

**The continuing persistence and biomagnification of
DDT and metabolites in American robin
(*Turdus migratorius*) fruit orchard food chains**

**by
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Abstract

DDT is an organochlorine insecticide that was widely used in fruit orchards in the South Okanagan Valley from the late 1940s and in the 1990s, this was documented to have caused extensive contamination of American robin (*Turdus migratorius*) food chains. Due to the environmental persistence of DDT and its metabolite, *p,p'*-DDE, the objective of this study was to re-sample previous orchards, as well as several new agricultural areas with the prediction that DDT and metabolite concentrations would significantly decline twenty-six years after a similar sample collection was conducted in 1993-1995. This was done by: 1) collecting soil, earthworms and American robin eggs from orchard and non-orchard areas in the South Okanagan Valley, 2) comparing previous and current contaminant burdens for DDE, DDT and DDD metabolites, and 3) calculating biomagnification factors for earthworms and robins on a lipid normalized basis. All robin eggs contained DDE, DDT and DDD, with the highest concentration being *p,p'*-DDE at 107 ug/g (wet weight), confirming that contamination is still present at similar and high levels relative to the 1990s. DDE and DDT levels in robins were significantly higher than *Aporrectodea* and *Lumbricidae* earthworms, and earthworm-robin regressions for DDE showed a significant positive relationship. Biomagnification factors were generally > 1 and were higher for DDE than DDT and DDD. Concentrations of *p,p'*-DDE in American robins in this study were comparable to and/or exceeded published levels in other migratory birds nesting in fruit orchards, including the eastern bluebird (*Sialia sialis*), where reproductive and immunostimulation effects were observed. The relatively high concentrations of DDE in the South Okanagan Valley may pose a health risk to local predators and birds of prey, such as Accipiter hawks and falcons, who often feed at higher trophic levels where DDE and other contaminants are biomagnified.

Keywords: DDT; orchards; American robins; robin eggs; earthworms; biomagnification

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List of Acronyms

ACTH	Adrenocorticotrophic hormone
AITC	Allyl isothiocyanate
AMRO	American robin
ASE	Accelerated solvent extraction
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BDNF	Brain-derived neurotrophic factor
BMF	Biomagnification factor
BSAF	Biota-soil accumulation factor
CARBs	Carbamates
CAS	Chemical Abstract Service Number
CRH	Corticotrophin releasing hormone
DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DDT-r	DDT-related compounds and/or DDT-residues
GABA	Gamma aminobutyric acid
GPC	Gel-permeation chromatography
HEWL	High-evaporative water loss
HPA	Hypothalamic-pituitary-adrenal
K _{OA}	Octanol-air partition coefficient
K _{OW}	Octanol-water partition coefficient
LD ₅₀	Lethal dose resulting in 50% mortality of test organisms
MDL	Method Detection Limit
MFO	Mixed function oxidase
MS-EI	Mass electron impact ionization
MW	Molecular weight
NWRC	National Wildlife Research Centre
OC	Organochlorine
OCS	Octachlorostyrene
OP	Organophosphate
PBDE	Polybrominated diphenyl ether

PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
RA	Robustus arcopallialis
SE	Standard error
SPE	Solid phase extraction
UV	Ultraviolet
VGSC	Voltage-gated sodium channel
VLDL	Very-low density lipoprotein
WHO	World Health Organization

Chapter 1.

Organochlorine (OC) insecticides and dichlorodiphenyltrichloroethane (DDT)

1.1. Introduction

Insecticides, which may be naturally derived (i.e. oil) or synthetically produced, are some of the most toxic chemicals released by humans into the environment (Chowanski et al., 2013; Relyea, 2005). Unlike most xenobiotics, insecticides are designed to kill, repel, or otherwise harm living organisms, and they are one of the few toxic substances that are intentionally applied to the environment, resulting in ubiquitous exposure (Cox & Surgan, 2006). By controlling pest outbreaks, insecticides offer several important economic and biological advantages, including increased crop yield, reduced soil disturbance, and prevention of waterborne and insect diseases (Chagnon et al., 2014; Evans, 1985). However, insecticides can undergo various long-term changes once released into the environment, including biotransformation to metabolites, volatilization into the atmosphere, regional transport, wet/dry deposition, runoff, groundwater discharge and global distillation (Williamson et al., 2013), thereby increasing their overall persistence. The effects of climate change (i.e. temperature increases and extreme weather events), are also believed to influence the long-term environmental fate of insecticides in various ways, including increased mobilization from reservoirs, increased airborne transport via wind and ocean currents, and delayed environmental degradation (Wiwanitkit, 2013), consequently impacting the composition and structure of food webs, as well as the source, transport, fate and accumulation of insecticides in biotic and abiotic samples. Accordingly, monitoring programs conducted in North America have found insecticides in one or more samples from almost every stream sampled, with over 70% of insecticides being detected in aquatic and terrestrial food chains based on stream water, ground water, bed-sediment and fish sampling (Gilliom & Hamilton, 2006).

Organochlorines (OCs) are a diverse class of insecticides that were originally developed in the 1930s for industrial and domestic purposes (Blus et al., 2006; Singh, 2016). The use of OC insecticides quickly surged in the late 1940s and 1950s during

The Green Revolution, which saw a drastic increase in population growth (Pingali, 2012) and the subsequent production of food grains and other agricultural crops, including rice, wheat, and corn (Pimentel et al., 2004). According to the World Health Organization (WHO) and the Stockholm Convention on Persistent Organic Pollutants (POPs), the most commonly used OC insecticides included the: 1) dichlorodiphenylethanes (DDT, dicofol, methoxychlor), 2) chlorinated cyclodienes (aldrin, dieldrin, endrin, chlordane, endosulfan, heptachlor), and 3) hexachlorocyclohexanes (mirex, lindane, oxaphene). In insects, these OC insecticides caused toxicity by either irreversibly binding to voltage-gated sodium channels (VGSCs), or in the case of the latter two OCs, by inhibiting gamma-aminobutyric acid (GABA) chloride-channel complexes, thereby disrupting the peripheral nervous system (Davies et al., 2007; Silver et al., 2014). Due to their widespread use and nonpoint-source contamination, OC insecticides have also caused non-target effects by interfering with the endocrine, immune and reproductive systems in vertebrates (Li et al., 2014; Takser et al., 2005; Tiemann, 2008). The intensification of agriculture and large-scale use of insecticides over the years has also led to unintended ecological impacts, including direct impacts on bird and pollinator populations (Bishop et al., 2020; Blacquièrè et al., 2012; Rundlöf et al., 2015), pest resistance, loss of habitat, degraded water quality and contaminated food sources (Dirbaba et al., 2018). In addition, many OC insecticides are formulated with various inactive ingredients that can increase the oral and dermal absorption of the insecticide(s), thus increasing their toxicity to various target and non-target organisms (Venail et al., 2015). As a result of their documented broad-spectrum toxicity, the production, use and release of OC insecticides is currently banned in many parts of the world, with the exception of DDT, which is currently listed in Annex B to the Stockholm Convention with its use restricted for the control of vector-borne diseases, such as malaria and leishmaniasis (Sarkar et al., 2008). However, DDT is known to persist at high concentrations even in areas of past intensive use (Bishop et al., 2000; Brogan et al., 2017; Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000), thereby increasing the risk of exposure to human and ecological receptors.

DDT is a colorless, tasteless and odorless compound that is synthesized by Friedel-Crafts reactions between trichloroacetaldehyde and two chlorobenzene rings, with a molecular weight (MW) equal to 354.51 g/mol and a chemical formula of $C_{14}H_9Cl_5$ (Wang et al., 2008). DDT was first synthesized in 1874 by Othmar Zeidler and was later

developed into a neurotoxic insecticide by Paul Hermann Müller in 1939 in order to control mosquitoes and other insects that carry typhus, malaria and dengue virus (Berg et al., 2017; Hurlbut et al., 1952; Wang et al., 2008). The use of DDT quickly expanded after World War II and eventually became a common agricultural, and commercial insecticide starting in the late 1940s, with approximately 80 million pounds (valued at more than \$20,000,000) of DDT being shipped abroad annually between 1959 to 1972 in North America (Sherman, 1977). To date, more than two billion tons of DDT has been used worldwide, with India, China, the Democratic People's Republic of Korea, and the United States being listed as the top exporters of DDT during the 20th century (Berg, 2009). DDT was manufactured in the United States for Canada up until 1985 and was primarily used to control codling moth (*Cydia pomonella*) infestations in fruit orchards (Environment Canada, 1995). Due to its efficacy and low cost of manufacturing, DDT was formulated and used in many different ways, including aerial sprays, as well as foliar application via powders, granules, and emulsifying concentrates (Berg, 2009). Technical-grade DDT (i.e. Gesarol, Sanex, Cezarex, Anofex, Chlorophenothane, Dicophane, Ixodex) was composed of different DDT compounds in para, para' (p,p' -) and ortho, para' (o,p' -) conformation, including o,p' -DDD and p,p' -DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane), as well as o,p' -DDE and p,p' -DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene). However, up to 80% of the DDT formulation consisted of the active ingredient p,p' -DDT, with the remainder of the mixture consisting of o,p' -DDT, o,p' -DDD, p,p' -DDD, o,p' -DDE, and/or p,p' -DDE isomers depending on the formulation (Wong et al., 2015). These DDT-related (DDT-r) compounds are commonly referred to as impurities or metabolites of DDT, and while they do not occur naturally, they continue to be detected at hazardous levels in air, water, sediment, soil and biota, especially DDE due to its long half-life, lipophilicity, and metabolic stability (Berg, 2009; Keller, 2010).

