BULK DEPOSITION OF PESTICIDE MIXTURES IN A CANADIAN PRAIRIE CITY AND THE INFLUENCE OF SOIL TEMPERATURE FLUCTUATIONS ON 17β-ESTRADIOL MINERALIZATION

by

Lindsey Amy Andronak

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MASTER OF SCIENCE

Department of Soil Science University of Manitoba Winnipeg, Manitoba

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ABSTRACT

Andronak, Lindsey Amy. M.Sc., The University of Manitoba, May, 2013. <u>Bulk</u> <u>deposition of pesticide mixtures in a Canadian Prairie city and the influence of soil</u> <u>temperature fluctuations on 17β-estradiol mineralization</u>. Major Professor: Annemieke Farenhorst.

Tests were conducted for 71 pesticides in weekly bulk (wet + dry) deposition samples collected from May 25 to September 21 over two years at two sites in the City of Winnipeg, Canada. Twenty-one pesticides and their metabolites were detected in this study and 99% of samples collected contained mixtures of two or more pesticides. Malathion and glyphosate were the largest contributors to bulk deposition in 2010 and 2011, respectively. A second study examined the mineralization of 2,4-D and 17βestradiol using a novel in-field soil microcosm study and a series of laboratory experiments under different temperature incubations. Results indicated that temperature fluctuations do not greatly affect the amount or rate of mineralization relative to the traditionally constant laboratory incubations of 20°C; however long-term freezing of soil reduced potential mineralization over time. This research advances scientific knowledge of agri-chemical fate and provides data for strengthening current environmental policy analysis in Canada.

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1. INTRODUCTION

1.1 Pesticide Use and Steroid Hormones

Pesticides are a wide group of substances used to repel or kill unwanted organisms. Globally, over 2.3 billion kilograms of pesticides are used each year (Grube et al., 2011). In Canada, annual agricultural pesticide use is approximately 35.4 million kilograms, of which 94% are herbicides, 4% are fungicides and 2% are insecticides (Cessna et al., 2010). Pesticides are also used in urban areas for the control of pests as part of lawn care for golf courses, schools and health centres, for clearances of right-of-ways and in other applications, and in protecting human health by reducing the potential transmission of diseases spread by insect vectors. Pesticides are believed to have many benefits to society including increased agricultural crop yields though lowering weed and insect populations and disease occurrence, increased human and livestock protection against diseases and reduced food spoilage (Cheng, 1990).

Pesticides applied either in agricultural or urban settings do not always reach their intended target and can move from their application area to the broader environment thereby contaminating the atmosphere, water and soil and potentially impacting nontarget organisms. In addition to the toxicity risks posed by a single pesticide, pesticides are often found as mixtures in the environment (Hoos et al., 2002; Waite et al., 2002; 2004; Donald et al., 2007; Messing et al., 2012). These pesticide mixtures have been

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shown to have additive or synergistic effects relative to a single chemical exposure (Hayes et al., 2006; Moser et al., 2006; Pape-Lindstrom and Lydy, 2009). In order to fully understand the risks that pesticides pose to organisms, the transport and transformation of pesticides in the environment must be understood. Transport and transformation processes are influenced by both the physio-chemical properties of the pesticide and the characteristics of the environmental compartment in which it is located (Cheng, 1990).

Steroid hormones are chemicals produced by an organism that have physiological effects on the development and growth of sex organs. Major groups of steroid hormones include corticoids, androgens, and estrogens and they are excreted by livestock and humans in urine and feces. Recent studies have demonstrated that models used to predict pesticide fate can also be used to model the fate of steroid hormones (Casey et al., 2003; Das et al., 2005).

1.2 Atmospheric Deposition of Pesticides

In urban environments, pesticide atmospheric deposition can be a source of non-point source pollution by contaminating gardens, sandboxes, swimming pools, and other urban features. Pesticides can enter the atmosphere through application drift, volatilization and wind eroded sediments (Coupe et al., 2000; Hill et al., 2003). Once in the atmosphere, it can be transformed into metabolites or transported and deposited by rain and dust particles onto land and surface waters. Several pesticides have the potential to be transported in the atmosphere across distances of over 2,000 km (Beyer et al., 2000), but the majority of atmospheric dispersal and deposition tends to be local or within the region

in which the pesticide is applied (Sanusi et al., 2000). Pesticide atmospheric deposition has been measured in rural areas (Goolsby et al., 1997; Hill et al., 2003; Yao et al., 2008; Messing et al., 2011) and urban environments (Nations and Hallberg, 1992; Waite et al., 1995, 2002; Hill et al., 2003). The number of pesticides included in these studies ranged from four (Rawn et al., 1999) to 83 (Yao et al., 2008) active ingredients (Table 1.1).

Nations and Hallberg (1992) conducted a comparative study between rural and urban sites and found that the pesticide detection frequency did not differ between sites; however, the greatest deposition concentrations occurred in the samples obtained from rural sites. 2,4-D, bromoxynil, dicamba, dichlorprop, MCPA and MCPP are among the most frequently detected pesticides in rain and atmospheric dust in the Canadian prairies These herbicides also tend to be detected in greater concentrations than most other pesticides examined (Waite et al., 1995, 2002 and 2005; Rawn et al., 1999; Hill et al., 2002, 2003).

There has been limited monitoring of pesticide deposition in urban areas, including in the Canadian Prairies where much of the agricultural land in Canada is situated in close proximity to urban centres. In Manitoba, the total amount of pesticides used in agriculture has remained constant over the past decades, but there have been significant changes to the types of pesticides applied. For example, between 1996 and 2006, glyphosate use increased from 16% to 45% of total herbicides used due to the adoption of glyphosate resistant crops (Wilson, 2012). There have been no studies in Manitoba that have monitored the atmospheric deposition of glyphosate, except for Messing et al. (2011) which monitored glyphosate bulk deposition in samplers installed in an agricultural field near Brandon, Manitoba.

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	Concer						
(ug/l or [ug/m2])							
Number of	Number of				Urban or	Type of	
pesticides detected	samples	Minimum	Maximum	Location	agricultural	Sampler	Reference
47	31	0.002	22.9	Mississippi	both	wet only	Coupe et al. 2000
12	5595	0.05	16	Midwestern US	both	wet only	Goolsby et al. 1997
19	508	0	30	Alberta	both	after	Hill et al. 2002
						rainfall	
4	166	unknown	26	Canadian Prairies	both	after	Hill et al. 2003
						rainfall	
83	7	0	[0.414]	Bratt's Lake, SK	agricultural	wet and dry	Yao et al. 2008
11	54	[0.6]	[97.8]	Saskatchewan	agricultural	bulk	Waite et al. 1995
2	45	0	[3.55]	Saskatchewan	agricultural	bulk	Waite et al. 2002
3	67	0	[2203]	Saskatchewan	agricultural	dry	Waite et al. 2002
5	45	0	[2350]	Saskatchewan	agricultural	bulk	Waite et al. 2004
10	6	0	0.15	Canadian Prairies	agricultural	wet and dry	Waite et al. 2005
12	36	0	[100]	Manitoba	agricultural	bulk	Messing et al. 2011
14	8	[0.009]	[2.3]	Manitoba	agricultural	wet and dry	Messing et al. 2012
14	325	0.1	40	Iowa	both	wet only	Nations and
							Hallberg 1992
4	unknown	0	0.44	Manitoba	agricultural	wet only	Rawn et al. 1999

Table 1.1 Published work measuring atmospheric deposition of pesticides in urban environments in North American and agricultural environments across the Canadian Prairies.

Another pesticide of particular interest is malathion which is an organophosphate insecticide. One of its formulated products is Malathion 95 ULV (ultra low volume application) which is applied in the City of Winnipeg in residential streets, rights-of-way, city parks, golf courses, and cemeteries for the control of adult mosquito populations. Malathion can also be used in agriculture to control insects such as alfalfa weevil larvae, armyworms and cereal leaf beetles in cereals and diamondback moths in canola. There have been no studies in Manitoba that have monitored the atmospheric deposition of malathion.

1.3 Pesticide and Steroid Hormone Fate in Soils

Agri-chemical contaminants, such as pesticides and steroid hormones, residing in surface soil can be transported over land surfaces and into surface waters when dissolved in water (e.g., snowmelt and rainfall runoff) or sorbed by soil particles (e.g., erosion). Contaminants near the soil surface can move to deeper depths in the soil through incorporation by tillage or through water infiltration with a continued downward movement resulting in groundwater contamination.

Transformation processes of contaminants may be physical, chemical or biological and generally involve degradation into metabolites. Contaminant mineralization into CO_2 and other inorganic compounds is a strictly biological transformation process that can only be achieved by microbial populations (Cheng, 1990). For most pesticide and steroid hormones, microbiological degradation including mineralization is a more predominant pathway of transformation than either physical or chemical processes (Munnecke et al.,

1982). The rate and amount of contaminant degradation by microorganisms is influenced by soil properties such as organic carbon content and environmental factors affecting soil moisture content and temperature (Walker, 1974; Ou, 1984; Bhanumurthy et al., 1989; Cattaneo et al., 1997; Soulas and Lagacherie, 2001). Soil is a dynamic environment and soil moisture content and temperature fluctuate spatially and temporally. For example, soil moisture content fluctuates with the timing of rainfall or irrigation events and soil temperature fluctuates diurnally and seasonally as a response to changes in solar radiation. Results from mineralization studies that are conducted at a constant temperature in the 10-30°C range (Bolan and Baskaran, 1996; Colucci et al., 2001; Picton and Farenhorst, 2004; Ying and Kookana, 2005; Lucas and Jones, 2006) may not be accurately reflecting in-field soil conditions. Temperature fluctuations, when compared with a constant environment, affect the abundance of microbial populations in soil which can subsequently impact pesticide mineralization rates and amounts (Howell et al., 1971; Biederbeck and Campbell, 1973). Veeh et al. (1996) found that maximum degradation amounts of 2,4-D increased with increasing soil temperatures and that the mineralization rate constant increased by a factor of two to four when the constant incubation temperatures were increased from 10 to 24°C. Very few studies have examined the impact of fluctuating temperatures on mineralization (Lavy et al., 1996).

Sorption is the measure of the retention between the chemical and soil particles. It can reduce the amount of pesticide movement through leaching and may affect pesticide transformation rates and amounts (Ogram et al., 1985; Greer and Sheldon, 1992). Sorption is affected by the physico-chemical properties of the chemical such as its water solubility and charge, as well as soil properties such as soil organic carbon content, pH and moisture content (Voos and Groffman, 1997; Bekbölet et al., 1999; Farenhorst et al., 2001; Picton and Farenhorst, 2004). Sorption and mineralization parameters are some of the most sensitive parameters in fate models (Boesten and van der Linden, 1991; Dubus et al., 2003; Dann et al., 2006). For example, Boesten and van der Linden (1991) found that changing the degradation rate of a pesticide by a factor of two changed the amount leached by a factor of ten. Hence, the lack of input data for sorption and mineralization parameters in contaminant fate models will lead to errors in the predictions of contaminant transport and persistence.

