to organic matter. Increasing concentrations of chemicals after biosolids application (for example, transformation of one hormone to another), and colloid-facilitated transport are beyond the scope of this model, but could be integrated in the future once the mechanisms are well-understood. Applications of the model show the utility in helping to identify management practices that result in lesser impacts to water quality.

Acknowledgment

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Notation

A=area to which biosolids are applied (cross-sectional area of unit volume) (m^2)
AWS=soil water storage capacity to saturation (initial abstraction – amount of rainfall before runoff or infiltration begins) (depth in cm or volume in L)
$B_{av(mix)}$=soil-biosolids mixture mass available to supplying chemical to the overland flow per unit volume of overland flow (extraction coefficient; kg)
B_b=biosolids mass available to supplying chemical to the overland flow per unit volume of overland flow (kg)
B_s= soil mass available to supplying chemical to the overland flow per unit volume of overland flow (kg)
$C_{av(mix)}$=runoff available concentration in surface layer of soil-biosolids mixture (mg/kg)
C_b=concentration in biosolids (mg/kg)
$C_{b(0)}$=concentration in biosolids on day of biosolids application (mg/kg)
$C_{b(t)}$=concentration in biosolids at time t after biosolids application (mg/kg)
C_{bi}= concentration of chemical in biosolids in subsurface layer i (mg/kg)
C_s=concentration in soil (mg/kg)
$C_{s(0)}$=concentration in soil on day of biosolids application (mg/kg)
$C_{s(t)}$=concentration in soil at time t after biosolids application (mg/kg)
C_{si}= concentration of chemical in soil in subsurface layer i (mg/kg)
C_w=concentration in water (mg/L)
C_{wi}= concentration of chemical in water in subsurface layer i (mg/L)
d_i=depth of incorporation of biosolids in soil (m)
E=enrichment ratio
f=water flux (L/h or cm/h)
$fOC_{b,f}(0)$= fraction of organic carbon that is fast-degrading at time 0
$fOC_{b,f}(t)$=fraction of organic carbon that is fast-degrading at time t
$fOC_{b,s}(0)$= fraction of organic carbon that is slow-degrading at time 0
$fOC_{b,s}(t)$= fraction of organic carbon that is slow-degrading at time t
$fOC_{b,r}(0)$= fraction of organic carbon that is recalcitrant at time 0
$fOC_{b,r}(t)$= fraction of organic carbon that is recalcitrant at time t
$k_{b,f}$=rate constant for degradation of fast-degrading organic carbon (d^{-1})
$k_{b,s}$=rate constant for degradation of slow-degrading organic carbon (d^{-1})
K_{db}=biosolids-water distribution coefficient (L/kg)
K_{ds}=soil-water distribution coefficient (L/kg)
K_{OCb}= organic carbon normalized biosolids-water distribution coefficient (L/kg)
K_{OCs}=organic carbon normalized soil-water distribution coefficient (L/kg)
M_b=mass of biosolids in unit volume of soil-biosolids mixture (kg)
M_{bi}= mass of biosolids in subsurface layer i (kg)
M_s=mass of soil in unit volume of soil-biosolids mixture (kg)
M_{si}= mass of soil in subsurface layer i (kg)
$MOC_b(0)$= mass of organic carbon in biosolids at time 0 (kg)
$MOC_b(t)$=mass of organic carbon in biosolids at time t (kg)
$MOC_{b,f}(0)$= mass of fast-degrading organic carbon in biosolids at time 0 (kg)
$MOC_{b,f}(t)$= mass of fast-degrading organic carbon in biosolids at time t (kg)
$MOC_{b,s}(0)$= mass of slow-degrading organic carbon in biosolids at time 0 (kg)