Like most persistent organic pollutants, DDT is a hydrophobic and lipophilic compound, allowing it to easily dissolve in fatty tissue and bind to organic particulates in soil and sediment (Environment Canada, 1995). These toxic properties allow DDT to build up, or bioaccumulate in organisms and affect higher trophic level species, through a process known as biomagnification, where the chemical in an organism exceeds that of its diet (Arnot & Gobas, 2006; Elliott et al., 2005; Fremlin et al., 2020; McIntyre & Beauchamp, 2007; Muir et al., 1999). The accumulation of DDT in an organism can be quantified by different metrics based on the route of exposure. Bioaccumulation factors

(BAFs) are measured under field conditions and include the uptake of a chemical from all routes, including diet, dermal and respiratory pathways, whereas bioconcentration factors (BCFs) include respiratory and dermal pathways only (i.e. no diet), and are typically measured under controlled laboratory conditions (Arnot & Gobas, 2006). Biomagnification factors (BMFs) can be determined under laboratory and field conditions and are calculated as the ratio of a chemical in a predator (expressed in units of mass of chemical per kg of the organism) to that of its diet or prey (Arnot & Gobas, 2006). Trophic magnification factors (TMFs) represent the average change in contamination concentrations per trophic level (using $\delta^{15}\text{N}$ isotopes) and usually involve multiple species and/or exposure pathways (Arnot & Gobas, 2006; Brink et al., 2015). These measurements can be expressed on a wet weight basis or dry weight basis, although are commonly expressed on a lipid normalized or lipid weight basis in order to allow for direct comparisons between different species (Arnot & Gobas, 2006). Diet and feeding/habitat range are considered the most important determinants of DDT exposure; however, the respiratory pathway can also be an important uptake/eliminate route affecting exposure in aquatic and terrestrial organisms (Kelly & Gobas, 2003; Kelly et al., 2007). As such, bioaccumulation and biomagnification metrics are often compared to either an octanol-water partition coefficient (K_{OW}), which partitions xenobiotics between an aqueous phase and lipid phase *in-vivo*, or an octanol-air partition coefficient (K_{OA}), which describes the partitioning of a chemical between the atmosphere and different environmental organic phases related to soil and sediment particulates (Kelly et al., 2007). Unlike water-respiring organisms that are capable of gill ventilation, the elimination of organic contaminants in terrestrial organisms is thought to be inversely related to a chemical's K_{OW} , with log K_{OW} 's between 2 to 5 and log K_{OA} 's between 6 to 12 generally resulting in biomagnification (Brink et al., 2015; Kelly et al., 2007). Growth dilution is also considered to be an important pseudo-elimination process, which can result in the dilution of a chemical's concentration due to an increase in the volume of tissue of an organism (Arnot & Gobas, 2006). The physio-chemical properties of DDT-r compounds and bioaccumulation potential, therefore, differs greatly between different species and trophic levels, reflecting differences in the pattern(s) of exposure, route(s) of uptake, and subsequent elimination of metabolites.

The environmental impacts of DDT were first brought to the attention of the broad public and to politicians in 1962 by Rachel Carson, a marine biologist and long-term

environmental activist. Carson is widely credited to citing some of the earliest anecdotal research of DDT, along with the reproductive toxicity of DDE metabolites in humans and wildlife, including birds, fish, amphibians and mammals (Carson, 1962). The link between DDT and egg-shell thinning in raptors was not discovered until after the publication of *Silent Spring* by British Nature Conservancy researcher, D.A. Ratcliffe, who conducted field surveys in the 1960s and reported a decrease in egg weight and an increased incidence of broken eggs in peregrine falcons (*Falco peregrinus*), sparrowhawks (*Accipiter nisus*) and golden eagles (*Aquila chrysaetos*) from different regions (Elliott, Bishop & Morrissey, 2011; Ratcliffe, 1967). Shortly thereafter, Hickey & Anderson (1968) revealed that eggshell thinning was occurring in several raptorial species in the United States, including bald eagles (*Haliaeetus leucocephalus*) and ospreys (*Pandion haliaetus*) that were feeding on DDT-contaminated prey, which weighed significantly in the decision to ban DDT in North America. Due to the environmental persistence of DDT, ongoing contamination continues to be observed in locally exposed, year-round resident populations of Accipiters in Canada (Elliott & Martin, 1994), including the Cooper's hawk (*Accipiter cooperii*) and sharp-shinned hawk (*Accipiter striatus*), in large part due to their preference for terrestrial birds and patchiness in soil contamination linked to their food chains (Bishop & Brogan, 2013; Brogan et al., 2017). Due to their trophic positions and diet preferences, raptors and aquatic birds are more vulnerable to the effects of eggshell quality compared to gallinaceous birds, gulls and passerines, who are more prone to the acute effects of direct ingestion of contaminated prey (Rattner et al., 1984; Walker, 1983). Yet, even decades after the ban and usage of DDT, several studies have reported high DDE residues in migratory thrushes and their eggs, including the eastern bluebird (*Sialia sialis*) and the American robin (*Turdus migratorius*) (Barker, 1958; Bishop et al., 2000; Dimond et al., 1970; Elliott et al., 1994; Fluetsch & Sparling, 1994; Harris et al., 2000; Hellou et al., 2013; Johnson et al., 1976; Smith, 2004; Stringer et al., 1974).

The American robin is a common breeding species found throughout North America that has adapted well to both natural and anthropogenic habitats (Cannings et al., 1987; Vanderhoff et al., 2014). American robins are an omnivorous species and have a highly variable diet throughout their annual cycle, shifting from soil invertebrates during the breeding season, to fruits and berries in the fall and winter (Sallabanks & James, 1999; Vanderhoff et al., 2014). Earthworms can form up to 80% of invertebrate biomass

in soil and can account for up to 40% of the overall diet of robins (Armitage & Gobas, 2007). During the spring and summer months, American robins can capture up to 20 worms per hour (Montgomerie & Weatherhead, 1997) and some have been reported to consume 14 feet of earthworms in a day (Sibley, 2020), either for self-feeding or nestling provisioning. Earthworm attack rates and foraging times are considered to be higher in fruit orchards, agricultural areas and other well-lit areas with saturated soils (Eiserer, 1980; Vanderhoff et al., 2008). In the Okanagan Valley, American robins readily use orchard habitat for nesting and feeding and could uptake significant amounts of DDT-r from earthworms, which are known to accumulate high levels of DDT-r from past intensive use (Elliott et al., 1994). American robins are also considered resident or non-migratory and many local populations overwinter in the Okanagan Valley (Campbell et al., 1997; Cannings et al., 1987). American robins in the Okanagan are therefore likely to accumulate greater concentrations of DDE during the breeding season as a result of their extensive orchard use and earthworm diet (Harris et al., 2000). Several soil-earthworm-robin exposure studies have been conducted; however, a limited number of prey samples were analyzed for DDT content (Barker, 1958; Dimond et al., 1970; Johnson et al., 1976), thereby lending uncertainty between DDT contamination in migrants and residents (Harris et al., 2000). As American robins continue to make extensive use of orchard habitat (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000), they provide a useful indication of the ongoing DDT exposure of other resident and migratory birds, including raptors (Elliott et al., 2005), who often feed at higher trophic levels, thereby increasing their toxicological risk.

1.2. Research objectives

The main objective of this study is to determine whether a legacy organochlorine, DDT, is persisting and biomagnifying in fruit orchards twenty-six years after a similar sample collection was conducted in 1993-1995 in the Okanagan Valley of B.C. This study focusses on a terrestrial food chain model that includes soil, earthworms and American robin eggs (with a focus on the latter two in this thesis) and uses a refined approach by assessing biomagnification factors (BMFs) as fugacity ratios, i.e. expressing chemical concentrations on a lipid normalized or lipid weight basis. In addition to assessing the current state and extent of DDT contamination in local biota, this research will improve our understanding of the underlying processes controlling

biomagnification in field studies and will aid in any current weight-of-evidence approaches involving terrestrial-based-food-web bioaccumulation models that can be used in the regulation of commercial chemicals, cleanup and remediation of contaminated sites, exposure assessments of current-use and emerging pesticides, ecotoxicological risk assessments and derivation of environmental quality criteria (Armitage & Gobas, 2007; Burkhard et al., 2011).

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Chapter 2.

The continuing persistence and biomagnification of DDT metabolites in American robin food chains

2.1. Introduction

DDT contamination continues to be an environmental issue globally, due to its long-term historical use, persistence in environmental media, ability to bioaccumulate and biomagnify through food chains, and toxic and endocrine disrupting effects in humans, and wildlife (Alexander & Maroli, 2003; Corsini et al., 2008; Elliott et al., 2018; Keifer & Firestone, 2007; Rogan & Chen, 2005; Wolff et al., 2000; Wong et al., 2015; Ye et al., 2013). In North America, DDT was a synthetic insecticide used from the late 1940s to control for various pests in agriculture, forestry and domestic areas (Environment Canada, 1995). Due to its broad-spectrum toxicity, the production, use and release of DDT for agricultural purposes was banned worldwide in 2004 by the Stockholm Convention on Persistent Organic Pollutants, a multilateral treaty aimed at protecting human health and the environment (Environment Canada, 1995). Even decades after its use, the environmental persistence of DDE metabolites, specifically *o,p'*-DDE and *p,p'*-DDE, in soil invertebrates and avian wildlife continues to be documented (Bishop et al., 2000; Blus et al., 1987; Currier et al., 2020; Elliott et al., 2009; Elliott, 2005; Elliott et al., 1994; Elliott & Martin, 1994; Fremlin et al., 2020; Gill et al., 2003; Harris et al., 2000; Richards et al., 2005; Schmitt et al., 2018). The trophic transfer, or biomagnification, of DDE metabolites in terrestrial food chains has been historically associated with eggshell quality effects and population declines of several raptors and aquatic birds, including peregrine falcons (*Falco peregrinus*), brown pelicans (*Pelecanus occidentalis*), double-crested cormorants (*Phalacrocorax auritus*), ospreys (*Pandion haliaetus*), bald eagles (*Haliaeetus leucocephalus*), American kestrels (*Falco sparverius*), sharp-shinned hawks (*Accipiter striatus*), black-crowned night herons (*Nycticorax nycticorax*), and white-faced ibis (*Plegadis chihi*) (DeWeese et al., 1986; Elliott et al., 1988; Elliott & Martin, 1994; Elliott & Shutt, 1993; Henny et al., 1985; Henny et al., 1983; Lincer, 1975; Wiemeyer et al., 1988; Yates et al., 2010). However, in the recent past, there has been a growing body of evidence documenting the persistence of DDE metabolites in migratory thrushes, including eastern bluebirds (*Sialia sialis*) in

Southern Ontario (Bishop et al., 2000), as well as American robins (*Turdus migratorius*) nesting in certain localized areas in the Okanagan Valley (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000; Iwaniuk et al., 2006; Smith, 2004).