1.4 2,4-D (2,4-(dichlorophenoxy) acetic acid) and 17β-estradiol

In addition to the 71 pesticides included in the atmospheric pesticide deposition study described in Chapter 2, the research in Chapter 3 focuses on the sorption and mineralization of two agri-chemicals in soil: 2,4-D (2,4-(dichlorophenoxy) acetic acid and 17 β -estradiol (E2). 2,4-D (Figure 1.1) is a widely used herbicide for post-emergent control of broadleaf weeds in cereal and other production systems. 2,4-D is the fifth most widely used pesticide in Manitoba with over 148,800 kilograms applied annually to agricultural land (Wilson, 2012). It has a moderate vapour pressure 1.87×10^{-5} Pa and a relatively high water solubility, 900 mg/l at 25°C (McKeague, 1998). 2,4-D residues are soil-active to provide for the beneficial extended control of later flushes of weeds, but 2,4-D in soil has also shown to negatively affect beneficial soil organisms such as mycorrhizal fungi (Estok et al., 1989). 2,4-D residues can be transported from agricultural soils into the broader environment such as surface and groundwater by processes such as surface runoff (Nicholaichuk and Grover, 1983) and leaching (Waite et al., 1992). 2,4-D also volatilizes in air from land surfaces (Maybank et al., 1978) and

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contaminates surface water through dry and wet deposition (Messing et al., 2012). 2,4-D has been detected in surface water and groundwater at concentrations up to 2.04 μ g/L (Waite et al., 1992; Rawn et al., 1999; Donald et al., 2001; Messing et al., 2011; Glozier et al., 2012;). Mixtures of pesticides including 2,4-D have been found to alter phytoplankton communities in wetlands (Sura et al., 2012). 2,4-D was linked to increased risk of non-Hodgkin's lymphoma in humans in several studies (Hoar et al., 1986; Wigle et al., 1990; Zahm et al., 1990).



Figure 1.1 Molecular structure of 2,4-D

E2 is an endocrine disrupting chemical which interferes with metabolism and growth control of vertebrates (Lai et al., 2002) and has been additionally classified by the U.S. Department of Health and Human Services (2000) as a human carcinogen due to its link to breast cancer. All vertebrates, including livestock and humans of both sexes excrete E2. Dairy cattle and farrowing sows can excrete 113,000 ng/kg and 1,215 μ g/kg, respectively of E2 in their manure (Williams, 2002). Canada has over 13.5 million head of cattle and 12.8 million head of hogs (Statistics Canada, 2012) and the application of this livestock manure on agricultural land can lead to higher levels E2 in soil. Because E2 has a low vapour pressure (3x10⁻⁸ Pa, Hanselman et al., 2003) and a low water solubility (272.4 g/mol at 20°C, Ying and Kookana, 2005), it is more likely to remain in the soil to

undergo the processes of sorption and biodegradation rather than being readily transported. However, E2 has been shown to move offsite as it has been found in rivers, occasionally at concentrations exceeding 10 ng/L, the lowest observable effect level for fish (Routledge et al., 1998; Miles-Richardson et al. 1999). Temperature has been found to affect mineralization of E2 in soil. In a study conducted by Xuan et al. (2008), the rate constant increased from 0.141 to 0.75 to 0.894 day⁻¹ with an increase in temperature from 15 to 25 to 35° C.



Figure 1.2 Molecular structure of 17β-estradiol

1.5 Research Objectives

The overall objective of this thesis was to determine the atmospheric contribution of pesticides into an urban environment and examine the mineralization of selected chemicals in the soil. The first study, described in Chapter 2, quantifies the concentrations of 71 pesticides in bulk atmospheric deposition samples collected from May 25 to September 21 in 2010 and 2011 at two sites in the city of Winnipeg, Manitoba. In the second study, described in Chapter 3, an in-field microcosm study was designed to determine the mineralization parameters of E2 and 2,4-D in a range of agricultural soils

under field conditions. For E2, mineralization experiments were also conducted in a laboratory incubator using temperature fluctuations or at a constant temperature of 20°C. The results of these studies are important for understanding which pesticides are present in our environment and for understanding the potential for soil temperature to influence the persistence of these chemicals in soil.

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2. BULK DEPOSITION OF PESTICIDE MIXTURES IN A CANADIAN PRAIRIE CITY DURING YEARS WITH AND WITHOUT MALATHION APPLICATION FOR MOSQUITO CONTROL

2.1 Abstract

Tests were conducted for 71 pesticides in weekly bulk (wet + dry) deposition samples collected from May 25 to September 21 over two years at two sites in the City of Winnipeg, Canada. A key difference between the years was the total amount of rainfall during the sampling seasons which was 487 mm in 2010 and 182 mm in 2011. Total pesticide deposition for the sampling season was on average 386 μ g /m² in 2010 and 426 μ g/m² in 2011. In the wetter summer of 2010, when the insecticide malathion was frequently used by city officials to control adult mosquito populations, 55% of the total mass of pesticides deposited in the sampling season was malathion (on average 213 µg $/m^2$ per season). In contrast, malathion was not sprayed by city officials in the drier summer of 2011 and the contribution of malathion to the total mass of pesticides deposited was only 0.1% (on average 0.4 μ g/m² per season). Total weekly pesticide deposition was significantly correlated to weekly total rainfall; however deposition by particulate matter only was also observed, particularly for glyphosate. Twenty-one other pesticides and their metabolites were detected in this study and 99% of samples collected contained mixtures of two or more pesticides. None of the Canadian Council of Ministers of the Environment drinking water quality guidelines for individual pesticides

were exceeded in the samples and but irrigation water quality guidelines were exceeded for dicamba in 63% of samples and for MCPA in 46% of samples. This study demonstrates that pesticide atmospheric deposition can be a source of non-point source pollution in contaminating gardens, sandboxes, outdoor swimming pools, and other urban features. This is the first study to demonstrate the deposition of glyphosate in North American urban environments, and to quantify the impact of nuisance vector control of adult mosquitoes on malathion concentrations in urban rain and dust deposition.

2.2 Introduction

Sources of pesticides entering the atmosphere arise from urban and rural environments, for example as a result of pesticide application drift and post-application vapour losses from plant, land and water surfaces (Coupe et al., 2000; Hill et al., 2003). Pesticide atmospheric deposition has been quantified particularly in rural areas (Waite et al., 2005; Yao et al., 2008; Messing et al., 2011) but urban environments may demonstrate the same frequency of pesticide detections in rainfall (Nations and Hallberg, 1992).

The City of Winnipeg, the capital and largest city in the Province of Manitoba, Canada, is surrounded by crop land most notably the Manitoba's Red River Valley to the South where grain production dominates. More than 2.5 million kilograms of pesticide active ingredients are applied each year to agricultural land in Manitoba (Wilson, 2012). 2,4-D, bromoxynil, dicamba, dichlorprop and MCPA are herbicides that are frequently applied in grain production. In environmental samples collected in rural environments of Canada,

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these herbicides are detected in greater concentrations than other pesticides (Waite et al., 1995, 2002, 2005; Rawn et al., 1999; Hill et al., 2002, 2003; Messing, 2012).

Due to the adoption of glyphosate resistant canola in Canadian agriculture since the late 1990s, glyphosate now accounts for more than 45% of the total herbicides used in Manitoba (Wilson, 2012). Glyphosate detection is traditionally more difficult than most other herbicides, however the herbicide was frequently detected in a number of recent rural studies, including one in Manitoba (Quaghebeur et al., 2004; Humphries et al. 2005; Chang et al., 2011; Messing et al., 2011) for which it was detected in 50 to 92% of the bulk deposition samples. Glyphosate was detected in 0 to 10% of the bulk deposition samples in the city of Nantes, France and the city of Flanders, Belgium (Quaghebeur et al., 2004; Lamprea and Ruban, 2011) but tests for its presence have not been conducted in any North American cities.

In the City of Winnipeg, malathion has been used for decades for nuisance adult mosquito population control (Health Canada, 2003). City officials and private citizens apply a wide range of other pesticides on land devoted to residential areas, golf courses, parks and school grounds (Welch, 2012). Malathion is also registered for use in North American agriculture to control insects such as alfalfa weevil larvae, armyworms and cereal leaf beetles in cereals and diamondback moths in canola. Malathion has been detected in bulk deposition samples collected in urban and rural sites (Jegier, 1969; Majewski et al., 1999; Coupe et al., 2000; Grynkiewicz et al., 2003; Raina et al., 2010).

Very few cities in Canada report on the use of malathion, but the insecticide is also registered for use in a range of smaller and larger cities throughout Canada to control

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mosquitoes for reducing risks for the transmission of West Nile virus to humans. The West Nile virus was detected in Canada in birds in 2000 with the first recorded human case in 2002 (Norris, 2009). The maximum number of recorded human cases in Canada was 2,401 in 2007 with 98% of those cases occurring in Manitoba and its two neighbouring provinces to the west, Saskatchewan and Alberta (Public Health Agency of Canada, 2012). In 2012, there were 450 recorded human cases, with 96% of the cases in Ontario, Quebec and Manitoba (Public Health Agency of Canada, 2012).

The objective of this study was to determine the concentrations of 71 pesticides in weekly bulk deposition at two sites in Winnipeg, Manitoba during a summer season in a year with an above average use (2010) and no malathion use (2011) by City Officials.

2.3 Materials and Methods

2.3.1 Sampling Locations

The study sites are two locations in the southern part of Winnipeg with site Whyte Ridge (Site Ridge) (49° 48'N latitude, 97° 12'W longitude) being located approximately 7 km west of site St. Vital (Site Vital) (49° 47'N latitude, 97° 6'W longitude). Winnipeg has annual temperature extremes ranging from –45.0 to 40.6 °C. During the sampling season in 2010, temperatures ranged from 4.6 to 31°C with 487 mm of total rainfall and a maximum of 113.8 mm in one week. During 2011, temperatures ranged from -3.7 to 37.2°C with 182 mm of total rainfall with a maximum of 46.3 mm in one week. The thirty year normal for May to September (1971-2000) for Winnipeg is 354 mm

(Environment Canada, 2012), therefore one year (2010) was above normal and one year was below normal (2011).

Weekly bulk deposition samples were collected for 18 weeks from May 25 to September 21, 2010 and 2011 adjacent to a meteorological station (WatchDog 2700 Weather Station, Spectrum Technologies Inc., Plainfield, IL) which recorded precipitation and temperature. Bulk deposition samples were collected with a 1 m by 1 m galvanized sheet metal pan sampler that emptied into a 23 L glass carboy shaded by plywood to minimize pesticide photodegradation. If no rainfall occurred during the week, the pan was rinsed with 500 mL of deionized water and the sample was considered a dry deposit. If there was precipitation during the week, the pan was also rinsed and the rainwater collected in the carboy was mixed thoroughly and a maximum volume of 1 L subsample was taken and stored at 4°C in an amber glass bottle with a Teflon sealed cap. No rinsing with deionized water was done when samples were collected when it was raining; this occurred two (11%) and three (17%) times at Site Ridge, and one (6%) and two (12%) times at Site Vital, in 2010 and 2011, respectively.

2.3.2 Chemical Extraction and Quantification

Samples were analyzed for 71 chemicals (44 herbicides, 20 insecticides and 7 fungicides) (Table 2.1) at Alberta Innovates Technology Futures (AITF), Vegreville, Alberta, Canada. Samples were shipped from Manitoba to Alberta in an ice packed cooler on the same day they were collected and stored in the fridge for analyses within 7 days. Pesticides were extracted and quantified according to AITF methods for analysis of pesticides in water using a multi-residue method accredited under the Canadian Association of Laboratory Accreditation. Briefly, samples were then acidified with

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phosphoric acid to a pH of 2. Each sample was extracted by liquid-liquid partitioning with methylene chloride and the extract was dried through acidified sodium sulphate. The extract was reduced to approximately 300 μ l under nitrogen gas. Any acidic compounds were esterified with diazomethane. The final volume (250 μ l) was analyzed on a Varian 2200 GC/Iontrap mass spectrometer with a Varian 3400 autosampler and a DB-5 30m column. As part of QA/QC protocols, for every batch of 11 samples, a laboratory blank was included and a standard curve was run for calibration. Deuterated surrogates were added to water samples to determine percent recovery. All compounds were identified and quantified using retention times and compound specific ions of specific mass and charge. Ion ratios are compared to certified standards (instruments are calibrated using certified standards at 4 calibration levels). One ion is used for quantification as compared to standards. Compounds identified must meet retention time and ion ratio criteria to be qualitatively identified. Minimum detection limits varied from 0.005 to 0.2 μ g/L (Table 2.1).