$MOC_{b,s}(t)$= mass of slow-degrading organic carbon in biosolids at time t (kg)
$MOC_{b,r}(0)$= mass of recalcitrant organic carbon in biosolids at time 0 (kg)
$MOC_{b,r}(t)$= mass of recalcitrant organic carbon in biosolids at time t (kg)
OC_s=% organic carbon in soil
OC_b=% organic carbon in biosolids
P=rainfall depth (depth in cm or volume in L)
$PERC_i$= volume of water percolated from subsurface layer i (L)
$PERCM_1$=mass of chemical lost via infiltration from the surface layer (mg)
$PERCM_i$= mass of chemical in percolate from subsurface layer i (mg)
PMS_i= mass of chemical in subsurface layer i (mg)
POR=porosity of soil-biosolids mixture
Q=surface runoff (depth in cm or volume in L)
QM=mass of aqueous chemical lost via runoff from the surface layer (mg)
r=application rate of biosolids (kg/m^2)
S=sediment loss (kg)
$S_{1/2s}$=half-life in soil phase (d)
$S_{1/2b}$=half-life in biosolids phase (d)
SM=mass of chemical sorbed to sediment lost via runoff from the surface layer (mg)
t=time (d)
T=time (storm duration) (h)
V=volume of water per unit volume of runoff interface
V_{fb}=volume of biosolids per unit volume of soil-biosolids mixture (L)
V_{fs}=volume of soil per unit volume of soil-biosolids mixture (L)
V_{fw}=volume of water per unit volume of saturated soil-biosolids mixture (L)
V_{wi}= volume of water in layer i (L)
z=concentration of chemical in surface layer (mg)
z_0=concentration of chemical in surface layer at the beginning of the storm (mg)
ρ_b=density of biosolids (kg/L)
ρ_s=density of soil (kg/L)

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Chapter 3

The Antimicrobial Triclocarban Stimulates Embryo Production in the Freshwater Mudsnail Potamopyrgus antipodarum

Abstract

Recent research has indicated that the antimicrobial chemical triclocarban (TCC) represents a new type of endocrine disruptor, amplifying the transcriptional activity of steroid hormones and their receptors while itself exhibiting little affinity for these receptors. The effects of TCC were studied in the freshwater mudsnail *Potamopyrgus antipodarum*. Specimens were exposed to concentrations ranging from 0.05 to 10.5 μg/L dissolved TCC and were removed and dissected, and embryos contained within the brood pouch were counted and classified as shelled or unshelled after 2 and 4 weeks of exposure. After 4 weeks, environmentally relevant TCC concentrations of 1.6 to 10.5 μg/L resulted in statistically significant increases in the number of unshelled embryos, while 0.2, 1.6, and 10.5 μg/L exposures significantly increased numbers of shelled embryos. The lowest observed effect concentration (LOEC) was 0.2 μg/L, the no observed effect concentration (NOEC) was 0.05 μg/L, and the 10% effective concentration (EC10) and median effective concentration (EC50) for unshelled effects were 0.5 μg/L and 2.5 μg/L, respectively. Given the widespread occurrence of TCC in the environment and effects shown at environmentally relevant concentrations, these results indicate that TCC may be causing reproductive effects in the environment. Furthermore, the present study indicates that environmental risk from a new class of endocrine disrupting chemicals (EDCs) is both qualitatively and quantitatively similar to risk from existing classes of EDCs.

1 Introduction

Many synthetic organic chemicals have been classified as endocrine disrupting chemicals (EDCs) due to their ability to interact with and alter endocrine systems and cause adverse health effects in organisms or their offspring. In humans, there is evidence and concern that these chemicals may be contributing to various types of cancer [1, 2], abnormal timing of the onset of puberty [3], and fetal abnormalities [4, 5]. In wildlife, effects range from feminization, hermaphroditism, and intersexuality [6, 7] to impacts on fertility and fecundity [8, 9] to behavioral effects [10], and in some cases, complete collapse of populations has been documented [11, 12].

There is a large body of literature concerning EDCs with estrogenic or androgenic potential [13]. Many of these studies address the question of whether a single chemical alone acts as an endocrine disruptor, generally as an agonist or antagonist to one of the steroid hormone receptors but also through non-receptor mediated modes of action. New research suggests that the chemical triclocarban (TCC; **Figure 3-1**), long suspected to interfere with reproduction in rats and rabbits [14], exhibits a novel form of endocrine disruption. Triclocarban alone exhibits little or no activity towards steroid hormone receptors but amplifies transcriptional activity of steroid sex hormones in the estrogen and androgen receptors, both in human cell lines [15]. In vivo, when added to a diet containing a high amount of testosterone, it significantly increased male sex organ weight relative to control diets, or those diets containing testosterone or TCC alone in castrated rats [16].

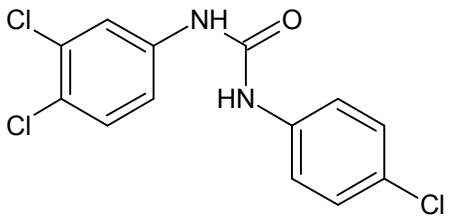

Figure 3-1. Chemical structure of triclocarban (TCC).