The Okanagan Valley is located in the south-central interior of British Columbia, Canada and is an intensive fruit growing region (past and present) that generates more than \$118 million in wholesale revenue and approximately \$800 million in economic activity (BC Fruit Growers' Association, 2010). The mild and semi-arid climate of the Okanagan Valley is thought to be associated with various biogeochemical conditions that encourage fruit set, kill overwintering pests and assist in the thinning of fruit, as a small percentage of buds typically suffer winter damage (BC Fruit Growers Association, 2010; Kuo et al., 2012). Commercial fruit orchards account for more than 10,000 hectares in the Okanagan-Similkameen and represent a significant component of BC's tree fruits, which includes apple, apricot, peach, pear, sweet cherry, and sour cherry crops (Kuo et al., 2012). Okanagan fruit orchards were historically treated with large amounts of DDT to control for codling moth (*Cydia pomonella*) infestations in apple and pear orchards, and some areas received up to 27 kg DDT/ha/year, which is approximately three times more than the recommended application rate in other provinces (Elliott et al., 1994; Harris et al., 2000). Codling moth damage occurs when larvae burrow inside fruits to feed and when second-generation larvae leave the fruit to pupate, which in both cases allows fungi and bacteria to enter the fruit, and cause fruit rot damage (BC Fruit Growers' Association, 2010). Although orchards are among the most heavily sprayed agricultural systems, orchards provide understory vegetation and complex structural habitat for a range of wildlife (Garcia et al., 2018). Orchards also provide significant migration corridors, such as hedgerows, grasslands, wood pasture, ponds and shrubs that can increase habitat connectivity in an otherwise fragmented landscape (Garcia et al., 2018), thus driving plant community composition and overall biodiversity. As a result, orchards are highly productive areas and are greatly utilized by birds for foraging and nesting habitat (Bishop et al., 2000). However, current-use pesticides and legacy residues can have significant direct and/or indirect impacts on birds, and several studies have documented high residues of DDT-r compounds in Okanagan orchards (Elliott et al., 1994; Harris et al., 2000, Iwaniuk et al., 2006; Smith, 2004), with *p,p'*-DDE concentrations reaching 302 ug/g (wet weight) in American robins

during the 1990s, the highest concentration ever detected in a robin in the region (Gill et al., 2003).

American robins are a common breeding species in the Okanagan and previous studies have documented their extensive use of orchard habitat (Cannings et al., 1987; Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000). Unlike other long-distance migratory species in the *Turdidae* family that migrate between their breeding areas and wintering grounds, American robins are considered short-distance migrants with some plasticity in migratory behaviour (Vanderhoff et al., 2020). Migration allows individuals to avoid uncertainties with temperatures, food availability and other factors influencing migration, such as inter- and intraspecific competition in saturated habitats, as well as avoiding predators and disease (Chin & Lentink, 2017; Fudickar et al., 2013; Gilroy et al., 2016; Hegemann et al., 2019; Lindenmayer et al., 2018). Migratory behaviour in birds may also be related to age or sex, in which for instance, socially dominant males force subordinate individuals (i.e. females and juveniles) to leave specific areas due to competition for resources (Gow & Wiebe, 2014). However, American robins overwintering in the Okanagan could benefit from different food items throughout their annual cycle, such as fruits, berries and earthworms, in which the latter are frequently contaminated with DDT (Cannings et al., 1987; Harris et al., 2000). Still, other robins travel just under a hundred kilometers from their breeding grounds and recent studies suggest that more and more populations of American robins are adopting a non-migratory strategy, possibly due to climate change (Vanderhoff et al., 2020). As a result, American robins in the Okanagan could accumulate greater concentrations of DDT-r due to their earthworm diet and because of the potential for migrants, and residents to use orchard habitat year-round (Campbell et al., 1997; Cannings et al., 1987).

American robins require extensive amounts of protein, especially when both sexes are molting, and when females are producing eggs (Vanderhoff et al., 2020). In the Okanagan, American robins frequently nest in orchards and will feed almost exclusively on earthworms near their nest sites (Harris et al., 2000; Howell, 1942; Sallabanks & James, 1999), which are known to accumulate high levels of DDT-r compounds as a result of historical use (Harris et al., 2000), as well as other mixed residues containing organochlorine insecticides (Fluetsch & Sparling, 1994). American robin eggs therefore provide a direct link of DDT contamination from the soil (Bishop et al., 2000) and can be used to represent adult exposure, since maternal concentrations

are mobilized and deposited directly into eggs during the breeding season (Elliott et al., 1994). Several field studies have documented the ongoing persistence of DDT-r compounds in soil, earthworms and robins (Barker, 1958; Dimond et al., 1970; Johnson et al., 1976; Knupp et al., 1976), with bioaccumulation factors (BAFs) indicating slight regional differences of contamination. However, a study published by Harris et al. (2000) confirmed that American robins were acquiring DDT-r burdens locally in Okanagan orchards, with reported average *p,p'*-DDE concentrations of 5.2 ug/g, 52 ug/g and 484 ug/g (dry weight) in soil, earthworm and robin eggs, respectively. Whether or not DDT-r compounds are persisting and biomagnifying in orchard food chains remains unclear, since many of the early studies had reported a high degree of variability among orchard sites, had small sample sizes and/or did not analyze sufficient earthworm communities for DDE content. Additionally, there was no significant relationship between DDE concentrations in earthworms and robins in the Harris et al. (2000) study, thereby lending uncertainty to the link between robin contamination and orchard soils.

Due to the environmental persistence of DDT and its main metabolite, *p,p'*-DDE, the objective of this study was to re-examine sites sampled in 1993-1995 by Harris et al. (2000), as well as several new agricultural areas in the South Okanagan Valley, with the prediction that DDT-r concentrations would significantly decline in soil, earthworms and American robin eggs. The research questions of this study are:

1. To determine the extent of DDT-r contamination in soil, earthworms and American robins twenty-six years after an intensive sample period for the same biota and soil
2. To determine local DDT-r burdens and assess any biomagnification trends in American robins nesting in the Okanagan
3. To determine the quantitative relationships among DDT-r concentrations in different earthworm species and robins

2.2. Methods

2.2.1. Sampling orchards and collection sites

Sampled orchards were located in the Okanagan River Valley, BC, Canada (approximate latitude 50°21'49"N, longitude 119°20'59"W) and were generally within a 20 km² radius of Penticton, Naramata and Summerland (Figure 1). Ten orchards were sampled, which included standard-size, semi-dwarf and dwarf trees of apple, cherry, and pear varieties. Three out of the ten orchards were part of the original Harris et al. (2000) study and many of the orchards sampled in previous field studies were no longer present, and/or were converted to vineyards. All orchards were confirmed to be intact since the 1950s when DDT was actively sprayed, including two apple orchards in Summerland, B.C., that were established in the 1920s and were not previously sampled. Non-orchard habitats included dry grassland, open pine forest and wetlands of the White Lake Grasslands Protected Area and had no known prior usage of DDT in the area.

2.2.2. Soil sampling

Three soil samples were collected within each orchard (n = 30) from May 13, 2019 to June 7, 2019. Soil samples were collected in preferred robin foraging locations and were collected at least ten metres from each subsample, with the assumption that there could be spatial differences in DDT-r contamination within the orchard (Stringer et al., 1974; Stringer et al., 1975). To be consistent with sampling techniques used by Harris et al. (2000), soil samples were collected by first digging out a block of soil approximately 5-10 cm deep using a trowel and/or bulb planter, which was thoroughly cleaned with 70% ethanol in between uses. Each soil sample was directly transferred to a separate 250 ml chemically rinsed jar (acetone and hexane), stored immediately on ice at the sample site and within 1-2 hours, stored frozen at -20 °C until thawed for chemical analysis. Chemical analyses for soil samples could not be completed at this time and will not be discussed in this thesis.

2.2.3. Earthworm sampling

Earthworm samples were collected on the same dates, and in close proximity, to the soil samples within each orchard. In order to account for any spatial and/or other

potential sampling discrepancies, earthworm samples were collected at three locations within each orchard that had high robin activity and/or saturated soil (i.e. plant and foliage litter). In areas where no foraging was observed, earthworm samples were collected in damp/moist areas, which would have represented ideal areas where robins could potentially forage. To be consistent with sampling techniques used by Harris et al. (2000), the area within a 60 cm² wooden quadrat at each site was first cleared of groundcover with a rake, followed by clipping, and removing grass and weeds, when present. Earthworms were collected via a combination of hand sorting and chemical expulsion, by first preparing a stock solution of 4 g/L of allyl isothiocyanate (AITC; Fisher Scientific, Ottawa, ON, CAN; CAS# 57-06-7), which was diluted with 4 L of isopropanol (Fisher Scientific, Ottawa, ON, CAN; CAS# 67-63-0). A final amount of 50 mg/L was mixed with 10 L of water and poured over each quadrat with a watering can at each sampling site.

Surfacing earthworms were collected and rinsed in clean water for approximately 60 seconds to remove any residual AITC solution and other surficial material. Earthworms were placed on moist paper towels in sealed aluminum trays for at least 48 hours to clear their gut contents (i.e. depuration). Individual earthworms were rinsed with water and were identified to species, weighed and tallied by site. Earthworms (n = 19; 437 individuals) were grouped by species from the same orchard and placed into 100 ml chemically rinsed jars (acetone and hexane) and refrigerated at -20 °C until thawed for subsampling and chemical analysis. In some cases, earthworm species were identified as either juveniles or adults. In very few cases where earthworms could not be identified to species (i.e. setae and/or pores not visible), earthworms were identified and grouped by genus.

2.2.4. American robin sampling

Identification of robin nests (n = 10 orchards; n = 2 non-orchard areas) and egg collections (n = 22 nests; n = 22 eggs; 1 egg per nest) were conducted in the Okanagan Valley (Penticton, Naramata and Summerland) from May 13, 2019 to June 7, 2019. American robin nests were located using a systematic row-by-row search in nine conventionally managed (pesticide sprayed) orchards and one certified organic (no pesticides used for at least 5 years) orchard. In some cases, robin nests were located in adjacent buildings or sheds within orchards. Robins at each nest found were observed

for approximately 10-15 minutes to ensure they were exclusively feeding in orchard habitat. Nest contents were examined with an extending mirror pole and also by climbing trees to better inspect nests. All nests were monitored daily and were considered for sampling if there were at least 3 eggs within each nest.

Within each orchard, one robin egg was arbitrarily collected from an active nest for up to 3 nests per orchard, and a wooden robin egg was simultaneously placed to reduce the risk of nest abandonment (Harris et al., 2000). Nests were subsequently monitored for at least one hour to ensure that robins returned to their own nests, and to ensure that there were no predators or human disturbances post egg removal. Upon collection, robin eggs were refrigerated (4 °C) for up to four weeks. To be consistent with sampling techniques used by Harris et al. (2000), robin eggs were first weighed and measured with a ruler to determine their length and width. Eggs were lightly scored along the circumference with a scalpel that was rinsed with 70% ethanol in between uses. Egg contents were stored in chemically rinsed jars (acetone and hexane) and frozen at -20 °C until thawed for subsampling and chemical analysis.

Developmental stages for any visible embryo was noted based on a chicken egg reference guide with development time being adjusted. Eggshells were stored at room temperature and dried for at least one month before eggshell thickness was measured. Handling and collection of American robin eggs was conducted under a Simon Fraser University Animal Care permit (1299B-19) following guidelines of the Canadian Council on Animal Care and was authorized by Environment and Climate Change Canada under a *Migratory Birds Convention Act* permit (SC-BC-2019-0008).