2.3.3 Urban and rural pesticide use estimates

The most recent comprehensive agricultural pesticide use database (2004-2006) of Manitoba (Wilson, 2012) was used to calculate the mass of herbicides, insecticides and fungicides applied to crop land within a 13 kilometre radius around the perimeter of the City of Winnipeg. Acres seeded within a 13 kilometer radius for 2010 and 2011 were obtained from Manitoba Agricultural Services Corporation (2013). Eleven crops were grown for a total of 472,163 acres seeded in 2010 and 445,089 acres seeded in 2011. For the 11 crops grown, differences in area seeded between 2010 and 2011 ranged from 163 acres for soybeans and 18,599 acres for canola. Urban pesticide use data were obtained

	MDL		MDL						
Pesticide	$(\mu g/L)$	Pesticide	$(\mu g/L)$	Pesticide	MDL (µg/L)				
Herbicides									
2,4-D	0.005	Diuron	0.02	MCPB	0.02				
2,4-DB	0.005	Ethalfluralin	0.005	MCPP	0.005				
2,4-DP	0.005	Ethofumesate	0.005	Metolachlor	0.005				
Aminopyralid	0.01	Fenoxaprop-P- ethyl	0.005	Metribuzin	0.01				
Atrazine	0.005	Fluazifop	0.04	Napropamide	0.02				
Bentazon	0.01	Fluroxypyr	0.01	Picloram	0.005				
Bromacil	0.03	Gylphosate	0.2	Quinclorac	0.005				
Bromoxynil	0.005	Hexaconazole	0.05	Quizalofop	0.03				
Clodinofop- propargyl	0.04	Imazamethabenz- methyl	0.05	Simazine	0.01				
Clopyralid	0.02	Imazamox	0.005	Triallate	0.005				
Cyanazine	0.05	Imazethapyr	0.02	Triclopyr	0.01				
Dicamba	0.005	Linuron	0.02	Trifluralin	0.005				
Diclofop- methyl	0.005	MCPA	0.005						
2		Herbicide M	letabolites	5					
2,4-	0.01	Aminomethyl	0.5	Desethyl	0.05				
Dichlorophenol		Phosphonic Acid		atrazine					
4-Chloro-2-	0.01	Clodinofop acid	0.02	Desisopropyl	0.05				
methylphenol				atrazine					
		Insectio	cides						
α-BHC	0.005	Dimethoate	0.005	p,p- Methoxychlor	0.03				
α-Endosulfan	0.005	Disulfoton	0.2	Parathion	0.01				
Aldicarb	0.1	Ethion	0.1	Phorate	0.005				
Aldrin	0.005	γ-BHC	0.005	Pyridaben	0.02				
Chlorpyrifos	0.005	Guthion	0.2	Terbufos	0.03				
Diazinon	0.005	Malathion	0.05	Thiamethoxam	0.05				
Dieldrin	0.005	Methomyl	0.1						
Fungicides									
Carbathiin	0.1	Metalaxyl-M	0.01	Propaconazole	0.05				
Chlorothalonil	0.005	Oxycarboxin	0.05	Vinclozolin	0.01				
Iprodione	0.02								

 Table 2.1 Minimum detection limits (MDL) of pesticides analyzed.

from Welch (2012) and consisted of records of the mass of herbicides, insecticides and fungicides applied on 3 post-secondary institutions, 4 school divisions, 17 golf courses, as well as miscellaneous government land in 2011. Pesticides applied in these agricultural and urban settings were compared to the types, frequencies and concentrations of pesticides detected in the bulk deposition samples collected in Winnipeg in 2010 and 2011. In addition, the City of Winnipeg (2012) provided detailed records of the frequency of malathion applications in each of the 51 Insect Management Areas (IMAs) within city limits. Malathion is applied by city officials by ground ULV (ultra low volume) fogging trucks. Rates may range from of 2.60 – 6.08 μ g of active ingredient/m² (Pest Management Regulatory Agency, 2003). The frequency of fogging in residential areas is variable from year to year, for example there were 237 applications in 2010 but no applications in 2011. In other recent years, the total number of applications for all IMAs was 103 in 2009, 171 in 2008 and 222 in 2007. The City of Winnipeg (2012) also indicated that it used 6,970 litres of Malathion 95 ULV in 2010.

2.3.4 Statistical analyses

Statistical analyses were performed in SAS for Windows version 9.2 (SAS Institute Inc. 2002 - 2008). Pearson's correlation analysis was used to determine correlations between the mass of pesticides deposited and pesticide physiochemical properties, rainfall amounts and air temperature. Even though the distribution of the variables used for the analysis failed the Shapiro-Wilk test for normality (normal distribution W=0.90; our data W=0.81 for 2010, W= 0.84 for 2011), non-transformed data was still used due to the

robustness of the Pearson analysis (Legendre and Legendre, 1998). Attempts to transform the data always produced a lower Shapiro-Wilk value. A regression analysis was also performed to estimate the relationship between the masses of pesticide deposited per week at Site Ridge and Site Whyte.

2.4 Results

A total of nineteen active pesticide ingredients (parent compounds) were detected over the two year study period, consisting of fourteen herbicides, three insecticides and two fungicides. In addition, four of the six target metabolites were detected: AMPA (Aminomethyl Phosphonic Acid) (the major metabolite of glyphosate), 4-chloro-2methylphenol (metabolite of MCPA and MCPP), desethyl atrazine and desisopropyl atrazine. Total seasonal bulk deposition was consistently greater at Site Vital (Table 2.2) than Site Ridge (Table 2.3). Site Vital generally had greater weekly deposition rates when compared with Site Ridge. Pesticide deposition did not follow a clear increasing or decreasing seasonal trend.

Metolachlor and diazinon were detected despite not being reported for use in Winnipeg or on its surrounding cropland (Table 2.4). Metolachlor was detected twice in 2010 and six times in 2011 (Tables 2.2 and 2.3). Diazinon was detected twice in 2010 and not in 2011 (Table 2.2). Diazinon is not registered for urban use by private citizens and has restricted agricultural use in Canada. All other pesticides detected have reported agricultural use on cropland around Winnipeg. Of these 2,4-DB, 2,4-DP, atrazine, bentazon, bromoxynil, clopyralid, fluoroxypyr and trifluralin had no reported use within city limits (Table 2.4). The other pesticides detected, 2,4-D, chlorothalonil, chlorpyrifos, dicamba, glyphosate, malathion, MCPP and propiconazole were applied both in Winnipeg and in the surrounding agricultural area (Table 2.4). Of these eight pesticides, the amount applied agriculturally exceeded the amount applied in Winnipeg, with the exception of chlorothalonil and malathion (Table 2.4). The mass of chlorothalonil applied in Winnipeg was over 140 times greater than the mass applied agriculturally in large part due to its use on golf courses (Table 2.4). However, chlorothalonil amounted to less than one percent of the total pesticide deposition during our two year study.

2011 had higher deposition amounts (on average 426 μ g /m² across the two sites) compared to 2010 (on average 385 μ g /m² across the two sites). Peaks observed in 2010 were due to malathion deposition and peaks observed in 2011 were due to glyphosate deposition (Figure 2.1 and 2.2). In 2010, at both sites, 56% of the total seasonal deposition was insecticides followed by herbicides (43%) then fungicides (1%). In 2011, at both sites 99% of the total seasonal deposition was herbicides followed by insecticides and fungicides.

Malathion was frequently applied by city officials in 2010, 7 times in the IMA of Site Vital and 4 times in the IMA of Site Ridge (Figure 2.3). Malathion was detected in 47% (Site Ridge) and 38% (Site Vital) of samples, with bulk deposition rates ranging from 0.34 to 107.7 μ g/m² (Figure 2.3). Total malathion deposition over the sampling period was 151 μ g/m² at Site Ridge (Table 2.3) and 274 μ g/m² at Site Vital (Table 2.2), or 54% and 56% of the total deposition observed for these sites respectively. Malathion was often detected in weeks that the insecticide was applied in the IMAs in which Site Ridge and

	Total						Propor	tion of
	Seasonal		Detection		Max.		Total Pesticide	
	Depos	sition	Frequ	lency	Concen	tration	Seas	onal
	(µg/	\mathbf{m}^2)	(%	(0)	$(\mu g/m^2)$	/week)	Depositi	ion (%)
Pesticide	2010	2011	2010^{a}	2011^b	2010	2011	2010	2011
Malathion	274	0	38	6	108	0	56	0
Glyphosate	80	356	71 ^b	100	24	104 ^c	16	82
2,4-D	33	33	100	100	7^{d}	13 ^e	7	8
Aminomethyl								
Phosphonic	22	0.8	6 ^b	18	22	0.4	5	0.2
Acid						c.		
MCPP	22	19	94	100	10	8^{t}	4	4
MCPA	17	10	69	82	4	4	4	2
Atrazine	13	5	38	47	10	3	3	1
Bromoxynil	6	3	50	59	2	1	1	0.6
Dicamba	6	5	88	88	2	2	1	1
Desethyl atrazine	5	1	6	24	5	0.6	1	0.2
Clopyralid	3	0.4	6	18	3	0.2	0.5	0.1
Diazinon	3	ND	13	ND	3	ND	0.6	ND
Chlorothalonil	0.7	ND	6	ND	0.7	ND	0.2	ND
Propiconazole	0.6	0.4	6	12	0.6	0.3	0.1	0.1
Bentazon	0.5	0.1	6	12	0.5	0.1	0.1	0.0
2,4- dichlorophenol	0.2	ND	6	ND	0.2	ND	0	ND
Fluroxypyr	0.2	0.4	6	29	0.2	0.2	0	0.1
Metolachlor	0.2	0.3	6	18	0.2	0.2	0	0.1
Chlorpyrifos	ND	0.3	ND	12	ND	0.2	ND	0.1
Desisopropyl atrazine	ND	0.4	ND	6	ND	0.4	ND	0.1
Trifluralin	ND	0.1	ND	6	ND	0.1	ND	0
Total	486	435						

 Table 2.2 Pesticides detected at Site Vital.