Triclocarban was introduced to commerce in the United States in 1957, and has been routinely added to cosmetics and personal care products since then. Annual production in the United States is estimated at between 500,000 and 1,000,000 lbs (225,000 to 450,000 kgs) per year [17]. Triclocarban is incompletely removed in wastewater treatment plants. Most partitions into sludge, but some is also discharged in effluent accompanying steroid hormones and other EDCs [18, 19], causing a potential risk to aquatic organisms downstream. It is estimated to be detectable in 60% of US streams with mean and median concentrations of 213 and 109 ng/L, respectively [18].

Given its characteristics as a new kind of endocrine disruptor and its widespread occurrence in the aquatic environment, there is a need to determine if TCC poses a demonstrated risk to aquatic species. The test species for these experiments is the freshwater mudsnail *Potamopyrgus antipodarum* (Gastropoda, Prosobranchia, Hydrobiidae), commonly called the New Zealand Mudsnail, which has previously been used in a whole organism bioassay for estrogenic and androgenic endocrine disrupting effects [20]. Native to New Zealand, it was introduced to Europe in the mid 1800s [21] and North America in the late 1980s [22]. Unlike its native range where males are present and it reproduces sexually, in its invasive range, the species is almost exclusively female and is ovoviviparous and parthenogenetic [22].

The objectives of the present study are to determine: (1) whether environmentally relevant concentrations of TCC impact reproductive output in *P. antipodarum*, and if so (2) whether TCC causes effects that are distinguishable from the effects of traditional estrogen receptor agonists in vivo. The hypothesis is that TCC will increase reproductive output in a dose-dependent manner. The reasoning behind this is that TCC acts by enhancing the transcriptional activity of endogenous estrogens present in the female, and this should lead to increased numbers of embryos within the brood pouch, as has been found in experiments with other exogenous estrogenic EDCs [23].

2 Methods and materials

2.1 Chemicals

Sea salt was obtained from Aquarium Pharmaceuticals (Chalfont, PA). Calcium carbonate, sodium bicarbonate, reagent alcohol, acetonitrile, methanol, ethyl acetate, acetone, and acetic acid were all obtained from Fisher Scientific (Pittsburgh, PA) and were the highest grade available. Triclocarban (3,4,4'-Trichlorocarbanilide, 99% purity) was obtained from Aldrich (St. Louis, MO). Deuterated triclocarban (TCC-d7) and carbon-13 labeled triclocarban ($^{13}C_6$-TCC) were obtained from Cambridge Isotope laboratories (Andover, MA).

2.2 Experimental methods

Specimens of the freshwater mudsnail *Potamopyrgus antipodarum* were collected from Putah Creek near Winters, CA on October 17, 2008 under the supervision of California Department of Fish and Game staff. Aquatic vegetation was collected in D-nets and snails were removed and kept in river water in 1 gallon buckets for transfer to the laboratory. Within 2 h, snails were transferred into 10 gallon aquaria filled with

artificial freshwater [Milli-Q water (Millipore, Billerica, MA) plus added salts at a rate of 5 g $CaCO_3$, 5 g Sea Salt, and 0.5 g $NaHCO_3$ per 10 gallons of water].

After 3 to 5 d-acclimation to laboratory conditions, 60 individuals with shell lengths greater than 3.0 mm were transferred to each individual 1-L jar filled with 800 ml of artificial freshwater. The jars were aerated through glass pipettes to keep dissolved oxygen near saturation.

Each jar was spiked with a solution of TCC dissolved in reagent alcohol to achieve five nominal target aqueous TCC concentrations (0.045, 0.14, 0.45, 1.4, 4.5, and 14.0 ug/L, in triplicate). Reagent alcohol always represented less than 0.003% of the final volume of solution. The control blank triplicates were also spiked with an equivalent volume of pure reagent alcohol. A pilot study was conducted that contained both water only and solvent controls, and results showed no significant differences in number of embryos between the treatments, suggesting that ethanol had no effect on embryo numbers. Therefore, no water only control was used during the duration of this experiment. The pilot study also indicated that large amounts of TCC (i.e. >10% of the total mass in the jar) were partitioned into the snail biomass within 5 d. In order to keep aqueous TCC concentrations relatively constant, water was replaced and re-spiked at the initial concentration level in each jar every 3 d.