2.2.5. Chemical analyses

Earthworm and robin egg samples were shipped frozen on dry ice to the National Wildlife Research Centre (NWRC) in Ottawa, ON and stored in -20 °C upon reception. Earthworm and robin egg contents were analyzed as whole homogenates and were cut into small pieces using a ball-mill (Retsch™ MM400 Mixer Mill, Fisher Scientific). Approximately 0.25–3.0 g of sample homogenate was ground with diatomaceous earth (J.T. Baker, NJ, U.S.A.), which was then spiked with 25 µL of a standard solution. Lipids from American robin and earthworm samples were extracted with a 50:50 dichloromethane:hexane (DCM:HEX) solvent mixture using an accelerated solvent

extraction system (ASE, Dionex ASE 350, CA, USA). DDT-r compounds and other chemicals in the extracts were then separated from the remaining lipids and analyzed using gel-permeation chromatography (GPC; GX-271 Liquid Handler, Gilson, Inc., WI, USA). Any residual lipids were removed with solid phase extraction (SPE). Final samples were concentrated to 100 μ L using nitrogen evaporation. All samples contained 400 μ L of iso-octane prior to subsequent instrumental analyses.

DDT-r and other compounds were analysed using an Agilent 7890 gas chromatograph (Agilent Technologies, CA, USA) coupled to a single quadrupole mass analyzer (Agilent 7000 MS) in electron impact ionization (MS-EI) mode. A 15 m DB-5MS column (0.25 mm ID, 0.25 μ m film thickness; J&W, Agilent Technologies) was used with the injector in splitless mode and held at 280 °C. The internal standards for the quantification of OCs and PCBs were carbon labelled and were selected to cover the range of tri- to octa-PCBs: 13C-PCB28, 13C-PCB52, 13C-PCB118, 13C-PCB153, 13C-PCB180 and 13C-PCB194. Congeners that co-elute are reported as a sum and are listed in the form PCB xx/xx (e.g. PCB 18/17 is the sum of the co-eluting congeners PCB-18 and PCB-17). Quantification of PCBs and PBDEs was determined with selected ion monitoring (MRM). The internal standards for the quantification of PBDEs were also carbon labelled and covered BDE-30, BDE-118, BDE-156, 13C-*syn*-Dechlorane Plus, 13C-*anti*-Dechlorane Plus and 13C-BDE-209. The following analyte pairs coelute: BDE-15 and -TBECH; BDE-154 and BB-153. These congener pair concentrations are reported as a sum, for example; BDE-154/BB-153.

2.2.6. Stable isotope analyses

Stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were analyzed at the Jan Veizer Stable Isotope Laboratory (formerly G. G. Hatch) at the University of Ottawa (Ottawa, ON, Canada). American robin egg contents and whole-body earthworms were stored in -20 °C upon reception. Samples were briefly freeze-dried, ground up with a polytron and ball-mill and weighed (~1 mg) into 6 mm tin capsules. Samples were combusted at 1800°C in a Vario EL Cube elemental analyzer (Elementar, Germany) interfaced to a Delta Advantage isotope ratio mass spectrometer (IRMS; Conflo III, Thermo Scientific, Germany).

The internal standards used for $\delta^{13}\text{C}$ (in ‰) included: C-51 Nicotiamide (0.07, -22.95), C-52 mix of ammonium sulphate and sucrose (16.58, -11.94), C-54 caffeine (-

16.6, -34.46), and blind standard C-55 glutamic acid (-3.98, -28.53). These standards cover the natural range and the data is reported in Delta notation δ , with units in per mil (‰) and are expressed as:

$$\delta X = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$$

where δX is ^{15}N or ^{13}C , and R is the ratio of the abundance of the heavy to the light isotope. All $\delta^{15}\text{N}$ is reported as ‰ vs. AIR and normalized to internal standards calibrated to International standards IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (-4.52‰) and USGS-41 (47.57‰). All $\delta^{13}\text{C}$ is reported as ‰ vs. V-PDB and normalized to internal standards calibrated to International standards IAEA-CH-6 (-10.4‰), NBS- 22 (-29.91‰), USGS-40 (-26.24‰) and USGS-41 (37.76‰).

2.2.7. Biomagnification factors (BMFs) and lipid-equivalent concentrations

Lipid contents were measured in American robin egg and earthworm samples using a gravimetric method. Approximately 1 mL (or 10%) of the extracted sample was transferred into a pre-weighed aluminum dish, which was allowed to air dry in a fume hood for approximately 30 minutes, and then re-weighed to calculate the lipid content on a wet weight basis.

In order to account for differences in lipid contents between robins and earthworms, chemical concentrations in wet weight were expressed in terms of lipid equivalent concentrations ($C_{\text{lipid equivalent}}$; ug/g of lipid equivalent) (Arnot & Gobas, 2006). Non-lipid organic matter was also incorporated into the lipid normalization to account for earthworm samples with low lipid content and high protein content. Therefore, lipid, protein, and non-lipid organic carbon content were included in the normalization for all samples. Lipid-equivalent concentrations were based on the following equation:

$$C_{\text{lipid equivalent}} = C_{\text{wet}} / [L_{\text{wet}} + P_{\text{wet}}(0.05) + OC_{\text{wet}}(0.1)]$$

in which L is the lipid fraction of the sampled tissue (g of lipid/g of wet tissue), P is the protein fraction estimated as the product of the percent of nitrogen measured during elemental analysis (g of N/g; converted to wet tissue) multiplied by a species-specific nitrogen:protein conversion factor (g of protein/g of wet tissue) (Janssen et al.,

2017; Sosulski & Imafidon, 1990) and OC is the fraction of non-lipid organic carbon, estimated as the percent of organic carbon measured during elemental analysis (i.e. g of C/g; converted to wet tissue) subtracted from the lipid content of the sample. The constant 0.05 represents that proteins exhibit 5% of the sorptive capacity of lipids, and the constant 0.1 assumes that non-lipid organic carbon behaves similar to carbohydrates and exhibits 10% of the sorptive capacity of lipids (Debruyn & Gobas, 2007). Biomagnification factors for *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD at each site were calculated by dividing the average lipid equivalent concentration of each DDT-r compound in American robin eggs by the average concentration in all earthworm species. BMF values greater than 1 indicate that a chemical is biomagnifying in the food-web, a BMF of less than 1 indicates trophic dilution and a BMF equal to 1 indicates that on average, the chemical is likely not biomagnifying (Arnot & Gobas, 2006; Burkhard et al., 2011).

2.2.8. Quality control and assurance

In both labs, method procedural blanks were processed with each extraction batch to monitor for background interferences and/or contamination. Accuracy and precision were evaluated by running an aliquot of a Certified Standard Reference Material (SRM; NIST 1947 Lake Michigan Fish Tissue), as well as duplicates of randomly selected egg or earthworm samples. For each contaminant, the method detection limit (MDL) was defined as the minimum measured concentration of analyte producing a peak with a signal to noise ratio (S/N) of 3. The MDLs for *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD were 0.0008 ug/g, 0.0012 ug/g and 0.0006 ug/g (wet weight), respectively. All samples were above the MDL (i.e. not censored). A sample field blank was also collected during soil, earthworm and egg sampling to monitor for possible contamination from handling. Sample field blank collection consisted of opening an empty chemically rinsed jar and closing it upon completion of sorting for each sampling activity and region. Chemical concentrations were blank corrected for all earthworm and robin samples.

2.2.9. Statistical analyses

All statistical analyses were conducted in R (RStudio Inc, version 1.2.5042). A mixed effects model was conducted to analyze stable nitrogen ($\delta^{15}\text{N}$) and carbon

($\delta^{13}\text{C}$) isotopes in American robin and earthworm samples, with species as a covariate and site as a random effect. All stable isotope graphs were created in R using the stable isotope mixing model ('simmr') and 'ggplot2' packages.

Factors related to American robin samples (% lipid, weight, length and developmental stage of each egg) and earthworms (density and biomass) were checked, and confirmed for normality using the Shapiro-Wilk Normality Test. An AIC (Akaike Information Criterion) model selection process was used to distinguish among a set of possible models describing the relationship between each DDT-r compound and different species. For robin samples, each DDT-r compound (wet weight) was analyzed separately as the dependent variable in a mixed effects model, with % lipid, developmental stage and the interaction term weight*length as main effects, and site as a random effect. To determine whether the presence of different earthworm species contributed to varying earthworm DDT-r residues, each DDT-r compound (wet weight) was also analyzed separately as the dependent variable in a mixed effects model, with earthworm species as a covariate, along with density and biomass as main effects, and site as a random effect. A Tukey's Multiple Comparisons Test was conducted if both mixed models identified any significant main effects or an interaction. To account for the skewness of DDT-r concentrations, Box-Cox methods were used to select the ideal transformation, which was a square-root transformation.

To be consistent with previous studies, the relationship between DDT-r concentrations in American robins and earthworms was also assessed using a simple linear regression. The linear regression was based on pooled robin and earthworm DDT-r data from each orchard, since there were unpaired robin and earthworm samples at some sites. Biomagnification factors (BMFs) were compared among different orchards using the non-parametric Wilcoxon-Signed Rank Test. The statistical significance of p-values for all models was assessed at $\alpha = 0.05$.

2.3. Results

2.3.1. Stable isotopes

Stable nitrogen and carbon isotopes were analysed in 22 American robin eggs and 19 earthworm samples (Figure 2). $\delta^{15}\text{N}$ varied with species group ($F_{3,27} = 74.87$, $P <$

0.001), with average $\delta^{15}\text{N}$ values ranging from 8.42 in American robins, to 4.65, 4.91 and 5.24 in *Lumbricus terrestris*, *Lumbricus rubellus*, and *Aporrectodea* spp., respectively (Figure 3). The average $\delta^{15}\text{N}$ value for all earthworm species was 4.93 (SE 0.39). By contrast, $\delta^{13}\text{C}$ did not vary with species group ($F_{3,28} = 1.41$, $P > 0.1$) and there was considerable overlap in the $\delta^{13}\text{C}$ values in our food chain, with average $\delta^{13}\text{C}$ values ranging from -26.31 in American robin eggs, to -25.95, -26.32 and -26.34 in *L. terrestris*, *L. rubellus*, and *Aporrectodea* spp., respectively (Figure 3). The average $\delta^{13}\text{C}$ value for all earthworm species was -26.28 (SE 0.48).

2.3.2. Concentrations of DDT-r compounds in earthworms

The species composition of earthworm communities did not differ substantially among orchard types. Earthworm communities were dominated by *Aporrectodea* spp. and *Lumbricidae* spp., which included the common leaf worm, *L. rubellus*, and the nightcrawler, *L. terrestris*, which has not been sampled in some of the previous earthworm-robin studies in the Okanagan. Concentrations of DDT-r in earthworms differed substantially among orchards and there was a 123-fold difference in *p,p'*-DDE, with concentrations ranging from 2.72 ug/g to 335 ug/g (lipid equivalent) (Table 1).