^aout of 17 samples, ^bout of 18 samples, minimum concentration detected ($\mu g/m^2/week$) ^c0.3, ^d0.03, ^e0.03, ^f0.008;ND = Not Detected

	To	tal					Propor	tion of
	Seas	onal	Dete	ction	Ma	IX.	Total P	esticide
	Depos	sition	Frequ	iency	Concen	tration	Seas	onal
	(µg/	\mathbf{m}^2)	(%	(0)	$(\mu g/m^2)$	/week)	Depositi	ion (%)
Pesticide	2010	2011	2010^a	2011 ^b	2010	2011	2010	2011
Malathion	151	0.7	47	11	88	0.4	54	0.2
Glyphosate	41	297	61 ^b	94	12	67	15	71
Aminomethyl								
Phosphonic	27	5	6 ^b	11	27	3	10	1
Acid								
2,4-D	21	39	88	100	4	$7^{\rm c}$	7	9
MCPA	10	21	53	89	2	8	4	5
MCPP	9	30	76.5	100	2	9 ^d	3	7
Bromoxynil	8	8	53	61	4	4	3	2
Atrazine	7	6	47	44	4	3	3	1
Dicamba	3	6	72	78	0.6	2	1	2
Desethyl	2	1	12	17	2	07	07	03
atrazine	2	1	12	17	2	0.7	0.7	0.5
Chlorothalonil	0.8	ND	12	ND	0.4	ND	0.3	ND
Propiconazole	0.5	0.4	6	11	0.5	0.3	0.2	0.1
2,4-	0.4	0.4	18	6	0.2	0.4	0.1	0.1
dichlorophenol	0.4	0.7	10	0	0.2	0.7	0.1	0.1
Bentazon	0.4	ND	6	ND	0.4	ND	0.1	ND
2,4-DP	0.2	0.6	6	6	0.2	0.6	0.1	0.1
Clopyralid	0.2	1.2	6	17	0.2	0.8	0.1	0.3
Fluroxypyr	0.2	1	5.9	28	0.2	0.5	0.1	0.2
Metolachlor	0.1	0.3	6	17	0.1	0.1	0.1	0.1
Desisopropyl	ND	0.2	ND	6	ND	0.2	ND	0.1
atrazine		0.2		0		0.2		0.1
Trifluralin	ND	0.1	ND	6	ND	0.1	ND	0
Total	282	419						

^aout of 17 samples, ^bout of 18 samples, minimum concentration detected ($\mu g/m^2/week$) ^c0.1, ^d0.2; ND = Not Detected

		Urban Use Ranking -	
	Agricultural Use	Golf Courses (% of	Urban Use Ranking
Pesticide	Mass (% of total)	total)	- Other (% of total)
2,4-D	3237 (<1)	154 (9)	758 (9)
2,4-DB	71 (<1)		
2,4-DP	721 (<1)		
Atrazine	121 (<1)		
Bentazon	35 (<1)		
Bromoxynil	5793 (<1)		
Chlorothalonil	7 (<1)	1009 (61)	30 (<1)
Chlorpyrifos	282 (<1)	1 (<1)	188 (2)
Clopyralid	582 (<1)		
Diazinon			
Dicamba	42074 (2)	19 (1)	70 (<1)
Fluroxypyr	298 (<1)		
Glyphosate	92894 (5)	48 (3)	418 (5)
Malathion	23 (<1)		6632 (74)
MCPA	820046 (41)		
MCPP	4380 (<1)	96 (6)	390 (4)
Metolachlor			
Propiconazole	1148 (<1)	162 (10)	2 (<1)
Trifluralin	441 (<1)		
Total	1979728	1649	8917

Table 2.4 Mass of active ingredients (kg) applied in a 13 km radius around the Winnipeg perimeter (2004 - 2006 average) (Wilson, 2012) and applied within Winnipeg (2012) (Welch, 2012).



Figure 2.1 Weekly total pesticide deposition in 2010 (top) and 2011 (bottom)


Figure 2.2 Glyphosate deposition and rainfall at Site Ridge and Site Vital in 2010 (top) and 2011(bottom)



Figure 2.3 Malathion deposition and rainfall in 2010 at Site Vital (top) and Site Ridge (bottom) Arrows show dates of malathion spraying by the city of Winnipeg

Site Vital are located. Malathion was detected on three occasions in weeks that there were no reported malathion applications by city officials in the IMA of Site Ridge (July 13 and August 31) or Site Vital (July 20). During the 2011 sampling period, relatively dry weather conditions resulted in lower adult mosquito populations such that malathion applications were deemed unnecessary by city officials, and malathion was only detected twice at Site Ridge and once at Site Vital with bulk deposition rates ranging from $0.26 - 0.41 \,\mu\text{g/m}^2$.

Deposition by particulate matter was observed during both sampling seasons, as glyphosate, 2,4-D, AMPA, MCPP and dicamba were all detected during the five sampling weeks without rain. The mass of pesticides deposited was significantly positively correlated with Koc (soil organic carbon-water partitioning coefficient) in 2011 (r= 0.85, P<0.0001) but not in 2010. When glyphosate was removed from the data in 2011, the relation between total seasonal pesticide deposition and Koc was no longer significant. Koc was the only physiochemical property with which total seasonal dry pesticide deposition was correlated (2011) (r= 0.86, p<0.0001). Although glyphosate deposition rates. The mass of pesticides deposited was not significantly correlated with pesticide Kow or water solubility values in either year.

The mass of pesticides deposited was significantly positively correlated to weekly total rainfall in both years, but, in 2010, only after removing malathion from the data set. Total weekly pesticide deposition rates showed a stronger correlation to rainfall in 2010 (r=0.70, p \leq 0.0001) than in 2011 (r=0.50, p \leq 0.002) which was a drier year. Although detected in weeks with rainfall, benzaton, chlorothalonil, chlorpyrifos, metolachlor,

propiconazole and trifluralin were not detected during sampling weeks without rain. Weekly deposition concentrations of atrazine and desethyl atrazine and trifluralin were the only pesticides significantly positively correlated with rainfall and weekly deposition concentrations of dicamba, glyphosate and 2,4-D were the only pesticides significantly negatively correlated with rainfall. In general, pesticides had higher average weekly deposition rates during weeks with rain than weeks without rain but there were exceptions. For example, AMPA (Site Ridge only) and 4-chloro-2-methylphenol were detected during weeks without rain but not in weeks with rain.

Eleven of the compounds screened for have vapour pressures exceeding 9 mPa and hence are considered relatively volatile. Only five of these compounds were detected during this two year study: diazinon, trifluralin, AMPA and desethyl and desisopropyl atrazine. The other eighteen compounds detected in this study have vapour pressures below 3 mPa. Total seasonal pesticide deposition was thus not significantly correlated with Henry's law constant or vapour pressure, nor with air temperature.

The insecticides detected were all organophosphates. Eight out of the 14 herbicides detected were auxinic growth regulators and included phenoxy acids (5), pyridine acids (2) and a benzoic acid (1). The remaining six herbicides each had a different mode of action; one microtubule assembly inhibitor, one photosynthetic inhibitor at Photosystem II Site A, two photosynthetic inhibitors at Photosystem II Site II, one inhibitor of EPSP (5-enolpyruvylshikimate-3-phosphate) synthesis and one inhibitor of cell growth and division. The two fungicides were a substituted aromatic and a triazole.

Of the pesticides detected, three pesticides have guidelines for the protection of aquatic life, six pesticides have irrigation guidelines, ten pesticides have livestock guidelines and eight pesticides have drinking water quality guidelines (CCME, 2012) (Table 2.5). Irrigation water quality guidelines were exceeded for dicamba in 44 samples and for MCPA in 32 samples. None of the guidelines for drinking water quality or livestock were exceeded. Over the two years this study was conducted, 98.5% of the samples had more than one pesticide detected and 4.4% of the samples had 13 pesticides (maximum detected per sample).

Quality Guidem				
	Freshwater			Drinking
	MAC ¹ Short	Irrigation MAC	Livestock MAC	Water MAC
Pesticide	Term (µg/L)	(µg/L)	(µg/L)	(µg/L)
2,4-D	4	N/A	100	100
Atrazine	N/A	10	5	5
Bromoxynil	N/A	0.33	11	5
Chlorothalonil	N/A	5.8	170	N/A
Chlorpyrifos	0.02	N/A	24	9
Dicamba	N/A	0.006	122	120
Glyphosate	27000	N/A	280	280
MCPA	N/A	0.025	25	100
Metolachlor	N/A	28	50	5
Trifluralin	N/A	N/A	45	N/A

 Table 2.5 Canadian Council of Ministers of the Environment Selected Water

 Ouality Guidelines 2012.

¹MAC – Maximum Allowable Concentration

2.5 Discussion

The area of cropland around Winnipeg (13-km radius) typically receives approximately

185 times the mass of pesticides compared to the city itself. However, the mass of

malathion applied in Winnipeg in 2010 was over 285 times greater than the mass applied agriculturally. In 2010, the majority of the mass of pesticides deposited was related to urban use as approximately 55% of total mass deposited was malathion. Malathion was detected in greater concentrations during weeks in which the insecticide was used by city officials in the Site Vital and Site Ridge IMAs. Nations and Hallberg (1992) also concluded that malathion deposition was due to urban sources as malathion was detected only in urban samples and not in rural samples in their study in

Eastern Iowa. Based on records provided by the City of Winnipeg, malathion was more frequently applied in the Site Vital region (7 times) than in the Site Ridge region (4 times) hence the greater total amount of malathion deposited at Site Vital in 2010 is a reflection of the greater amount of malathion locally applied in this region of the city.

Malathion was detected (21.8 μ g/m²) in one week without rainfall (Site Vital – July 13, 2010). Malathion had been sprayed by the City on July 11, 2010 in the Site Vital IMA. Recommended fogging rates are 2.60 – 6.08 μ g of active ingredient/m² (Pest Management Regulatory Agency, 2003); hence deposition could be due to direct spray drift as the insecticide is applied and the deposition of particulate matter containing malathion. Malathion has a high Koc value (1800 mg/g) (Pesticide Properties Database, 2011) and has been found to persist in the atmosphere associated with particulate matter (Borrás et al., 2011).

In total, 6,632 kg of malathion was applied within city limits as part of the mosquito control program in 2010. The estimated amount of malathion deposited per spraying event was relatively similar at Site Vital (39.1 μ g/m²) and Site Whyte (37.8 μ g/m²). Therefore, the average mass of pesticides deposited per spraying event across the two

study sites was $38.4 \ \mu g/m^2$, and multiplying this by the 229 spraying events across all 51 IMAs, the calculated total pesticide deposition in Winnipeg is approximately 85 kg, representing approximately 1.3% of the total mass applied. Although there are sources of uncertainty in this estimation such as whether the estimated amount of malathion deposited per spraying event is consistent across IMAs, it suggests that a large majority of malathion is either being transported outside city limits or degraded. The primary metabolite of malathion is malaoxon. The United States Environmental Protection Agency (2006) has determined that malaoxon is 61 times more toxic to humans than malathion.

In 2011, glyphosate represented 77% of the total mass deposited at both sites (Figure 2.3). Glyphosate is the third most widely applied agricultural pesticide in the area around the city of Winnipeg (Wilson, 2012) as well as the third most intensively used pesticide in the city of Winnipeg (Welch, 2012). Both agricultural and urban uses are likely sources contributing to glyphosate deposition in urban rain and dust. 2011 was a drier year and had increased deposition by particulate matter. Glyphosate has a high Koc value (21,699 mg/g) (Wilson, 2012) and is transported with particulate matter (Humphries et al., 2005), which is likely the reason for the more frequent and larger masses detected in 2011 than 2010.

Diazinon, an organophosphate insecticide, was detected twice in 2010 despite being banned for use by the public and having restricted agricultural use. Diazinon has been previously detected in air samples from the Canadian prairies and it was thought that the concentrations detected were background levels from urban use (Raina et al., 2010).

Both dicamba and MCPA exceeded agricultural irrigation quality guidelines, and many plants in urban gardens have higher sensitivities when compared to agricultural crops. For example, *Allium sepa* (onion), *Cucumis sativa* (cucumber), *Lactuca sativa* (lettuce), and *Lycopersicon esculentum* (tomato) are at least ten times less tolerant to bromoxynil, dicamba and glyphosate when compared to agricultural crops such as *Avena sativa* (oats) and *Zea mays* (corn) (Boutin et al., 2004). The impact of atmospheric deposition by pesticides on urban gardens is currently unknown, with respect to both the impact of garden plant growth and the absorption of pesticides into parts of the produce that are edible.