The experiments were conducted at 14 ± 0.7°C under a light:dark rhythm of 16:8 h. Snails were fed ground TetraMin (Tetra, Melle, Germany) every day or every other day at an approximate rate of 0.1 mg per day. At t=0, 2, and 4 weeks, 15 specimens were removed from each jar and narcotized for 1 h in 2.5% $MgCl_2$ solution. Photographs of the specimens were taken and the length of the shell measured using image processing

software. Shells were cracked in a vice and dissection took place under a dissecting microscope. To measure reproductive output, embryos were counted, making a distinction between those with shells (i.e. older, more developed embryos) and those without (i.e. newer embryos). The identical procedure was performed at the same time points on 15 individuals that had been kept in 10 L aquaria, and was repeated for snails kept in aquaria at 6 and 8 weeks as well. Mortality in the treatments was recorded every 3 d-and dead snails were removed.

All data were analyzed using JMP 8.0 (SAS institute, Cary, NC). Using absolute embryo numbers, means and standard errors were calculated, followed by one way analysis of variance (ANOVA) (n=3) and comparison of treatment means to the control using Dunnett's method (α=0.05). All ANOVA assumptions were verified through standard tests of residuals. Using percentage responses relative to the control, non-linear regressions were calculated using a three-parameter logistic model and were used to calculate an 10% effective concentration (EC10) and median effective concentration (EC50) for each, where EC10 and EC50 are the concentrations causing a 10 and 50% increase in embryo numbers relative to the solvent control, respectively. All effects are referenced to time-weighted mean concentrations as determined analytically rather than nominal concentrations.

2.3 Water chemistry

At day 1, 4, 10, 16, and 25, water samples were taken from one randomly chosen replicate of each of the exposure concentrations immediately before and shortly after the water renewal and re-spiking procedure. These samples were measured for dissolved oxygen and pH, then analyzed for dissolved TCC concentration. The pH was

measured using a Mettler Toledo (Columbus, OH) pH meter. Dissolved oxygen was measured using a YSI (Yellow Springs, OH) dissolved oxygen meter. At the same intervals, additional samples were taken to measure nitrite/nitrate and ammonia using Aquarium Pharmaceuticals (Chalfont, PA) test kits.

For the determination of dissolved TCC, samples were first acidified to pH 2 using hydrochloric acid. Deuterated surrogate (TCC-d7) was spiked into the samples, followed by solid phase extraction. The extraction was carried out on Waters (Milford, MA) OASIS HLB 6cc disposable cartridges on a Supelco (St. Louis, MO) Visiprep DL manifold. Each cartridge was conditioned with 5 ml 75:25 ethyl acetate/acetone mixture followed by 5 ml methanol and then 5 ml Milli-Q water. Samples of 10 ml were loaded at a rate of between 1 and 2 ml/min and then dried for 10 min at 40 mmHg. Cartridges were eluted with 8 ml of 75:25 ethyl acetate/acetone. Eluates were then evaporated to dryness under a gentle stream of nitrogen at 65°C. Finally, extracts were redissolved in 200 µl or 1 ml of 90:10 acetonitrile/Milli-Q water containing $^{13}C_6$-TCC as an internal standard.

Extracts were analyzed via liquid chromatography with tandem mass spectrometry. Injection volume was 10 µl, and separation was achieved on a Phenomenex (Torrance, CA) Prodigy ODS 100A 100 x 2.0 mm column at 40°C. The binary mobile phase consisted of 0.5 ml/min of A: 90:10 Milli-Q:acetonitrile with 2 mM acetic acid, and B: 50:50 methanol:acetonitrile with 10 mM acetic acid. The gradient was as follows: 20% B rising to 80% B over 16.5 min, then rising to 100% B over 2.5 min, followed by 1 min at 100% B. Detection was achieved using an Agilent (Santa Clara, CA) 1100 series LC/MSD ion trap with electrospray ionization in negative mode and multiple reaction

monitoring. The drying gas flow rate was 12 l/min, the drying gas temperature was 350°C, and the nebulizer pressure was 35 psi. All other instrument parameters were optimized for the detection of TCC.

The response was corrected by recovery of the surrogate TCC-d7 and normalized to the response of the internal standard $^{13}C_6$-TCC. Calibration was via seven external standards which were analyzed before and after every set of samples and the linear regression fit to the averages of each pair of responses. A summary of the chemicals analyzed is shown in **Table 3-1**. All data were analyzed using Bruker Daltonik DataAnalysis v.2.1 software (Bremen, Germany).