All earthworm samples contained detectable levels of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD (Table 1). The mean concentration of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD in all earthworms was 1.60 ug/g, 0.299 ug/g and 0.0613 ug/g (wet weight), respectively or 117 ug/g, 23.1 ug/g and 4.07 ug/g (lipid equivalent), respectively. Earthworms had higher concentrations of *p,p'*-DDE compared to *p,p'*-DDT and *p,p'*-DDD, and the highest concentration of *p,p'*-DDE detected was 5.06 ug/g (wet weight) or 335 ug/g (lipid equivalent) in a *L. terrestris* sample collected from an apple orchard that was established in the 1950s.

Based on a multiple comparisons test, concentrations of *p,p'*-DDE were significantly higher in *L. terrestris* earthworms compared to *L. rubellus* ($P < 0.05$). There were no significant differences in *p,p'*-DDE concentrations between *Aporrectodea* spp., *L. rubellus* ($P = 0.912$; SE 0.41) or *L. terrestris* ($P = 0.08$; SE 0.57) (Figure 3). Concentrations of *p,p'*-DDT were not significantly different between *Aporrectodea* spp., *L. rubellus* ($P = 0.806$; SE 0.096), or *L. terrestris* ($P = 0.313$; SE 0.131). There were also no significant differences in *p,p'*-DDT concentrations among *Lumbricidae* earthworms (P

= 0.177; SE 0.14) (Figure 4). Concentrations of *p,p'*-DDD were significantly higher in *L. terrestris* earthworms compared to *Aporrectodea* spp. ($P = 0.0269$; SE 0.04), but not higher than *L. rubellus* ($P = 0.06$; SE 0.045). Concentrations of *p,p'*-DDD were not significantly different between *Aporrectodea* spp. and *L. rubellus* species ($P = 0.933$; SE 0.03) (Figure 5).

There was a limited effect of earthworm biomass on DDT-r concentrations; biomass had no significant effect for either *p,p'*-DDE ($F_{1,5} = 3.58$, $P > 0.1$) or *p,p'*-DDT ($F_{1,7} = 1.13$, $P > 0.1$) concentrations; however did have a significant effect on *p,p'*-DDD ($F_{1,5} = 10.4$; $P < 0.05$) concentrations. There was a significant effect of earthworm density on *p,p'*-DDE ($F_{1,5} = 6.82$, $P < 0.05$) and *p,p'*-DDD ($F_{1,5} = 8.21$, $P < 0.05$) concentrations, but not for *p,p'*-DDT ($F_{1,6} = 3.79$, $P < 0.1$) concentrations. The ratios of DDE to DDT in earthworms ranged from 2.84 to 16.0 in orchard areas and there was no significant main effect of earthworm species on the ratios ($F_{2,5} = 0.467$, ns).

2.3.3. Concentrations of DDT-r compounds in American robins

All 22 robin eggs contained detectable levels of *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD (Table 2). The mean concentrations of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD in robin eggs collected from orchards was 36.6 ug/g, 1.14 ug/g, and 0.156 ug/g, respectively (wet weight) or 632 ug/g, 19.8 ug/g and 2.74 ug/g (lipid equivalent), respectively. The mean concentrations of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD at reference sites was 0.495 ug/g, 0.0257 ug/g and 0.00160 ug/g (wet weight), respectively or 7.62 ug/g, 0.395 ug/g and 0.0246 ug/g (lipid equivalent), respectively. The lowest *p,p'*-DDE concentration detected was 3.28 ug/g wet weight (56.7 ug/g lipid equivalent) and the maximum concentration was 107 ug/g wet weight (1890 ug/g lipid equivalent), both detected at different apple orchards in the Summerland area. Concentrations of *p,p'*-DDE in robin eggs from orchards were consistently higher than *p,p'*-DDT and *p,p'*-DDD metabolites.

The average weight and length of robin eggs collected from orchards was 6.44 g and 2.74 cm (respectively), and 7.15 g and 2.85 cm in non-orchard areas (respectively). The % lipid of egg samples had no significant effect for *p,p'*-DDE ($F_{1,8} = 0.15$, $P > 0.1$) or *p,p'*-DDD ($F_{1,3} = 0.0005$, $P > 0.1$) concentrations, however did have a significant effect for *p,p'*-DDT ($F_{1,9} = 16606$, $P < 0.05$) concentrations. There was no significant effect of the weight*length interaction for *p,p'*-DDE ($F_{1,8} = 2.26$, $P > 0.1$) or *p,p'*-DDD ($F_{1,2} = 2.72$,

$P > 0.1$), although there was a significant effect for p,p' -DDT ($F_{1,9} = 4690$, $P < 0.05$) concentrations. The ratio of DDE to DDT was significantly higher in the eggs of orchard-nesting versus non-orchard nesting robins ($F_{24,7} = 4948$, $P < 0.001$), with ratios ranging from 18 to 624 in orchards and 12 to 21 in non-orchard areas. There was considerable variation in the ages reported for each egg and developmental stages generally included; no visible development (i.e. yolk and albumen), eyes evident, distinct head region, limb buds developing, feather follicles, toenail development and an enlarged embryo.

2.3.4. Biomagnification factors (BMFs) for earthworms and American robins

Based on a mixed effects model that included American robin and earthworm species as covariates for each DDT-r compound (lipid-equivalent), there was a significant effect of species on p,p' -DDE ($F_{3,29} = 15$, $P < 0.001$) concentrations (Figure 4), but not for p,p' -DDD ($F_{3,30} = 2.30$, $P < 0.1$) (Figure 5) or p,p' -DDT ($F_{3,30} = 0.605$; $P > 0.1$) (Figure 6). Concentrations of p,p' -DDE were significantly higher in American robins compared to *Aporrectodea* spp. ($P < 0.001$), *L. rubellus* ($P < 0.001$) and *L. terrestris* ($P < 0.001$) species (Figure 4). Earthworm-robin regressions for p,p' -DDE showed a significant positive relationship ($F_{1,8} = 11.62$, $P = 0.0092$, $R^2 = 0.60$) (Figure 7); however these results were based on pooled data and a small sample size, thus minimizing statistical power.

The mean BMF for p,p' -DDE across all orchards was 6.53 (SE 2.12), with values ranging from 0.77 to 24.32 (Table 3). The mean BMF for p,p' -DDT was 1.12 (SE 0.326), with values ranging from 0.16 to 2.98 and the mean BMF for p,p' -DDD was 0.65 (SE 0.148), with values ranging from 0.18 to 1.33. BMF values for p,p' -DDD were the lowest out of all the metabolites and were greater than 1 in only three out of the ten orchards, whereas BMFs for p,p' -DDT were greater than 1 in four out of the ten orchards. By contrast, the BMFs for p,p' -DDE were greater than 1 in nine out of the ten orchards. A Wilcoxon Signed-Ranks Test indicated that BMFs for p,p' -DDE were significantly different among orchards ($V = 53$, $P = 0.0059$), but not for p,p' -DDD ($V = 9$, $P = 0.064$) or p,p' -DDT ($V = 26$, $P = 0.92$).

2.3.5. Other halogenated contaminant concentrations

Several other legacy pollutants were detected in American robin eggs at low levels, including polybrominated diphenyl ethers (PBDEs; flame retardants) and polychlorinated biphenyls (PCBs; Table 4). Σ BDE consisted of 15 congeners, including 15, 17, 28, 48, 49, 66, 85, 99, 100, 138, 153, 154/153, 183, 190, and 209. Out of all the flame retardants, BDE-99, and BDE-100 were detected in 100% of robin eggs, with BDE-99 having the highest value of 17.4 ng/g (wet weight). Σ PCB consisted of 35 congeners, with most samples containing PCB-138, 153 and 187. Out of all PCBs, congener PCB-138 was detected in 100% of robin egg samples, which averaged 0.0020 ug/g (wet weight). Egg and earthworm homogenates were also analyzed for other legacy organochlorines, including chlorobenzenes (Σ CBz = 1,2,4,5-tetrachlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene and hexachlorobenzene), hexachlorocyclohexanes (Σ HCH = α -, β - and γ - hexachlorocyclohexane), chlordane-related compounds (Σ CHLOR = oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor and heptachlor epoxide), octachlorostyrene (OCS), mirex (Σ Mirex = photomirex and mirex), and dieldrin. Out of all these OCs, concentrations for hexachlorobenzene, dieldrin, oxychlordane and *trans*-nonachlor were the highest in robin eggs, although none of the compounds exceeded 0.05 ug/g (wet weight).

2.4. Discussion

2.4.1. American robins as a bioindicator species & sampling eggs

American robins have many characteristics that make them an ideal sentinel species for monitoring DDT-r contamination. Unlike other migratory species with unique foraging behaviors and/or regional distributions, American robins are a common overwintering species with a fairly localized distribution in the Okanagan Valley, and readily nest within orchards (Campbell et al., 1997; Cannings et al., 1987), making them suitable for assessing DDT exposure at fine geographic scales. During the breeding season, American robins forage near their nest sites and feed almost exclusively on soil earthworms (Johnson et al., 1976; Sallabanks & James, 1999; Sibley, 2020; Wheelwright et al., 1986), which are known to accumulate high levels of DDT-r compounds (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000), thereby providing a direct link of contamination from the soil (Bishop et al., 2000). The food chain for

American robins and other passerines is also relatively short and less complicated than other species (Elliott, Bishop & Morrissey, 2011), which further facilitates the linear transfer of DDT (and other POPs) up the food chain (Fremlin et al., 2020), making them suitable indicator species for risk assessment purposes and other local contaminant monitoring programs.

Eggs were chosen to represent the maternal transfer of DDT and have been previously used to represent population-level exposure in birds (Bargar et al., 2001; Bishop et al., 1995; Bishop et al., 2000; Drouillard & Norstrom, 2001; Eens et al., 2013; Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000; Van den Steen et al., 2009; Verreault et al., 2006). Due to their lipophilicity, American robin eggs are expected to reflect DDT-r contamination more accurately and precisely than other pesticides with different modes of action (Borges et al., 2013), and can therefore be directly compared to DDT toxicity guidelines, including impaired reproduction. Robin eggs can also be used to assess any potential contamination trends from the local area, since female robins will acquire body burdens locally during the breeding season, prior to and during egg laying (Elliott et al., 1994). In addition to being easily homogenized, robin eggs tend to accumulate greater concentrations of DDT-r than other sampling matrices, such as whole body tissue and/or feathers, that either have low lipid content, a low degree of hydrophobic partitioning, degrade easily, are too invasive to collect, and/or are simply not representative of bioaccumulation (Jaspers et al., 2019). American robin eggs are also easy to identify in the field and the removal of a single egg from a clutch is expected to have little population impact (Furness, 1993) as American robins lay 3-4 eggs per clutch on average, and can have up to 3 broods per breeding season (Cannings et al., 1987), further facilitating a large sample size in a wild species. Previous research has shown that monitoring robin nests and handling eggs does not interfere with overall nest success (Furness, 1993; Ortega et al., 1997).