For some of the pesticides detected in our study, relative to a single exposure, their mixtures have been shown to demonstrate additive or synergistic effects to aquatic midges (Pape-Lindstrom and Lydy, 2009), frogs (Hayes, 2006), rats (Moser et al., 2006) and salmon (Laetz et al., 2009). About 99% of our atmospheric deposition samples contained pesticide mixtures, which is in agreement with a rural atmospheric deposition study in Manitoba by Messing et al. (2012). Very few studies have considered pesticide mixtures in ecological risk assessments but pesticide mixtures are also typically found in surface waters that become contaminated because of atmospheric deposition and pathways such as runoff (Hoos et al., 2002; Waite et al., 2002; 2004; Donald et al., 2007). It is important that studies continue to monitor levels of pesticide mixtures in the environment, combined with improving knowledge of the risks that these pesticides pose both individually and in a mixture to human and environmental health.

2.6 Conclusion

Nineteen active pesticide ingredients were detected in an urban city across a sampling period ranging from May to September, including fourteen herbicides, three insecticides and two fungicides. Four herbicide metabolites were also detected. 99% of the samples contained at least two pesticides. The pesticide with the highest total seasonal deposition was malathion in 2010 and glyphosate in 2011. The herbicides 2,4-D and MCPP were other pesticides most frequently found. Pesticide deposition was positively correlated with Koc and weekly rainfall. 2,4-D, dicamba, MCPP and glyphosate were all detected during sampling weeks without rain, suggesting that dry deposition contributes to total atmospheric deposition. Sources of pesticides were from both urban and agricultural use.

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3. 17β-ESTRADIOL MINERALIZATION IN FIELD AND LABORATORY MICROCOSM STUDIES

3.1 Abstract

Mineralization studies of natural steroid hormones and pesticides are usually performed in laboratory incubators at a constant temperature. This research established a novel infield microcosm design to quantify the mineralization of 17β -estradiol (E2, a natural steroid estrogen) and 2,4-D (a weakly-acidic herbicide) across two field seasons in soils ranging in texture from sandy loam to heavy clay. Probes installed in the soil microcosms and at similar depth in adjacent soil were used to collect hourly temperature data in each year, and these data demonstrated small diurnal and seasonal fluctuations in temperature during the growing seasons. Representative of the minimum, mean and maximum temperature conditions observed during intervals across the growing seasons, additional laboratory microcosm incubations were set up in both years to examine relative to incubations at a constant 20°C, the impact of simulated temperature changes on E2 mineralization in the soils. Results for most soils indicated that the temperature treatment in laboratory incubations had no significant impact on the value of E2 mineralization parameters. E2 and 2,4-D sorption and mineralization parameters were significantly associated with a wide range of soil properties resulting in strong predictive models as determined by Partial Least Square regression analysis. Regardless of the soil texture, the duration of freezing soils at -25°C decreased the value of mineralization parameters in the field and laboratory studies over time. This indicates the need for a greater discussion for a more consistent approach to the duration and temperature of storage conditions for soils prior to mineralization experiments.

3.2 Introduction

Livestock excrete 17β -estradiol (E2) and other steroid hormones in urine and feces. Steroid hormone residues are detected in soil at elevated concentrations after manure is applied to agricultural land (Lange et al., 2002). The Province of Manitoba is an area in Canada where manure is frequently applied on agricultural land with more than 2.6 million hogs and 1.1 million cattle contributing to the livestock sector in this province (Statistics Canada, 2012).

E2 residues can be transported from agricultural soils into the broader environment by processes such as surface runoff (Nichols et al., 1997; Finlay-Moore et al., 2000) and leaching (Kjær et al., 2007). E2 has been detected in rivers at concentrations exceeding the lowest observable effect level for fish (Routledge et al., 1998; Tyler et al., 1998; Miles-Richardson, 1999). The major effect of E2 is the interference of the normal activities of hormones involved in animal metabolism and growth control (Lai et al., 2002). E2 has also been classified as a human carcinogen due to its expected link to increased risk of breast cancer (U.S. Department of Health and Human Services, 2000). Reported half-lives of E2 in soil have ranged from 0.17 to 9.7 days (Lee et al. 2003; Xuan et al. 2008). The final step of E2 degradation in soil is mineralization (ring-cleavage)

controlled by microorganisms such as *Rhodococcus* sp. and *Sphingomonas* sp. (Xuan et al., 2008; Stumpe and Marschner, 2009; Kurisu et al., 2010). Prior to mineralization experiments in the laboratory, soils are air-dried or left at their field moisture content and then commonly stored for various lengths of time by one of three methods: stored at room temperature (Parker and Doxtader, 1983; Ferrell and Vencill, 2003), refrigerated at 4°C (Entry et al., 1994; Völkel et al., 1994) or frozen (Topp and Starratt, 2000; Colucci et al., 2001). Stenberg et al. (1998) found that soil microbial communities decrease after soil has been frozen when compared to fresh soil; however they did not decrease as much when compared to soils that had been refrigerated. Several studies have found no impact of freezing on the amounts and rates of chemical mineralization (Stenberg et al., 1998; Pesaro et al., 2003).

Mineralization studies are typically performed at a constant temperature in the 10-30°C range (Bolan and Baskaran, 1996; Colucci et al., 2001; Picton and Farenhorst, 2004; Ying and Kookana, 2005; Lucas and Jones, 2006; Stumpe and Marschner, 2009; Shymko et al., 2008; Caron et al., 2010a). The mineralization rate constant of E2 (k) in soil increased from 0.141 to 0.750 to 0.894 day⁻¹ with an increase in constant temperature from 15 to 25 to 35°C (Xuan et al., 2008). Soil temperature can affect mineralization through its influence on soil microbial activity and populations (Veeh et al., 1996; Willems et al., 1996; Xuan et al., 2008) as well as its influence of on soil-chemical sorption reactions (DiVincenzo and Sparks, 2001). Soil temperatures in the field fluctuate during the growing season and diurnally. There have been no studies on the impact of temperature changes during experiments on steroid hormone mineralization.

The objective of this study was to quantify the impact of incubation temperature changes on E2 mineralization parameters in ten agricultural soils ranging in texture from sandy loam to heavy clay. In this research, we first designed an in-field microcosm protocol to quantify E2 mineralization in the soils during two field seasons and to monitor hourly temperature data in the soils over time. Based on the temperature fluctuations observed in the field, scenarios of controlled temperature changes in the laboratory were established for comparison to traditional incubations at a constant 20°C. The herbicide 2,4-(dichlorophenoxy)acetic acid (2,4-D) was also included to test whether the design of the novel in-field microcosm experiment would work for a chemical that is known to be difficult to study under field conditions because of its relatively rapid mineralization in soil (Lavy et al., 1996). 2,4-D is a herbicide for the post-emergent control of broadleaf weeds in cereal and other production systems. 2,4-D is the fifth most widely used pesticide in Manitoba with over 148,800 kilograms applied annually to agricultural land (Wilson, 2012).

3.3 Materials and Methods

3.3.1 Chemicals and Soil Properties

Chemicals in sorption and mineralization experiments were analytical grade 17β-estradiol (98% chemical purity) and 2,4-D (95% chemical purity) from Sigma Aldrich Chemical Company, St. Louis, MO. Chemicals in the mineralization experiments also included 4-

¹⁴C-ring labeled 2,4-D (99% radiochemical purity; specific activity 50 mCi/mmol) from American Radiolabeled Chemicals Incorporated, St. Louis, MO. Sorption experiments used the same ¹⁴C-ring labeled 2,4-D as well as 6,7-³H labeled 17β-estradiol (99% radiochemical purity; specific activity 40 mCi/mmol) from American Radiolabeled Chemicals Incorporated, St. Louis, MO.

Ten surface soils (0-10 cm) were sampled in June 2010 to account for a variety of textures, organic carbon contents and nutrient concentrations typical of agricultural soils in Manitoba (Table 3.1). Soils were air-dried, sieved (< 2 mm) and frozen at $-25 \pm 2^{\circ}$ C until for use in mineralization experiments throughout 2010 to 2012 (Table 3.2). Time frozen ranged from 1 to 17 months (Table 3.2).

Soil pH was measured with a 1:1 soil:water and CaCl₂ slurry with a pH meter (Jones, 2001). Soil organic matter content (SOM) was determined using the Loss of Weight on Ignition (LOI) procedure at 360°C and 105°C adapted from Konen et al. (2002). NO₃-N was extracted using the Cadmium Reduction Method described by Maynard et al. (2008). P was extracted using the Olsen (NaHCO₃) test as described by Schoenau and O'Halloran (2008). K, Ca and Mg were extracted using 1M ammonium acetate at pH 7 (Hendershot et al., 2008) and those measurements were used to calculate cation exchange capacity (CEC). Zn was extracted with DTPA (diethylenetriaminepentaacetic acid) (Lindsay and Norvell, 1978). Particle size analysis was performed using the pipette method as described by Kroetsch and Wang (2008). Field capacity was determined using laboratory leaching columns (11 cm in height, 2.7 cm radius) to determine the amount of gravity retained soil moisture after 96 hours.

Sorption was determined using batch-equilibrium procedures using a 1:2 soil to solution ratio in 10 mL Teflon tubes for 2,4-D and in 10 mL glass tubes for E2. A 0.01M CaCl₂ solution was used instead of water to minimize disruption of the soil mineral environment (Wauchope et al., 2002). Solution concentrations for both E2 and 2,4-D were 0.5, 1, 2, 4 and 8 mg chemical/L and with a radioactivity of 830, 1,700, 3,300, 6,700 and 13,300 Bq/mL, respectively. In the E2 sorption experiments, all equipment and soils were autoclaved at 121°C for 30 minutes before use as recommended by Caron et al. (2010b).

Tubes were rotated for 24 hours in the dark to establish equilibrium. The slurry was centrifuged for 10 minutes at 7,000 rev min⁻¹ after which 1 mL sub-samples of supernatant (duplicates) were transferred to 8 mL scintillation vials with 5 mL 30% Scintisafe scintillation cocktail (Fisher Scientific, Fairlawn, NJ). The amount of radioactivity in solutions and experimental samples were quantified by Liquid Scintillation Counting (LSC) with automated quench correction (#H method) (Tri Carb 2100TR, Beckman Instruments, Fullerton, CA) and using a maximum counting time of 10 minutes. To quantify desorption, the remaining supernatant was removed from the tubes and disposed. 9 ml of 0.01 M CaCl₂ solution was added to each tube and the tubes were rotated in the dark for an additional two hours. Again, 1 ml sub-samples of supernatant (duplicates) were removed and radioactivity levels in samples were determined as described above. The Freundlich sorption coefficient, $K_f [\mu g^{1-1/n} g^{-1} m L^{1/n}]$, was calculated by linear regression using the empirical Freundlich equation (log transformed): $\log C_s = \log k_f + (1/n) \log C_e$, where where C_s is the concentration sorbed to soil $[\mu g g^{-1}]$ and C_e is the concentration remaining in the solution at equilibrium $[\mu g m L^{-1}]$

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															Kf(µ n	$\mathfrak{lg}^{1-1/n}\mathfrak{g}^{-1}$ nl ^{1/n)}
Soil Series	Latitude (N)	Longitud e (W)	sand (%)	clay (%)	silt (%)	pН	OC (%)	N mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	Zn mg/kg	CEC (meq)	E2	2,4-D
Denham	49° 30'	98° 02'	72	16	12	5.6	2.0	15.7	21.7	305.7	1338	294	1.9	9.9	6.96	0.63
Dezwood	49° 21'	98° 23'	57	29	15	7.0	2.3	38.2	49.3	316.3	2547	478	1.8	17.6	6.92	0.41
Halboro	49° 55'	99° 22'	85	9	6	4.4	1.1	7.2	54.0	241.0	511	106	1.9	4.1	6.12	0.48
Hibsin	49° 30'	98° 01'	65	19	17	6.8	3.3	18.5	39.3	458.3	3217	1137	3.6	26.8	8.26	0.90
Isafold	50° 10'	97° 52'	43	27	29	7.9	4.1	56.2	31.7	141.0	4768	1412	2.4	35.9	7.12	0.50
LaSalle	49° 30'	98° 02'	42	29	29	7.7	2.2	28.8	27.3	331.3	4533	519	2.9	27.8	8.47	0.44
Newdale ³	50° 03'	99° 55'	32^{2}	24 ²	44^{2}	7.5	3.2	13.5	11.7	315.3	3565	614	0.9	23.8	nd	nd
Pembina	49° 20'	98° 23'	26	34	40	6.6	4.8	36.5	41.7	344.7	2554	385	4.7	16.8	9.00	1.17
Ramada	49° 54'	99° 21'	39	30	31	5.7	3.5	185.3	30.3	177.7	2701	632	4.2	19.2	8.67	0.89
St. Norbert	49° 41'	97° 07'	4	57	39	6.9	5.2	94.7	62.3	696.0	4494	1263	3.5	34.8	8.54	0.87

Table 3.1. Selected soil properties¹ and sorption parameters from Ap horizon.