Table 3-1. Selected Analytical Parameters

Chemical	Triclocarban (TCC)	Deuterated Triclocarban (TCC-d7)	C13 labeled Triclocarban ($^{13}C_6$-TCC)
Purpose	Analyte	Surrogate	Internal Standard
Molecular Weight	315.6	322.6	321.5
Precursor ion	313	320	319
Fragment	160	163	160
Instrument Detection Limit	0.35 µg/L	0.10 µg/L	n/a
Instrument Quantitation Limit	0.80 µg/L	0.25 µg/L	n/a
Limit of Detection	10 ng/L	2.9 ng/L	n/a
Limit of Quantitation	23 ng/L	7.0 ng/L	n/a

3 Results and Discussion

Dissolved oxygen was always above 95% saturation and pH was 7.9 ± 0.4. Nitrite, nitrate, and total ammonia were always below the detection limits of the tests, which were 0.1 mg/L, 5 mg/L, and 0.5 mg/L, respectively. Dissolved TCC concentrations decreased by 5 to 50% over the course of each 3 to 9 d period between analyses. The rate of disappearance decreased over each interval. Preliminary experiments indicated

that significant amounts of TCC partitioned into snail biomass within days, so the decreasing rate of TCC disappearance is likely because the applied concentrations were approaching equilibrium with the TCC that had partitioned into the biomass of the snails. Even so, time weighted mean concentrations in general showed good agreement with nominal concentrations; the concentrations (in μg/L) were determined to be 0.05 (0.04 nominal), 0.22 (0.14), 0.47 (0.45), 1.6 (1.4), 4.1 (4.5), and 10.5 (14.0). The highest measured concentration (10.5) was probably lower than the nominal value (14.0) because of insolubility of TCC in the alcohol stock or the water. Halden and Paull predict a solubility of TCC in water of 0.65-1.55 mg/L at 25°C [18]; however, the actual solubility is probably much lower based on experience preparing aqueous TCC solutions. In addition, this experiment was conducted at 14°C where the solubility would be expected to be lower than literature values referenced to 25°C. Calculated recoveries for the analytical procedure averaged 72% with a standard deviation of 18%.

After 2 weeks exposure, the number of embryos showed no significant differences from the controls. After 4 weeks, significant increases were found for numbers of unshelled, shelled, and total embryos, as shown in **Figure 3-2**. Exposures of 1.6, 4.1, and 10.5 μg/L exhibited significantly elevated numbers of unshelled embryos, reaching 217% of the control numbers. Exposures of 0.2, 1.6, and 10.5 μg/L resulted in significantly more shelled embryos, up to 167% of the control numbers. Total embryos were significantly greater than controls in snails exposed to 0.2, 1.6, 4.1, and 10.5 μg/L, up to 184% of the controls. The LOEC was therefore 0.2 μg/L, and the NOEC was 0.05 μg/L. The EC10 and EC50 for unshelled effects were 0.5 and 2.5 μg/L, respectively (r^2=0.59). The regressions did not show good fit with the unusual dose-response curves

for shelled and total embryo numbers, and therefore an EC10 and EC50 were not calculated. The most logical reason why numbers of unshelled embryos showed the greatest increase is because by 4 weeks, most if not all unshelled embryos have likely been formed since the start of the exposure, and therefore best represent effects of exposure conditions.