Egg laying order effects and intra-clutch variation have been reported for some birds, which might have implications for the interpretation of contaminant data (Ackerman et al., 2016; Van den Steen et al., 2009). For example, Braune et al. (2018) reported that *p,p'*-DDE concentrations were 41% higher in replacement eggs compared with first-laid eggs in wild populations of the thick-billed murre (*Uria lomvia*), a species often used in Canadian Arctic contaminant monitoring programs. Other examples of intra-clutch variation in DDT contamination have been reported in the common tern

(*Sterna hirundo*), great crested grebe (*Podiceps cristatus*), glaucous gull (*Larus hyperboreus*) and herring gull (*Larus argentatus*; Nisbet, 1982; Lukowski, 1978; Mineau, 1982), possibly due to their high trophic level positions and lipid-rich diets (Van den Steen et al., 2009). Similarly, Morrissey et al. (2010) investigated egg contaminant loading in European dippers (*Cinclus cinclus*) and American dippers (*Cinclus mexicanus*) and reported that female dippers increased their foraging rate as breeding approached by shifting their diets to higher trophic level organisms, including fish, plecopterans, and coleopterans. Morrissey et al. (2010) also reported higher levels of DDT-r, as well as other organochlorines, in these dipper eggs and argued that the availability of prey and type of diet shift (i.e. towards lipid rich prey or higher trophic levels) could be influencing contaminant exposure in piscivorous birds.

Studies investigating DDT residues in passerine birds, including European starlings (*Sturnus vulgaris*), prothonotary warblers (*Protonotaria citrea*) and tree swallows (*Tachycineta bicolor*), generally found that eggs within a clutch tend to have similar DDT-r concentrations, and that a single egg can be used to statistically represent the entire clutch (Eens et al., 2013; Reynolds et al., 2004; Verreault et al., 2006). These findings are in agreement with other field studies investigating DDT-r levels in migratory birds, including the black-crowned night heron (Custer et al., 1998), double-crested cormorant (Custer et al., 1997), British peregrine (Newton et al., 2008), white-tailed sea eagle (Helander et al., 1982) and brown pelican (Blus, 2011), in which DDT-r concentrations were not significantly different within clutches. Yet, a study conducted on a small songbird, the blue tit (*Cyanistes caeruleus*), showed that females experienced up to a 45% decrease in *p,p'*-DDE levels in relation to the laying order of eggs, although in closing, variation in contamination was reported to be higher among clutches, rather than within clutches (Van den Steen et al., 2009). Blue tits are known for having a highly variable clutch size and females can lay anywhere up to sixteen eggs in a single clutch (Van den Steen et al., 2009). Eggs collected from blue tits and potentially other passerines could also have differences in contamination sources (i.e. urban and/or rural areas), thus influencing the contamination profile between first-laid eggs and after clutch completion (Van den Steen et al., 2010). This contrasts with a study by Eng et al. (2012), who conducted a 21-day dosing study of a polybrominated diphenyl ether (PBDE) congener, BDE-99, on adult zebra finches (*Taeniopygia guttata*) and reported a significant decrease in maternal plasma contaminant concentrations at the first egg laid,

the third egg laid and at clutch completion, with no significant differences in maternal yolk precursor (i.e. very-low-density lipoprotein; VLDL) levels with egg BDE-99. Eng et al. (2012) argued that maternal and egg contaminant levels could be highly variable depending on egg or follicle size in some birds. Although this study did not investigate maternal plasma levels or intra-clutch variation, egg size and percent lipid in robin egg samples had no significant effect on *p,p'*-DDE or *p,p'*-DDD concentrations.

The maternal transfer and deposition of DDT into passerine eggs is thought to be influenced by various physio-chemical and biological processes (Bargar et al., 2001; Drouillard & Norstrom, 2001). Due to their hydrophobic nature, DDT-r compounds can freely diffuse across biological membranes, such as the oocyte plasma membrane, and therefore be mobilized and deposited along with yolk proteins directly into eggs (Eng et al., 2012). However, this assumption relies on the fugacity principle, which describes the tendency of a chemical to move from one compartment to another based on a diffusion gradient (Gobas et al., 2015; Gobas et al., 1993). In other words, the fugacity principle assumes a constant overall flux or equilibrium state where egg:maternal contaminant concentrations would be equal to 1, which has not always been the case in some passerines (Eng et al., 2012). On the other hand, the deposition of DDT-r compounds into robin eggs could also be an active process, in which DDT-r compounds could bind to other hydrophobic/lipophilic molecules and egg yolk proteins synthesized in the liver, such as vitellogenin, and then be actively transported into the yolk via receptor-mediated endocytosis (Eng et al., 2012; b). The extent of DDT contamination between eggs is also thought to be related to clutch size and egg laying period (Drouillard & Norstrom, 2001; Eng et al., 2012). For instance, Bourgault et al. (2007) reported a significant decline in yolk lipid content and fatty acids throughout the laying sequence of different populations of blue tits and argued that some birds could invest in different quantities of nutrients, and lipids into the egg relative to their body weight, thereby affecting the overall contamination profile between different individuals. In precocial birds, eggs laid earlier can be larger and have proportionally more water or yolk lipids, and therefore higher DDT-r concentrations (Ackerman et al., 2016). In other species that lay fewer broods and larger eggs, maternal body burdens could also increase due to fewer opportunities to excrete contaminants, particularly those with long-half lives, such as DDT (Bargar et al., 2009). This is particularly true for pelagic seabirds, such as terns and gulls, which lay fewer, but relatively larger eggs compared to robins, accounting for up to 16% of their

total maternal body weight (Lemmetyinen et al., 1982). By comparison, the weight of robin eggs in the present study averaged approximately 6.44 g, which accounts for a little more than 8% of a robin's body weight. The weight of robin eggs in this study did not drastically vary among individuals and did not have a significant effect on DDT-r concentrations in eggs.

In most passerines, the ovary will release a mature ovum (i.e. yolk) at different daily intervals until a complete clutch of eggs is laid (Perrins, 2008). In the case of American robins, females will lay on average a new egg each day and must therefore invest in enough dietary resources (i.e. income breeder) for the production of eggs during the short egg-laying period (Houston et al., 2008; Morrissey et al., 2010). Subtle differences in DDT-r levels within American robin clutches (and other passerines) might therefore be a function of the heterogeneity of contaminant distribution on the sites where eggs were collected, in which foraging locations and/or food items consumed could have varied among individuals (Reynolds et al., 2004). Conversely, similar DDT-r levels within American robin clutches might be related to individual females foraging in areas containing similar concentrations of DDT (Reynolds et al., 2004), which could potentially result in a constant mobilization and deposition of maternal lipids, and their associated DDT residues into eggs (Braune et al., 2018; Van den Steen et al., 2009; Verreault et al., 2006), thereby resulting in similar levels within each clutch. Furthermore, many robins will rear a second brood after the first have fledged and depending on local conditions, sometimes even a third brood within the same nesting area (Sibley, 2020), which could result in varying degrees of within- and intra-clutch variability of contaminant concentrations (Reynolds et al., 2004). In any case, American robin eggs are likely suitable sampling matrices for DDT-r contamination, as these will represent a broader spectrum of the breeding population, which is particularly the case for the large number of overwintering and breeding American robins in the Okanagan (Cannings et al., 1987).

2.4.2. DDT-r contamination in earthworms

Concentrations of DDT-r compounds in earthworms differed substantially among orchards and there was a 123-fold increase in *p,p'*-DDE, with values ranging from 2.72 to 335 ug/g (lipid equivalent). Compared to previous field studies in the Okanagan, there was considerable variation in the species composition, as well as in the density and biomass of earthworm samples, which might be due to differences in sampling

techniques. For instance, Harris et al. (2000) used a modified formalin extraction method (50 ml of 37% formaldehyde in water) to retrieve earthworms from Okanagan orchards, while the present study used a 4 g/L stock solution of allyl isothiocyanate (AITC) mixed with isopropanol and water. AITC is a natural breakdown product of glucosinolates found in *Cruciferae* flowering plants and is considered to be a safer and eco-friendly alternative to formalin and other chemical extractants, such as potassium permanganate, which do not recover all earthworm species equally and can have non-target effects on soil microorganisms, plants, and wildlife (Čoja et al., 2008; Eichinger et al., 2007). AITC is also more effective in sampling epigeic (litter dwellers), endogeic (top soil), as well as anecic and other large classes of earthworms that can escape to deeper layers, compared to hand digging alone (Zaborksi et al., 2002), which generally only retrieves epigeic and endogeic worms, thereby potentially resulting in under-estimates of the large anecic earthworms (Pelosi et al., 2008). Total biomass and density of earthworms has also been shown to increase with increasing AITC concentration (Zaborksi et al., 2002), which might explain why there was a limited effect of biomass and density on *p,p'*-DDE, and *p,p'*-DDD concentrations in this study. Nonetheless, AITC has been successfully used to sample earthworms foraged by local American robins (Fremlin et al., 2000) and is considered to be a safer and economical sampling method, since it is low cost, and biodegrades easily (Pelosi et al., 2009).