 $\frac{51.100011}{1} \text{ mean of three replicates; }^{2} \text{ estimate (Ehrlich et al., 1956); }^{3} \text{ included only in the 2011 E2 incubator studies; nd= not determined .}$

Table 3.2. Start and end date of each	mineralization experi	iment and length of froz	en sample storage.
Tuble 5.2. Stult and the date of cath	i miner anzanon esperi	ment and length of mor	ch sumple storage.

Experiment	Date Started	Date Completed	Duration of Frozen Sample Storage
2010 field season	August 25, 2010	October 10, 2010	1 month
2011 E2 incubator	March 2, 2011	June 2011	6 months
2011 field season	July 11, 2011	October 19, 2011	11 months
2012 E2 incubator	January 16, 2012	April 25, 2012	17 months

and 1/n is the dimensionless Freundlich constant describing nonlinearity. Units were chosen so all isotherm lines crossed $C_e=1$, which is important when determining K_f (Bowman, 1982). Percent desorption was calculated by dividing the total mass of chemical (E2 or 2,4-D) in the supernatant after the desorption step by the calculated total mass of chemical sorbed to the soil fraction after the sorption step, and then multiplying by 100.

3.3.3 Mineralization

In all experiments, soil microcosms were pre-incubated for 7 days at 20°C to stimulate microbial growth and then spiked with E2 or 2,4-D at environmentally relevant rates. E2 was applied at a concentration of 0.091 g m⁻³ soil with 66.7 Bq g⁻¹ soil, which was also the concentration used by Caron et al. (2010a). 2.4-D was applied at an agronomic rate of 0.62 g m^{-3} soil with 310 Bq g⁻¹ soil, which is a concentration previously used in Shymko and Farenhorst (2008). The microcosm consisted of a 500-mL Mason jar which contained a 50-mL glass jar with 25 g of soil (on an oven dried weight basis). The moisture content of each thawed soil was determined gravimetrically and distilled water was added to bring the soil to 80% of field capacity minus the liquid volume added from applying chemical solutions. A glass vial containing 5 mL of acidified water (pH of 3 using 6 N HCl) was inserted into the microcosm to keep the environment moist throughout the experiment. In addition, a 20-mL scintillation vial containing 5 mL of 0.5M NaOH was used to trap the ¹⁴CO₂ evolved during incubation. Traps were changed every 2 to 4 days until day 20 and then approximately every 10 days until cumulative 14 CO₂ production leveled off. Scintillation cocktail (8 mL) was added to traps and

radioactivity was quantified as described above to calculate the amounts of ¹⁴CO₂ evolved. The mineralization rate constants and maximum mineralization at time infinity were calculated in SigmaPlot for Windows, Version 11.0 (Systat Software Inc. 2008) assuming first-order kinetics: $max_t = max(1-e^{-kt})$ where $max_t = mineralization$ (as % of applied) at time t; max = amount of herbicide mineralized (as % of applied) at time infinity; k = first-order mineralization rate constant [day⁻¹]; and t = time in days [day].

3.3.4 Experimental Design

Field experiments were conducted on land near Winnipeg, Manitoba, Canada at the Kelburn Farm (49° 41' N, 97° 07'W) in 2010 and at the University of Manitoba Point Research Station (49° 28'N, 97° 06'W) in 2011. Each field experiment was set up as a randomized block design with five replicates per soil type. Soil microcosms were installed approximately 20 cm beneath the soil surface in a field plot (7.2 by 7.2 meter) and all microcosms were covered with 5 cm of soil flush with the soil surface (Figure 3.1).



Figure 3.1. In field experimental design a) view from above b) side view diagram.

An additional 44 microcosms were not treated with a chemical solution but installed with temperature probes (Thermochron® iButton, Maxim Integrated Products, San Jose, CA) to provide in-field temperature data in duplicated microcosms per soil type. In addition, 12 temperature probes (6 probes at 10 cm and 6 probes at 20 cm) were installed in the soil adjacent to these microcosms to allow comparison between temperatures inside and outside the microcosms. Finally, 12 temperature probes (6 probes at 20 cm) were installed throughout the plot to measure the spatial variability in soil temperatures within the experimental plot. All probes were encased in capsules (iButton Capsules, Maxim Integrated Products, San Jose, CA) to protect them against water damage.

Probes installed inside microcosms and directly in the soil demonstrated statistically similar minimum, mean and maximum temperature readings at a comparable soil depth. A series of laboratory experiments were set up to study the impact of simulated temperature changes on E2 mineralization in the soils using minimum, mean and maximum temperature conditions observed in soil microcosms representative of the 2010 and 2011 growing seasons in (Table 2). The three temperature factors considered in the laboratory experiments in each year were (Figure 3.2): 1) a diurnally fluctuating temperature (flux) reflecting the mean day and night temperatures during the growing seasons of 2010 or 2011, 2) a diurnally fluctuating temperature which included both high and low extremes observed during the growing seasons of 2010 or 2011 (eflux) and 3) a constant temperature (20°C) control. The duration of the day and night time was determined by assessing the point of inflection on the temperature curves with the day time hours corresponding to the temperatures above the point of inflection and the night

time hours corresponding to the temperatures below the point of inflection. In the second year, there were two distinct temperature intervals in the field, and hence the flux treatment in the laboratory was divided accordingly (Figure 3.2 see I and II).

3.3.5 Statistical Analyses

Statistical analyses were performed in SAS for Windows version 9.2 (SAS Institute Inc. 2002 - 2008). All data with a Shapiro-Wilk value below 0.9 were transformed when necessary to respect normality except for the correlation analysis in which nontransformed data can be used (Legendre and Legendre, 1998). For the field experiments in 2010 and 2011, two-way ANOVAs were used to determine significant differences in max values among soil series and chemical (E2 versus 2,4-D) treatments. Statistics on k values were omitted as 2,4-D mineralization showed an approximate 12 day time lag in 2011. For the E2 incubator experiments, two-way ANOVAs were used to determine significant differences in k and max values among soil series and the three temperature treatments in each year. For the E2 incubator experiments at a constant temperature (20°C), two-way ANOVAs were also used to determine significant differences in k and max values among soil series and time (2011 versus 2012). Pearson correlation coefficients (P < 0.05) were determined among soil properties, mineralization parameters, Freundlich sorption coefficients and percent desorption. Correlations were performed using the data from all experiments combined and data from each individual experiment (e.g., 2010 field season only). Similar trends in correlations were observed for the individual experiments, so correlations using the data from all experiments combined are presented in the results. Principal Component Analysis and Partial Least Squares





(PLS) regression in SAS for Windows version 9.2 were used to develop predictive models for the mineralization parameters. Due to collinearity, PLS was selected for the final models. Initially, all soil properties and sorption parameters were included as predictor variables. Soil properties and sorption parameters included in the final models had variable importance on prediction (VIP) over 1.

3.4 Results

Sorption data showed a good fit to the Freundlich equation with r^2 values ranging from 0.83 - 0.99 for E2, and from 0.92 - 1.0 for 2,4-D. For both E2 and 2,4-D, soil series had a significant effect on K_f, but not on the slope. The slope of the isotherms were well below unity for E2 (1/n ranging from 0.65 to 0.72) indicating that the saturation of sorption sites progressively reduced further sorption as the E2 concentration increased in the solution (L-type in Giles et al., 1960). 1/n was close to unity for 2,4-D, ranging from 0.92 to 1.08.

E2 was more strongly sorbed by soil than 2,4-D as K_f ranged from 6.12 to 9.00 μ g^{1-1/n} g⁻¹ ml^{1/n} for E2 but from 0.41 to 1.17 μ g^{1-1/n} g⁻¹ ml^{1/n} for 2,4-D (Table 3.1). For both E2 and 2,4-D, percent desorbed was significantly negatively correlated with K_f values. Desorption ranged from 2.1 to 17.3% for E2 but from 47 to 100% for 2,4-D. In two-thirds of the batch-equilibrium sorption samples, 2,4-D sorption was completely reversible after 2 hours. The E2 and 2,4-D sorption and desorption values were within range of that reported by other studies (Bekbölet et al., 1999; Karnjanapiboonwong et al., 2010).

	max E2	k E2	K _f E2	%D E2	max4D	k 4D	K _f 4D	sand	silt	clay	pН	OC	N	Р	K	Ca	Mg
k E2	0.14 ^c																
$K_f E2$	0.60^c	ns															
%D-Е2	0.38°	ns	0.57^{a}														
max4D	ns	ns	ns	ns													
k4D	ns	ns	ns	ns	0.40^{a}												
$K_f 4D$	ns	ns	0.73 ^c	ns	ns	0.48 ^c											
sand	ns	ns	0.73 ^a	0.57^{b}	ns	ns	0.48 ^c										
silt	ns	ns	0.79^{a}	0.55 ^b	0.20^c	0.22^c	0.54^{b}	0.95 ^a									
clay	ns	ns	0.61^{b}	0.54 ^c	ns	ns	0.38 ^c	0.96 ^a	0.82^{a}								
pН	0.21 ^b	ns	0.42°	ns	0.35 ^b	ns	ns	0.54 ^a	0.54^{a}	0.50^{a}							
OC	0.18 ^b	0.10 ^b	0.68^{b}	0.35 ^b	ns	0.21^c	0.69^{a}	0.87^{a}	0.86^{a}	0.80^{a}	0.48^{a}						
Ν	0.11 ^c	ns	0.45°	0.38 ^b	ns	ns	ns	0.52^{a}	0.50^{a}	0.49^{a}	ns	0.43^{a}					
Р	0.22^{a}	ns	ns	ns	ns	ns	ns	0.22^{c}	ns	0.38^{b}	ns	ns	ns				
Κ	ns	0.15 ^b	0.38°	ns	ns	ns	0.38°	0.46^{a}	0.26 ^c	0.60^{a}	ns	0.41^{b}	ns	0.55^{a}			
Ca	ns	ns	0.53^{b}	0.46^c	0.29^b	ns	ns	0.70^{a}	0.68^{a}	0.65^{a}	0.91 ^a	0.60^{a}	0.26°	ns	0.26°		
Mg	0.13 ^c	ns	ns	ns	ns	ns	ns	0.48^{a}	0.43^{a}	0.49^{a}	0.66^{a}	0.65^{a}	0.27°	ns	0.30^{b}	0.79^{a}	
Zn	ns	0.16 ^b	0.87^{a}	0.49^b	ns	0.22^c	0.87^{a}	0.65^{a}	0.76^{a}	0.50^{a}	ns	0.72^{a}	0.51^{a}	ns	0.25°	0.29^{b}	0.22^{c}
CEC	0.11^c	ns	0.48°	ns	0.25 ^b	ns	ns	0.66^{a}	0.62^{a}	0.66 ^a	0.86^{a}	0.66^{a}	0.27^{c}	ns	0.33^{b}	0.97^{a}	0.91 ^a

Table 3.3. Pearson Correlation Coefficients among E2 and 2,4-D mineralization and sorption values and soil properties for all field and laboratory experiments combined. Values in **bold** font are negative.