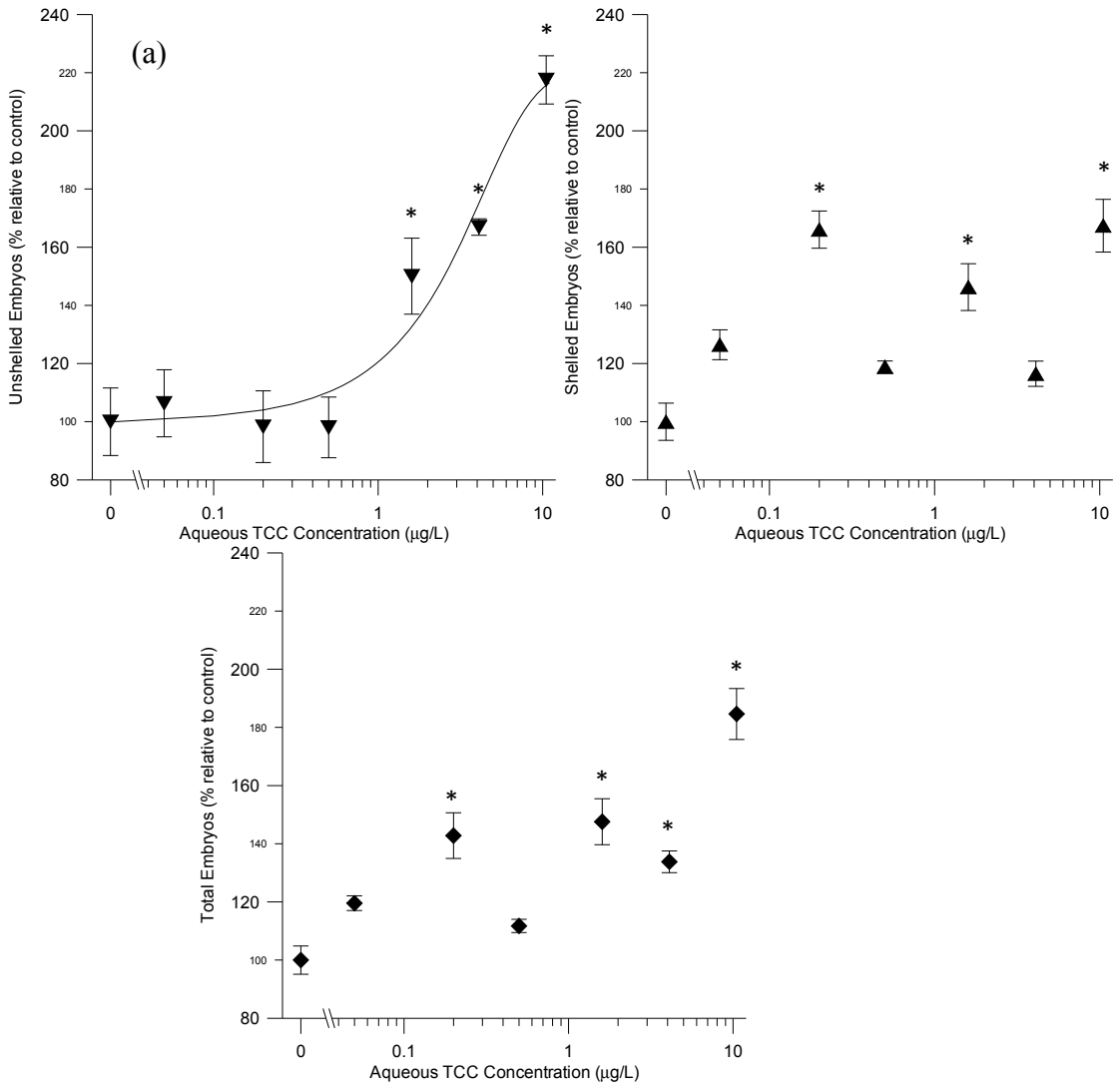

Figure 3-2. Effects of dissolved Triclocarban (TCC) exposures on embryo numbers of *Potamopyrgus antipodarum* at 4 weeks exposure in percentage of the solvent control (mean ± standard error of the mean, n = 15) for (a) unshelled embryos [▼], (b) shelled embryos [▲], and (c) total embryos [◆]. Logistic regression line for unshelled embryos is shown. *Significantly greater than solvent controls at p<0.05.

No effects on shell length were detected. Shell length was determined not to be a cofactor. If the exposures had been extended to 6 or 8 weeks, it would be possible that effects on embryo numbers would have been seen at even lower exposures, following the trend that was seen in [23]. Exposures were limited to 4 weeks for the sake of expedience and because preliminary experiments indicated that 4 weeks was sufficient to detect the effects. Mortality was less than 10% in all exposures except one jar of 0.5

µg/L and one jar of 4.1 µg/L, for which mortality reached 20% and 17% by the end of the 4 weeks, respectively. These jars had visible fungal growth on their bottoms at between 3 and 4 weeks, likely due to overfeeding, and this most likely led to higher mortality.

Embryo numbers in all treatments, including the controls, decreased substantially during the course of the experiment (see **Figure 3-3**). Levels found in snails housed in the aquarium declined in a similar fashion, but at a slower rate. The results of a prior pilot experiment that took place several months earlier indicate a similar decline, suggesting that transferring the organisms to laboratory conditions caused their reproduction to slow down during the course of the experiment. Anecdotal evidence from other labs suggests this to be a common effect of bringing wild-caught mudsnails into the lab.