The foraging behaviour of American robins is strongly correlated with the presence of non-native earthworms (Cameron & Bayne, 2012) and robins often rely on various sensory cues to locate different worms (Vanderhoff et al., 2020). A common earthworm foraged by robins during the breeding season is *L. terrestris*, an anecic earthworm that feeds on surface residues and forms permanent burrows in the soil (Pitkänen & Nuutinen, 1997). Due to its unique feeding and burrowing behaviour, *L. terrestris* earthworms are capable of translocating up to 60% of pesticide residues into the soil matrix (Farenhorst et al., 2000). Burrow linings created by *L. terrestris* also contain a greater percentage of organic carbon, nutrients and heavy metals compared to the surrounding soil matrix (Tomlin et al., 1992), which increases soil fertility and could then influence the long-term persistence of DDT-r compounds in soil. Gaw et al. (2012) conducted a 28-day bioassay using large classes of earthworms and found a significant positive relationship between heavy metal concentrations in soil (As, Cd, Cu and Pb) and earthworm tissue concentrations for *p,p'*-DDE, and *p,p'*-DDT in New Zealand

orchards, and argued that aged orchard soils could increase the bioavailability of DDT metabolites to certain earthworms. Edwards & Jeffs (1974) exposed *L. terrestris* earthworms in soil to 1 ug/g of DDT and showed that after six months, nearly all the DDT residues in *L. terrestris* tissues were converted to *p,p'*-DDE, with a significant portion having been mixed in the upper 15 cm of soil. Similarly, Bailey et al. (1974) collected earthworms in a British apple orchard during the 1970s when DDT was still used and found that although *p,p'*-DDT, and *p,p'*-DDE levels were high in soil, levels of *p,p'*-DDE were 3-5 fold greater in earthworms. Earthworms exposed to DDT-r under laboratory conditions are also capable of converting *p,p'*-DDT residues into *p,p'*-DDD and *p,p'*-DDE (Beyer & Gish, 1980), although at much lower levels, which in addition to a long latency period of DDT use, might explain why *p,p'*-DDD levels were so low in this study. The data from the present study also revealed that DDE and DDE residues were significantly higher in *L. terrestris* compared to small endogeic earthworms, such as *L. rubellus*, which generally feed on or beneath the surface and occupy only the first 10-15 cm of soil layer (Miglani & Bisht, 2019). However, other field studies conducted in orchards have found no significant differences in DDT-r among earthworm species (Harris et al., 2000; Thompson, 1973) and there is speculation that all earthworms, regardless of species or age, could be feeding on DDT-r residues near surface soils (Beyer & Gish, 1980; Stringer et al., 1974). Depending on local conditions, American robins could be foraging on a combination of small and/or large earthworms, thus influencing their overall exposure to DDT.

The extent of DDT-r contamination in earthworms is influenced by various land management practices and biochemical factors (Bahar, 2015). Harris et al. (2000) reported that earthworm DDE values increased with increasing soil organic matter in Okanagan orchards, but not in Ontario orchards and argued that low moisture levels in the B.C interior during the winter months could inhibit the abiotic breakdown of DDT residues, thereby increasing the uptake of DDT-r in earthworms. The Okanagan Valley is a semi-arid region and historical records show that very little precipitation occurred between 1941-1990 when DDT was actively used (Harris et al., 2000; Kuo et al., 2012). Temperature regimes in recent years could have also influenced the density and biomass of earthworms sampled. For example, Harris et al. (2000) sampled for earthworms in the Okanagan Valley between 1993 and 1995, with mean temperatures ranging from 10.6°C to 23.2°C between May and June (Environment Canada, 2020).

Meanwhile, mean temperatures in the Okanagan in 2019 (present study) ranged from 6°C to 30.5°C during the same time period (Environment Canada, 2020), which could have increased earthworm burrowing activity, and therefore greater individuals sampled in the present study (Perreault & Whalen, 2006). Burrow length and burrow depth generally increase with increasing temperature, and burrows may act as preferential flow pathways for DDT-r compounds and other organic substrates (Bernard et al., 2011). Earthworm burrows can also increase soil porosity, allowing more air and water to infiltrate and percolate through the soil, providing more of an aerobic environment for the photochemical degradation and/or dehydrochlorination of DDT to DDE (Edwards et al., 1993; Shuster et al., 2004). In addition, different earthworm species could indirectly stimulate the biotic transformation of DDT in the drilosphere (i.e. portion of the soil that consists of earthworm mucus secretions, burrowing holes and castings) by increasing the pH of acidic soils, increasing organic carbon content and increasing moisture levels (Tomlin et al., 1992; Xu et al., 2020). Okanagan orchards are typically associated with acidic pH's (Harris et al., 2000) and increases in pH via earthworm activity could promote the abiotic hydrolysis of DDT to DDE (Nash et al., 1977). These findings are further corroborated by laboratory-based studies which show that soil modification via earthworms strongly promotes the growth of several micro-organisms, including, Gram-positive and Gram-negative bacteria, which can aid in the overall degradation of DDT (Xu et al., 2020). Earthworms are therefore capable of transforming DDT-r through various abiotic and biotic pathways, thereby influencing the amount of DDT-r in the soil and the amount acquired by robins, and other wildlife.

Concentrations of DDT-r in earthworms in this study were generally lower than previous field studies. In Maryland orchards where DDT was still used, earthworms contained 2.66 ug/g DDE, 6.28 ug/g DDT and 3.57 ug/g DDD (dry weight) (Gish, 1970). Interestingly, Kuhr et al. (1974) collected earthworms in New York apple orchards after twelve years of DDT use and found that some species contained lethal concentrations of 106 ug/g DDT, suggesting that earthworms could have more DDT residues than those during the DDT era. In Okanagan orchards where DDT applications were discontinued for approximately twenty years, earthworms contained 43.5 ug/g of *p,p'*-DDE, 17.2 ug/g of *p,p'*-DDT and 2.2 ug/g of *p,p'*-DDD (dry weight) (Harris et al., 2000). Earthworms in these Okanagan orchards also had a low average DDE:DDT ratio of 2.56, whereas ratios in this study ranged from 2.87 to 16.7, confirming that DDT degradation in

earthworms progressed further in the Okanagan. Hoy (1955) observed earthworm mortality at 15 ug DDT/g soil, however, did not report any differences in the density or biomass of earthworms between treated and non-treated plots. Similarly, Thompson (1970) recorded an initial drop in earthworm abundance of approximately 33-36% in laboratory DDT-treated plots, although asserted that mortality rates in the wild could be far less due to earthworm avoidance and other environmental factors influencing earthworm activity, such as soil moisture, temperature, and nutrient availability. The exposure levels of DDT in earthworms in the wild may also be too small to cause any acute toxicity (WHO, 1989) and earthworms have been shown to develop physiological, biochemical, and behavioural resistance to DDT-r compounds (Tomlin et al., 1992). Therefore, it seems highly unlikely that earthworm communities in Okanagan orchards are still being affected by DDT residues, but rather are serving as a significant exposure source to American robins and other wildlife that feed on them.

2.4.3. DDT-r contamination in American robins & other birds

In this study, DDT-r compounds were detected in 100% of robin eggs and were generally lower compared to other robin studies conducted in the Okanagan. For example, concentrations of *p,p'*-DDE in robin eggs in this study were in the range of 3.28-107 ug/g (wet weight) in orchards and 0.141-0.848 ug/g (wet weight) in non-orchard areas. Mean concentrations of *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD in robin eggs in this study were 36.6 ug/g, 1.14 ug/g, and 0.156 ug/g (wet weight), respectively, which was considerably lower than the concentrations by Harris et al. (2000), who reported a mean average of 85.1 ug/g of *p,p'*-DDE, 13 ug/g of *p,p'*-DDT and 1.1 ug/g of *p,p'*-DDD (wet weight) in robin eggs collected between 1993-1995 in the same region. Additionally, robin eggs collected from orchards in the Okanagan between 1997-1998 averaged 64 ug/g DDE (Gill et al., 2003), 51.7 ug/g DDE (Smith, 2004) and 55.7 ug/g DDE (Iwaniuk et al., 2006), indicating that while contamination is still common in the Okanagan, DDT-r concentrations in orchard nesting robins have decreased over the years (Figure 8).

Elliott et al. (1994) collected bird eggs from various areas in the Okanagan Valley from 1990-1991 and found that DDE concentrations were the highest in American robins, ranging from 68 to 103 ug/g (wet weight) in orchards and 1.53 ug/g (wet weight) in non-orchard areas based on pooled samples. Comparatively, mean concentrations of DDE in these robin eggs were 18 to 3,500 times greater than eggs of neotropical migrant

species nesting in the same orchards, including the tree swallow (*T. bicolor*), barn swallow (*Hirundo rustica*) and house wren (*Troglodytes aedon*). Mean concentrations of DDE were also 100 times higher in robins compared to other non-migratory year-round residents nesting in Okanagan orchards, including the California quail (*Callipepla californica*; species mean 1.19 ug/g wet weight) and the predatory black-billed magpie (*Pica pica*; species mean 11 ug/g wet weight), which indicated that robins and other Okanagan birds were acquiring DDT burdens locally, and not on their wintering grounds (Elliott et al., 1994). Quail are primarily granivorous and magpies are highly opportunistic, often feeding on a wide range of invertebrates, seeds and carrion, which might explain why DDT-r levels were so low in these species compared to robins (Elliott et al., 1994). Reinforcing this finding, Enderson et al. (1982) found that mean DDE residues ranged from 0.14-5.8 ug/g in granivore, omnivore and insectivore prey species in the diet of peregrine falcons (*F. peregrinus*), further acknowledging that the lower trophic position of granivores limits their DDT biomagnification potential (Elliott et al., 1994). In further comparisons, the levels of DDT in American robins in this study were higher compared to other parts of North America. For instance, the mean concentration of *p,p'*-DDE in robin eggs in this study was 36 ug/g (wet weight), whereas mean DDE levels were 11.1 ug/g in robin eggs from orchards in Washington State, and 17.3-25.7 ug/g in robin eggs from various orchards in southwestern Ontario (Blus et al., 1987; Harris et al., 2000; Hebert et al., 1994). Concentrations of DDE in robin eggs in this study were also higher than red-winged blackbird (*Agelaius phoeniceus*) and tree swallow (*T. bicolor*) eggs collected from wetland sites in Ontario from 1990-1991, which contained up to 3.1 and 4.4 ug/g DDE, respectively (Bishop et al., 1995). These findings, along with the present study, indicate that DDT-r burdens continue to be higher in American robins and other ground-foraging birds in the Okanagan that thrive on an omnivorous diet.

Harris et al. (2000) pooled their DDT-r concentrations in earthworms and robin eggs from Okanagan orchards and calculated an average BMF of 41.52 for *p,p'*-DDE, and 60.05 for *p,p'*-DDT. These authors also reported an average BMF of 15.10 for *p,p'*-DDE and 6.42 for *p,p'*-DDT in earthworms, and robin eggs collected from Simcoe orchards in Ontario (Harris et al., 2000), which was higher than the BMFs reported in this study, which averaged 6.53 for *p,p'*-DDE, 1.12 for *p,p'*-DDT, and 0.65 for *p,p'*-DDD. The BMFs in this study were refined by incorporating lipid-normalized concentrations of DDT-

r in earthworms and robins, which provides a better estimate of biomagnification in food webs, since differences in lipid content are accounted for (Hebert et al., 1994). The earthworm-robin regressions for *p,p'*-DDE in this study also showed a significant positive relationship compared to the regressions conducted by Harris et al. (2000), which might have been related to an increased sampling effort and/or high variability among species. The square-root transformation of the linear regression in this study also suggests that the relationship between DDE in earthworms and robins may not be linear. This means that robins at the most contaminated sites may be feeding not only on earthworms (or other prey) in those contaminated areas, but also in areas with lower DDT concentrations, thus leading to an overestimate of the concentration of DDE at the most contaminated area. Furthermore, there were several instances of trophic dilution, or biodilution, at some orchards (BMF < 1), which could have meant that robins were foraging in either non-orchard habitat, foraging in areas with little or no DDT-r contamination, or foraging on other insects or prey with low DDT levels (Harris et al., 2000). Stomach sample records show that some American robins consumed soil invertebrates from over 100 different families (Wheelwright, 1986), including *Lepidoptera* (moths and butterflies), *Carabidae* (ground beetles) and *Curculionidae* (weevils) (Wheelwright, 1986; Wilson, 1949). Therefore, although earthworms are a common food item for robins during the breeding season, individuals could be foraging on different food items within orchard and/or non-orchard areas, thereby influencing their overall DDT exposure.