^a significant at <0.0001; ^b significant at < 0.01; ^c significant at < 0.05; ns not significant.

max - amount of herbicide mineralized; k - first-order mineralization rate constant; 4D -2,4-D; K_f - Freundlich sorption coefficient; %D – percent desorption; OC – percent soil organic carbon.

E2 and 2,4-D Kf values were significantly positively associated with % OC, clay and silt (Table 3.3) as previously found (Bekbölet et al., 1999; Caron et al., 2010b). E2 and 2.4-D Kf values were significantly positively associated with a range of other soil properties, with K and Zn being common to both (Table 3.3). Greater K levels in soil can reduce clay interlayer spacing and increase the sorption of a wide range of pesticides by organoclay complexes (Li et al., 2006). The significant positive association between 2,4-D and/or E2 K_f values with Zn, Ca, Mg and N was likely due to the observed strong association of these ions with OC.

Mineralization of E2 and 2,4-D demonstrated an excellent to adequate fit to the first order equation with r^2 ranging from 0.783 – 0.996 for E2 and from 0.436 – 0.999 for 2.4-D. E2k and 2,4-D-k were significantly negatively correlated with SOC; both E2-max 2,4-Dmax were significantly negatively correlated with soil pH; and 2,4-D-k was negatively correlated with 2,4-D-K_f, and E2-max was significantly negatively correlated with E2-K_f (Table 3.3). Increased sorption, including due to increased SOC and/or decreased soil pH, reduces the bioavailability of E2 and 2,4-D for mineralization in soil (Smith et al., 1992; Guerin and Boyd, 1997; Lee et al., 2003). 2,4-D-K_f, E2-K_f, E2-k, 2,4-D-k, E2-max and 2,4-D-max all showed strong predictive PLS models with soil properties (Table 3.4).

In the field experiment, the interaction between soil series and chemical (E2 versus 2,4-D) was significant in both years. 2,4-D max values were significantly greater than E2 max in six out of nine soils in year one but in only three out of nine soils in year two. E2 max values were significantly greater than 2,4-D max in one soil in 2010, but three soils in 2011 (Figure 3.3).

Soil	PLS regression equation	r^2
parameter		
E2 K _f	$4.8 + 2.7 \times 10^{-1} (\text{Zn}) + 6.1 \times 10^{-2} (\% \text{ OC}) + 1.3 \times 10^{-2} (\% \text{ silt}) - 4.0 \times 10^{-3}$	0.85
	(% sand)	
2,4-D K _f	$-1.4 + 2.2 \times 10^{-1} (Zn) + 1.0 \times 10^{-1} (\% \text{ OC}) - 1.6 \times 10^{-2} (pH) - 6.0 \times 10^{-5}$	0.84
	(Ca)	
E2 %	$1.2 \text{ x}10^{-1} - 2.0 \text{ x}10^{-2}(\text{Zn}) - 2.0 \text{ x}10^{-3}(\% \text{OC}) - 2.0 \text{ x}10^{-4}(\% \text{ clay}) - 2.0 \text{ x}10^{-1}(\% \text{ clay}) - 2.0 \text{ x}10^{-1}(\%$	0.72
desorption	2.0×10^{-4} (% silt) + 1.0×10^{-4} (% sand) - 1.0×10^{-6} (Ca)	
E2-k	$7.8 \times 10^{-2} - 9.0 \times 10^{-3} (\% \text{OC}) + 9.0 \times 10^{-5} (\text{P}) - 6.0 \times 10^{-5} (\text{K})$	0.89
2.4-D k	$-6.3 + 2.0 \text{ x}10^{-2}$ (% clay) + 5.9 x 10^{-1} (pH) + 1.0 x 10^{-2} N) + 3.0 x 10^{-2}	0.83
	(P)	
E2-max	$7.8 \times 10^{-2} - 6.8(\text{pH}) - 3.2(\%\text{OC}) + 1.0 \times 10^{-1}(\text{P}) - 3.0 \times 10^{-3}(\text{K}) +$	0.90
	3.0×10^{-3} (Ca)	
2,4-D max	$2.4 \times 10^{1} - 1.5 (\text{pH}) + 2.0 \times 10^{-2} (\text{N}) - 1.1 \times 10^{-1} (\text{P}) - 1.0 \times 10^{-3} (\text{Ca})$	0.84

Table 3.4. Partial Least Squares (PLS) regression equations for mineralization and sorption parameters.

For both 2,4-D and E2, and in all soils, max values were numerically greater in year 1 than year 2 (Figure 3.3), despite the warmer soil temperatures and longer duration of the field experiment in year two. In the laboratory under 20°C incubations, E2 max and/or k were significantly greater in year 1 than year 2 in most cases regardless of soil texture: in the Denham sandy loam, Halboro loamy sand, Hibsin sandy loam (E2 k only), Isafold clay loam, LaSalle clay loam (E2 max only), Ramada clay loam (E2 max only), Pembina clay loam (E2 k only) and St. Norbert clay (Figure 3.4). The main difference in the 2010 and 2011 results is the longer duration of time for which the soil samples were frozen (Table 3.2).

Neither soil series nor the temperature fluctuation treatment had a significant effect on E2-k in the laboratory in year one. Temperature fluctuation had no significant effect on E2-k in soils in year two, except that the eflux treatment for the Isafold clay loam showed a significantly greater E2-k than the constant treatment (Figure 3.5). Temperature fluctuations had no significant influence on E2 max in 7 soil series in year one and in 5

soil series in year two (Figure 3.5). For the other soils in either year one or two, there were always two temperature treatments showing statistically similar E2 max values (Figure 3.5).



Figure 3.3. Maximum mineralization values for E2 and 2,4-D under field conditions in 2010 (top) and 2011 (bottom).

Error bars represent standard error; * represents statistical significance between chemicals within a soil series





Error bars represent standard error; * represents statistical significance between years within a soil series







Figure 3.5. Maximum (max) (top) in 2010 and mineralization rates (k) (middle) and max (bottom) in 2011 for E2 under three temperature treatments (flux = diurnal fluctuations; eflux = diurnal fluctuations with extremes; constant = 20° C). Temperature treatments were based on field experiments conducted.

Error bars represent standard error; * represents statistical significance among temperature treatments within a soil series.

3.5. Discussion

E2-k and E2-max values obtained in the field and laboratory studies were within the range of those reported for soils under constant incubation temperatures in the 10 to 40°C range, with previous laboratory studies reporting a E2-k between 0.01 and 0.47 day⁻¹ and a E2-max between 2 and 35% (Colucci et al., 2001; Lucas and Jones, 2006; Fan et al., 2007; Xuan et al., 2008; Stumpe and Marschner, 2009; Caron et al., 2010a). This is the first field experiment that used soil microcosms containing radiolabeled chemicals in its experimental design. Radiolabeled pesticides were previously used in the field by Lavy et al. (1996) but their method measured long term leaching and degradation of herbicides

and is not appropriate for pesticides with a fast rate of mineralization. Soil temperatures in microcosms were similar to soil temperatures measured outside microcosms, indicating that the glass jar incubations had no impact on the field soil temperatures. Few challenges in the design were encountered, except for a few cases of rodents nesting in the installed PVC cylinders and temporary water logging in PVC cylinders following intense rainfall events.

The duration of frozen storage for the samples is shown in Table 3.2. The impact of the length of freezing is illustrated in Figure 3.4. This study clearly shows that long-term freezing significantly reduces the potential for 2,4-D and E2 mineralization in soil, possibly due to a reduction in soil microbial communities over time. E2 and 2,4-D degradation and mineralization is facilitated by a host of microbial communities such as Proactinomyces sp. and Nitrosomonas europaea for E2 (Pauwels et al., 2008) and Sphingomonas sp., Pseudomonas sp. and Alcaligenes sp. for 2.4-D (Ka et al., 1994) and freezing may impact microbial communities differentially (Stenberg et al., 1998). Many mineralization studies involve the freezing of soil for different lengths of time (Zelles et al., 1991; Stenberg et al., 1998; Colucci et al., 2001; Shymko et al., 2008; Caron et al., 2010a), but the effects of short-term (days) to long-term (years) freezing on soil properties and chemical mineralization are seldom determined. Pesaro et al. (2003) froze soil for four days and found that degradation of methidathion was not affected, even though population of microbial communities in the soil decreased as measured by respiration, cell cultures and DNA techniques. Storage at -20°C for 13 months showed little to no effect on the degradation rate of linuron when compared to fresh soils

(Stenberg et al., 1998). However, mineralization of glyphosate was reduced under freeze-thaw conditions when compared with incubation at 5°C (Stenrød et al., 2005).

Under laboratory conditions, in 78% and 94% of soils across two years combined, temperature fluctuations had no significant impact on E2-k and max, respectively. In 2010, the overall mean temperatures of the diurnal flux and eflux treatments were 13.3 and 14.1°C, respectively, and well below the 20°C constant incubations, but max values in 7 out of 9 soil series and k values in all soil series were significantly similar, suggesting that overall lower seasonal temperatures do not affect max or k values. Willems et al. (1996) found that although microbial activity was reduced at 10°C when compared with 20°C, the greatest reduction in activity occurred between 4 and 10°C. Although previous studies have demonstrated that the populations of bacteria under diurnally fluctuating temperature treatments could be reduced by as much as 40% relative to populations kept at a constant temperature (Howell et al., 1971; Biederbeck and Campbell, 1973), our study results suggest that temperature fluctuations in soils have little impact on E2 mineralization in soils.

Incubators can be expensive investments for a laboratory, particularly in low-income countries. Our results suggest that some mineralization experiments can be conducted at the ambient temperature in a laboratory room because small temperature fluctuations will not influence the mineralization results in soils with a wide range of properties. The ability to conduct experiments at ambient temperature without the need to carefully maintain a constant temperature may also lead to the development of larger-scale mineralization experiments for which incubator space could otherwise be a limiting factor, and these larger-scale experiments would allow for concurrently investigating the

impact of many factors on mineralization, including the impact of moisture content, nutrient content, chemical co-applications and any combination thereof in a wide range of soils.

3.6. Conclusion

An in-field mineralization microcosm study was designed to measure 2,4-D and E2 mineralization. Mineralization was also measured under fluctuating temperatures in the laboratory. k and max values measured in the wide range of soils investigated were generally consistent with the mineralization results reported in previous studies. Simulated temperature fluctuations in laboratory incubators did not significantly impact the amount and rate of mineralization in most soils, relative to a constant temperature of 20°C, suggesting that mineralization studies can be conducted at ambient temperatures in laboratory rooms. In contrast, long-term freezing of soil reduced E2 max and k values under constant temperature of 20°C by 19 and 42%, respectively, highlighting the need for a greater discussion for a more consistent approach to the duration and nature of storage conditions of soils prior to the onset of mineralization experiments.