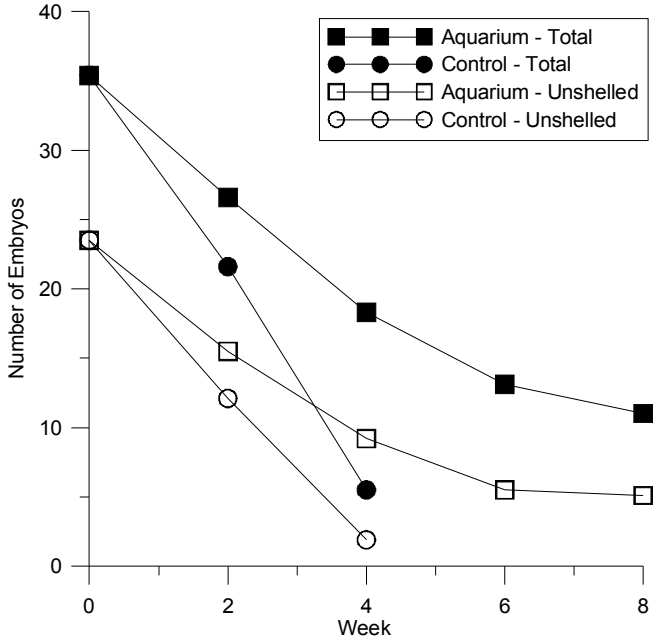

Figure 3-3. Embryo numbers in the control and in an aquarium declined substantially over the duration of the experiment. The decline in the aquarium was slower and not as pronounced, and leveled off at 8 weeks.

The specific ecological impact of the effects seen in this experiment is not clear, but it is likely that if the same effects are occurring in the environment, populations would be impacted. As Duft points out, increases in embryo production during seasonal minima in the reproductive cycle means more juveniles entering the environment at times when the environment is unfavorable for survival. Furthermore, limitations in the overall energy budget may then contribute to lower fecundity and lower survival rates in the seasonal maxima [23]. Multi-month exposures to examine effects of TCC on survival and multi-generational exposures to examine effects of populations in microcosms would enhance the understanding of the expected effects in the environment.

While many studies have examined acute and chronic effects of TCC on aquatic organisms, few have found effects at such low levels. The NOEC for chronic toxicity to Daphnia magna has been reported at 0.5 to 1.0 μg/L. The most sensitive endpoint for TCC on aquatic organisms found in the literature is a NOEC of 0.101 μg/L for decreased numbers of young in Americamysis bahia, a saltwater crustacean. The most sensitive study results for molluscs found in the literature were reduced viability (to 20% of control) in clam larvae at 10 μg/L TCC, and decreased larval length as low as 5 μg/L [24], 50 times higher than the LOEC of 0.2 μg/L found in the present study. While TCC concentrations downstream of wastewater treatment plants have only rarely been found to be above 5 μg/L, levels above 0.2 μg/L are quite common. It is estimated that 30 to 40% of samples described in the literature [18, 25] are above the LOEC for the present study.

Others have found similar effects as the present study on embryo production in *P. antipodarum* when exposed to the known environmental estrogens bisphenol A (BPA), octylphenol (OP), nonylphenol (NP), and ethynylestradiol (EE2) in both sediment and water [20, 23]. In water, the NOECs have been determined to be 1 μg/L for BPA and OP and 5 μg/L for NP [20]. The mechanism of action (MOA) of TCC on *P. antipodarum* is not known, but it is possible that it acts similarly to experiments done with the mammalian estrogen receptor in vitro [16]—that is, amplifying the binding affinity and consequently increasing the transcriptional activity of naturally present estrogen to the estrogen receptor. However, some evidence indicates that estrogenic compounds act via a different route than binding to the vertebrate-like estrogen receptor in mollusks [26]. In fact, it is not clear at this point what the precise MOA of estrogen analogues are in mollusks. While the possibility exists that TCC shares a common MOA in mollusks with already identified estrogenic EDCs, molecular evidence from vertebrate studies suggest that the MOAs differ. If this is true, this experiment shows that chemicals with different mechanisms of action produce nearly identical results in vivo. It also highlights the need for both in vitro and in vivo studies, especially for chemical-by-chemical screening programs. In vivo studies may not distinguish between different mechanisms of EDC, while in vitro studies based on the current single chemical testing paradigm (e.g., Tier 1 of the U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program) may miss potentially hazardous EDCs.

The present study represents a first step in characterizing risk to aquatic organisms of a new class of EDC. By showing that TCC, a chemical that exhibits little to no affinity for the estrogen receptor alone, causes reproductive effects that match those

caused by known estrogen receptor agonists, the present study indicates that a chemical not currently addressed by the current paradigm in EDC screening methodologies can exhibit equally problematic environmental risk.

Acknowledgement

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Appendix A

Supporting Information for Chapter 2

Table A-1. Input Climate Parameters for the Three Scenarios Used for Calibrating the Model.