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4. OVERALL SYNTHESIS

4.1 Summary of Research

Environmental contamination by pesticides and steroidal hormones can occur due to point source pollution at the site of application such as contamination resulting from a pesticide or manure tank spill. Pesticides and steroidal hormones can also enter the broader environment through non-source pollution as a result of transport processes such as chemical volatilization, leaching and runoff. The level of contamination of air, water and soil determines, in part, the risk the chemical is posing to organisms, including humans. Pesticide contamination has been linked to an overall reduction in ecosystem biodiversity, as well as reduced crop pollination and loss of crop yields in agricultural production systems (Pimentel et al., 1992). Steroidal hormones contamination in rivers has been shown to induce vitellogenin production in male fish (Tyler et al., 1998).

Prairie wetlands, rivers and dugouts in agricultural regions tend to be contaminated with a suite of pesticides and atmospheric bulk deposition is a likely source for this contamination (Rawn et al., 1999; Cessna and Elliott, 2004; Donald et al., 2007). The impact of atmospheric deposition by pesticides on the contamination of urban features, such as sandboxes and standing water in bird baths and swimming pools, is less known. Chapter 2 of this thesis focused on the types and amounts of pesticides deposited by rain and dust particles in the City of Winnipeg, Manitoba. Bulk atmospheric deposition was

monitored weekly for 71 pesticides at two sites in from May 25 to September 21, 2010 and 2011. A total of nineteen active pesticide ingredients (fourteen herbicides, three insecticides and two fungicides) and four metabolites were detected over the two year study period.

Atmospheric deposition is a source of non-point source contamination of pesticides in urban areas and, based on the types of pesticides detected in this study, originates from both urban and agricultural sources. Total seasonal bulk deposition across the four site-years ranged from 282 to 486 μ g/m² with 99% of seventy bulk deposition samples containing mixtures of two or more pesticides. Dicamba and MCPA concentrations exceeded the Canadian Council of Ministers of the Environment (CCME) irrigation water guidelines in 63 and 43% of the samples, respectively. Major differences between the two study years influencing the types and amounts of pesticides detected in bulk deposition were the differential amount of rainfall (487 mm in 2010 and 182 mm in 2011) and the use of malathion by City of Winnipeg officials. Malathion was sprayed 11 times in our study area in 2010, but not applied by city officials in 2011. Malathion and glyphosate had the highest seasonal deposition amounts in 2010 and 2011, respectively. Total weekly deposition rates were significantly related to weekly total rainfall in both 2010 (r=0.70, p ≤0.0001) and 2011 (r=0.50, p ≤0.002).

Approximately 32% of people in the Canadian prairies use groundwater as their source of drinking water (Environment Canada, 2011). A study conducted in Saskatchewan (Waite et al., 1992) found that over 60% of groundwater samples contained at least one pesticide. Fiore et al. (1996) found a significantly reduced immune response in women who had chronically ingested low levels of a pesticide (aldicarb) in contaminated

groundwater. The movement of a chemical to groundwater is largely influenced by the retention of the chemical by soil constituents and its half-life in soil (Cheng, 1990). Chapter 3 of this thesis focused on the K_f , k and max values of 2,4-D and E2 in ten different Manitoba soil series under field and laboratory conditions. E2- K_f , E2-k, 2,4-D-k, E2 max and 2,4-D max all showed strong predictive PLS models with soil properties. This research developed and designed a novel in-field soil microcosm study that was undertaken over the course of two field seasons. Temperature data recorded during the two field studies were used to simulate the in-field temperature conditions in laboratory incubators. The duration of soil freezing for storage purposes significantly impacted the k and max for both 2,4-D and E2, with reduced mineralization in soils that had been frozen longer.

Three temperature treatments were used in the laboratory incubator studies: mean diurnal temperatures, mean diurnal temperatures including high and low extremes observed in the field and a constant 20°C temperature. The k and max for E2, as well as 2,4-D max and k values were in range with those observed for soils used in previous studies (Yadav and Reddy, 1993; Veeh et al., 1996; Colucci et al., 2001; Picton and Farenhorst 2004; Lucas and Jones, 2006; de Lipthay et al., 2007; Fan et al., 2007; Shymko and Farenhorst, 2008; Xuan et al., 2008; Stumpe and Marschner, 2009; Caron et al., 2010). Fluctuating temperatures during the experiment had little to no effect on the rate of E2 mineralization nor the total amount mineralized in most soil series.

Results from the chapters combined demonstrate the impact of environmental factors on the deposition of the chemicals as well as their environmental fate. Monitoring studies should consist of multiple years to account for the variation within environmental conditions such as those observed in our chemical deposition studies. When determining environmental chemical fate in a laboratory, the duration of the soil in storage under frozen conditions also exhibits a large effect on experimental results and hence a need for more consistent storage conditions among mineralization studies is required.

4.2 Practical Implications

Based on the study results, there should be greater discussion in Winnipeg on the risks that atmospheric deposition by pesticides poses to the urban ecosystem. Some pesticides, such as dicamba, were detected in the deposition samples with over 50% of the samples exceeded the maximum contaminant level as specified by the CCME agricultural irrigation water quality guidelines. Common garden plants such as *Allium sepa* (onion), *Cucumis sativa* (cucumber), *Lactuca sativa* (lettuce), and *Lycopersicon esculentum* (tomato) are at least ten times less tolerant to dicamba when compared to certain agricultural crops (Boutin et al., 2004) and hence the levels detected in the bulk deposition could have negative effects on urban garden plants.

99% of our samples contained a mixture of pesticides. Pesticide mixtures can haveadditive and synergistic effects on aquatic invertebrates (Pape-Lindstrom and Lydy,2009) and frogs (Hayes et al., 2006) found in urban environments. There are currently no

regulations in Canada pertaining to mixtures of pesticides in water; the only regulations that exist are for single pesticides in potable water to protect human health, in surface water to protect aquatic organisms and in irrigation water to protect agricultural productivity. Further toxicological studies or other assessments are needed to determine the risks posed by pesticide mixtures on organism and ecosystem health and the Canadian government needs to develop new regulations concerning levels of pesticides mixtures in water.

In chapter 3 of this thesis, the newly designed in-field microcosm study was successful in measuring mineralization of 2,4-D and E2 and this methodology could be implemented by other researchers. The research also demonstrated that, relative to incubations at a contact temperature of 20°C, incubations with fluctuating temperatures typical of what was observed during the growing season in Manitoba did not have a significant effect on the amount and rate of mineralization of E2 in soil. Not all scientists have equal access to adequate amounts of laboratory incubators, such as researchers conducting complex experiments that would require excessive incubator space or many researchers in developing countries. The results of this study suggest that these researchers can effectively conduct mineralization experiments in a laboratory at room temperature as minor temperature fluctuations will not affect the overall results. Without the space limitations in incubators, larger scale mineralization studies can be undertaken, allowing for more treatments such as different soil types, moisture contents, nutrient and organic matter contents or any combination of these factors. Even temperature fluctuations which may occur in an incubator, due to breakdowns or power outages, are likely not as concerning as typically believed within the scientific community, and the experimental

results would most likely still be valid. Scientists can also use the in-field method to study mineralization conditions without the need for incubators.

Results of this thesis research can be used to further refine the value of sensitive input parameters in pesticide and steroidal hormone fate models. Specifically, the study provides for a better understanding of how different soil properties and temporal temperature fluctuations affect the rate and amount overall amount of chemical mineralization, and this new knowledge provides for the opportunity to improve environmental policy assessments that rely heavily on these predictions of chemical fate parameters in soil. Furthermore, current environmental policy analysis in Canada do not consider atmospheric deposition in its assessment (Cessna et al., 2010) and information collected from the bulk atmospheric deposition study in Chapter 2 can be used to help determine those concentrations.

4.3 Recommendations for Further Study

Further scientific research is needed to explore questions raised by the studies that are described in this thesis. Increasing the number of study sites in future atmospheric deposition studies will strengthen knowledge of the source, persistence and transport of pesticides in the atmosphere which is important for a full evaluation of pesticide deposition in urban environments. Concurrent studies run in both city centers as well as the surrounding rural environment will help to determine if there are differences between these two environments in the types and amounts of pesticides deposited by wet and dry

deposition. The majority of pesticide atmospheric deposition studies include only parent molecules and not their metabolites (Hill et al., 2002; Hill et al., 2003; Waite et al., 2005; Messing et al., 2011; Messing et al., 2012). Tests for the presence of six herbicide metabolites were conducted, and four were detected at frequencies up to 18%. In 2010, AMPA, a metabolite of glyphosate, had the fourth highest total seasonal deposition. This study shows that metabolites significantly contribute to total pesticide deposition. Since atmospheric deposition studies rarely analyze for pesticide metabolites and those that do monitor for only a few metabolites, more studies that monitor for metabolites are needed, particularly for those whose parent molecules have a high rate of use in Winnipeg, such as malathion.

A major limitation for this study was the lack of available current data on pesticide use in both urban and agricultural data. Manitoba Agricultural Services Corporation (MASC), which provided the raw data for agricultural pesticide use, no longer collects these data and there are no other organizations that collect these data. This is not only the case for Manitoba, but most other provinces in Canada as well. The same limitation occurs with the urban pesticide use data as there is no method of determining pesticide use by private citizens and data from applications by city officials are limited except for good records on the application of malathion or other agents used in mosquito control by the City of Winnipeg. Consequently, this study relied heavily on only one year of data available for urban pesticide use from Welch (2012). It is important to provide protocols and programs that ensure accurate data on pesticide use in rural and urban environments in Canada as this will allow for better assessments and programming for sustainable pesticide use in agricultural and human health protection applications.

The low amount of malathion dry and wet deposition in comparison to the amount sprayed leads to additional questions related to the overall fate of malathion applied in Winnipeg in most years, such as which portion of the applied malathion is transported out of the application area, and which portion is degraded into metabolites such as malaoxon which is 61 times more toxic to humans compared to malathion (United States Environmental Protection Agency, 2006). Analyzing samples in future studies for malaoxon may help determine malathion's fate as well as providing information on the concentration of malaoxon in atmospheric deposition. Additional studies on the toxicity of pesticides to non-target organisms, including urban garden plants and plants and animals found in urban ecosystems are needed. Such toxicological assessments and measures will need to include pesticide mixtures that are commonly detected in environmental samples. Continued monitoring of pesticide deposition in urban environments is important for determining short and long term trends as influenced by environmental factors and improved pest control management strategies.

As freezing the soil significantly affected the mineralization experiments in Chapter 3, further research is needed on how storage conditions for soils used in mineralization experiments alter the chemical or biological properties of the soil after it is sampled in the field. Many chemicals, like the ones used in our study, are mineralized through biological processes, therefore the effects of different storage methods on microorganism communities should be further investigated.

Steroidal hormones enter the soil not as single compounds but as part of manure. Manure contains a wide variety of nutrients and microorganisms which are likely to affect mineralization. As the next step, mineralization studies should be conducted using

different types and rates of manure. Some studies have been conducted to investigate these factors (Colucci et al., 2001; Lucas and Jones, 2006; Caron et al., 2010); however they are limited and were all conducted at a constant temperature in the laboratory.

Our microcosm studies were a closed system and did not account for potential leaching, surface runoff, incorporation into soil biota and volatilization that would occur in field soils; these factors must be taken into account when applying these mineralization data to models. In addition, under field conditions soil moisture content can fluctuate along with temperature; therefore the effects of moisture content fluctuations on mineralization should also be studied.

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5. APPENDIX

I. Example of E2 (top) and 2,4-D (bottom) Freundlich Sorption Isotherms for Pembina Clay Loam from Chapter 3