Parameter	Description	Units	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
			Sabourin et al., 2009 [a]											
TEMPX	Mean monthly maximum temperature in each month	deg C	-1.88	-0.22	4.96	13.13	20.02	25.39	27.78	26.73	22.62	16.26	8.01	0.94
TEMPN	Mean monthly minimum temperature in each month	deg C	-10	-9.42	-4.93	1.23	6.57	12.06	14.62	13.9	10.17	4.68	-0.57	-6.49
RAD	Mean monthly solar radiation for each month	MJ/sq cm	5.1	8.41	12.68	14.43	20.04	22.59	22.51	19.46	14.39	10.67	5.61	4.44
WIND	Mean monthly wind movement for each month	km/d	714.5	712.9	708.1	693.6	643.7	590.6	548.8	555.2	592.2	624.4	684	682.3
DEWPT	Mean monthly dew point temperature for each month	deg C	-8.41	-8.22	-4.72	1.17	6.88	12.42	14.78	14.78	11.06	5.78	-0.17	-5.92
			Giudice and Young, 2011 [b]											
TEMPX	Mean monthly maximum temperature in each month	deg C	13.00	17.00	19.00	23.00	28.00	32.00	34.00	34.00	32.00	26.00	18.00	13.00
TEMPN	Mean monthly minimum temperature in each month	deg C	5.00	7.00	8.00	10.00	12.00	14.00	16.00	16.00	15.00	12.00	8.00	4.00
RAD	Mean monthly solar radiation for each month	MJ/sq cm	7.41	11.25	15.61	22.43	25.61	28.53	29.16	26.15	20.17	14.27	9.62	6.19
WIND	Mean monthly wind movement for each month	km/d	278.0	285.8	328.2	332.1	351.4	374.6	343.7	328.2	285.8	247.1	231.7	255.0
DEWPT	Mean monthly dew point temperature for each month	deg C	4.68	6.53	7.11	7.88	10.52	12.67	14.29	14.41	12.76	10.32	7.40	4.65
			Yang et al., 2012 [c]											
TEMPX	Mean monthly maximum temperature in each month	deg C	3.65	6.08	9.68	15.9	21.19	27.28	31.38	30.41	25.25	18.82	9.9	5.09
TEMPN	Mean monthly minimum temperature in each month	deg C	-10.34	-8.12	-5.22	0.2	5.83	11.11	14.74	13.79	8.48	2.21	-4.91	-8.85
RAD	Mean monthly solar radiation for each month	MJ/sq cm	8.41	11.25	16.4	18.91	19.75	22.34	22.43	19	17.24	12.97	13.1	7.53
WIND	Mean monthly wind movement for each month	km/d	867.4	885.1	992.9	951.1	893.2	822.4	815.9	782.1	835.2	807.9	914.1	907.6
DEWPT	Mean monthly dew point temperature for each month	deg C	-10.86	-8.41	-7.55	-3.11	3.03	7.51	10.09	9.37	4.18	-1.41	-6.61	-9.22

[a] – Experiments conducted near London, Ontario, Canada. Geographically closest US Station in GLEAMS climate database (Sandusky, MI) used.
[b] – Experiments conducted in Davis, CA. Geographically closest US Station in GLEAMS climate database (Sacramento, CA) used.
[c] – Experiments conducted near Keenesburg, CO. Geographically closest US Station in GLEAMS climate database (Akron, CO) used.

Input Precipitation for Sabourin et al., 2009, Used for Calibrating the Model.

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SABOURIN	15.7	.	15.7	.	.	0.15	15.7	.	.	.	1
SABOURIN	1.13	0.07	.	.	.	0.08	0.64	0.63	0.34	0.64	1
SABOURIN	15.7	.	.	.	0.85	0.06	.	.	1.78	0.06	1
SABOURIN	.	.	.	.	2.51	5.31	15.7	.	.	.	1
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Input Precipitation for Giudice and Young, 2011, Used for Calibrating the Model.

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Input Precipitation for Yang et al., 2012, Used for Calibrating the Model.

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YANG	.	.	.	4.73	.	0.03	.	.	.	6.07	1
YANG	1.22	0.76	.	.	.	.	0.56	3.00	0.03	0.30	1
YANG	.	.	0.86	0.08	0.13	.	.	.	.	.	1
YANG	.	.	.	.	.	0.43	0.48	.	.	.	1
YANG	.	.	0.41	4.48	0.18	0.61	.	.	0.03	.	1
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