The highest earthworm-robin BMF and *p,p'*-DDE concentration in this study was 24.32 and 107 ug/g (wet weight), respectively, and there are several possible explanations for why DDE residues could be high in some robins. Field studies have shown that approximately 90% of DDT residues in orchards are found in the top 10 cm of soil, with maximum levels centered around tree trunks compared to the surrounding herbage (Cooke & Stringer, 1982; Stringer et al., 1974). Female robins will often seek out calcium- and protein-rich food sources for egg formation, and so a key pathway for DDT exposure to robins could be the consumption of earthworms that accumulated higher DDT-r levels through feeding on leaf fall near orchard trees (Baker & Skerrett, 1958). Inter-individual variation in egg concentrations of DDT could also be related to age (Eng et al., 2014). For instance, maternal body burdens could be higher in females who are laying their first clutch in which there might not have been enough time for DDT-

r metabolites to reach a pharmacokinetic equilibrium (Arnot & Gobas, 2006; Elliott et al., 1996). In other words, female robins laying their first clutch could have had fewer opportunities to eliminate (i.e. metabolize and excrete) their body burdens, thus increasing the amount of DDT-r deposited into their eggs (Gobas et al., 1993). However, older and experienced females could also have higher maternal body burdens, especially those who have had more time to feed and acquire DDT-r from their diet, consequently resulting in more DDT-r being deposited into their eggs (Bargar et al., 2001; Knupp et al., 1976). Dimond et al. (1970) collected adult robins and earthworms from Maine forests that were sprayed with DDT for spruce budworm, and reported significant differences between age classes, with adult robins containing 2.6 more DDT residues than some of the younger robins. Harris et al. (2000) also reported higher *p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD levels in female robins (mean 26 ug/g wet weight) compared to younger robins (mean 9.92 ug/g wet weight) collected from Ontario orchards. These findings are further supported by various thermodynamic studies which show that the fugacity (i.e. escape of a chemical from one compartment to another) of DDT increases as more food is digested and absorbed by an animal's gastrointestinal tract, causing the non-metabolizable chemical(s) to dissociate from lipid and become more bioavailable in the body, thus resulting in bioaccumulation (Gobas et al., 1993a; Gobas et al., 1993b).

American robins are considered a short-distance migratory bird and high concentrations of DDT-r in individuals could be related to migratory status. American robins that were contaminated at their wintering grounds between 1966-1973 lost nearly 90% of their DDT body burdens when females nested in uncontaminated forests, which was evidenced by the reduced DDT-r loads in eggs, nestlings and fledglings compared to reference sites (Knupp et al., 1976; Mahoney, 1975). Södergren & Ulfstrand (1972) simulated the stress encountered in long migratory flights by subjecting caged robins to a period of starvation following an administration of 10.5 ug of *p,p'*-DDT via diet for fifteen days and found that *p,p'*-DDT and *p,p'*-DDE concentrations (wet weight) were significantly higher in the brain, and in the breast muscles compared to reference birds. Other experiments have found that American robins can lose a significant amount of mass (more than 3.0 g) even during short flights, with more than 70% of their energy being derived from fat (Gerson & Guglielmo, 2013). Wind tunnel experiments conducted on a long-distance migratory Swainson's thrush, a closely related species to American

robins, have also shown that individuals flying long hours (up to 12 h) under humid conditions (i.e. high evaporative water loss; HEWL) catabolize a greater amount of fat and protein, which is thought to augment endogenous water production, and maintain water balance during flight (Groom et al., 2019). Although water and energy expenditures in birds is influenced by various environmental conditions and/or physiological traits (Fudickar et al., 2013; Guglielmo, 2002; McFarlan et al., 2009), migrating robins could have fewer fat reserves, which could result in a higher maternal body burden, and subsequently a higher amount of DDT-r excreted into eggs. Large die-offs of American robins were reported during the 1950s when DDT was used to control Dutch elm disease and males appeared to be more susceptible to DDT poisoning, which according to some, was attributed to the seasonal loss of fat reserves (Beaver, 1980; Hunt, 1969). Elliott et al. (1996) reported a significant negative relationship between DDE levels and the numeric scoring of body condition of bald eagles (*H. leucocephalus*) and argued that migration and starvation could cause DDT-r to move out of fat storage and into systemic circulation, thereby resulting in a higher DDE body burden. Comparing plasma contaminant levels in actively migrating and non-migrating robins would be an interesting follow-up study.

The migratory status of American robins could be influenced by various factors, such as the seasonal availability of different food items, thus influencing overwintering numbers from year to year (Vanderhoff et al., 2020). Western populations of American robins (*Turdus migratorius caurinus* and *Turdus migratorius propinquus*) could winter in parts of Washington, Oregon and California, and some argue that the acquisition of DDT-r in those areas would be negligible compared to the tissue concentrations detected in the Okanagan Valley (Blus et al., 1987; Harris et al., 2000). According to a local banding station in the Okanagan, there have been several incidences of AMRO (American robin) recaptures in the past twenty years of standardized fall migration monitoring, as well as an increase in the number of robin flocks seen during the fall, with an average count of 428 robins per season (M. Bieber, personal communication). Recent analyses from the U.S Geological Survey Bird Banding Laboratory also indicate that American robins are travelling less than 100 km from their breeding grounds, with more and more robins adopting a non-migratory strategy, possibly due to climate change (Vanderhoff et al., 2020). The expansion of fruit orchards and other agricultural areas in the Okanagan, coupled with the cultivation of exotic fruit trees and spread of invasive

plants near orchards and valley bottoms, could also provide robins with winter resources much farther north, thereby potentially increasing their residency behavior (Harris et al., 2000; Sibley, 2020; Vanderhoff et al., 2020). American robins with different migration frequencies, distances and directions could therefore have varying degrees of DDT exposure and overall body burdens.

American robins nesting in Okanagan orchards may experience growth, developmental and neurobehavioral effects due to persistent *p,p'*-DDE residues. Ten-day old robin nestlings that were collected from Okanagan orchards in 1997 that were not exposed to any current-use pesticides had significantly shorter middle toes and shorter tarsi, compared to nestlings collected from reference sites at 2, 5 and 9 months of age (Smith, 2004). Similarly, Iwaniuk et al. (2006) investigated whether *in-ovo* exposure of DDT in American robins led to changes in the sizes of different brain regions by collecting eighteen robin nestlings from various Okanagan orchards in July 1997. Iwaniuk's paper powerfully demonstrated that when robins were exposed to increased *p,p'*-DDE levels (up to 160 ug/g wet weight), individuals had significantly smaller brains, smaller forebrains, as well as reductions of the nucleus robustus arcopallialis (RA) and nucleus intercollicularis (ICo), which are part of the song system. The most notable reductions in brain size were in male robins, who experienced a 15% reduction in brain volume and a 13% reduction in relative forebrain size, concomitant with neuron reductions in the ICo. Iwaniuk et al. (2006) also reported that DDT concentrations were significantly higher in males than females, suggesting that male robins may be more sensitive to DDT toxicity due to hormone sensitivity and/or due to the maternal transfer of DDT-r in eggs, thereby decreasing the females body burden. Stress and direct neurotoxicity were also acknowledged as possible factors affecting brain changes in robins, and certain DDT metabolites could display various androgenic, anti-androgenic, estrogenic and anti-estrogenic properties *in-vitro* (Iwaniuk et al., 2006). The DDT metabolite, *p,p'*-DDE, is thought to act as an androgen receptor antagonist by preventing androgens (i.e. testosterone and dihydrotestosterone) from binding to its receptor, which could result in smaller brains, and song nuclei (Smith, 2004). DDE metabolites could also disrupt Ca^{2+} uptake in cholinergic neurons and reduce the expression of brain-derived neurotrophic factor (BDNF) found throughout the songbird brain, thereby disrupting signal transduction, and brain development in juvenile robins (Iwaniuk et al., 2006).

Smith (2004) collected 10-day old robin nestlings from various Okanagan orchards and reference sites and found significantly higher heterophil to lymphocyte ratios compared to reference birds. However, glucocorticoid levels play an important role in reducing inflammatory responses and are known to fluctuate in birds at different times of the day, as well as during different seasons, which could influence H:L ratios (Crisp et al., 1998; Gross & Siegel, 1983; Jong et al., 2002; Smith, 2004; Smits & Williams, 1999). Additionally, differences in heterophil and lymphocyte levels could have been attributed to either bacteria and/or virus infections (Siegel, 1980), respectively, which coincidentally were observed in both orchard-nesting and reference robins in the study conducted by Smith (2004). This contrasts with a study by Bishop et al. (1998), who collected tree swallow (*T. bicolor*) eggs from apple orchards in Southern Ontario from 1994-1995 and reported a mean *p,p'*-DDE value of 12.05 ug/g (wet weight) among sprayed orchards and 1.06 ug/g (wet weight) among non-sprayed sites. According to Bishop et al. (1998), tree swallow nestlings in sprayed orchards had a significant adaptive immune response compared to nestlings from non-sprayed areas, which was evidenced by the increase in B- and T-lymphocytes that was correlated with increased DDE exposure. Although a small number of these nestlings had parasites, Bishop et al. (1998) argued that body mass did not vary in nestlings among sites and concluded that such infestations likely had a minimal impact on the overall health of nestlings. A follow up study by Mayne et al. (2005) demonstrated that tree swallow nestlings had significantly higher plasma thyroxine (T_4) concentrations and hypertrophic thyroids with collapsed follicles, compared to reference chicks. Tree swallow and eastern bluebird nestlings collected from similar orchards that were co-exposed to *p,p'*-DDE and other current-use pesticides had significantly higher levels of adrenocorticotrophic hormone (ACTH), and qualitative histopathological evaluations revealed that these chicks had greater thymic lymphocyte densities and B-cell hyperplasia of the spleen compared to reference chicks (Mayne et al., 2004), suggesting that nestlings in contaminated orchards may have a slightly active and/or more compromised immune systems. These findings are consistent with results obtained in a laboratory study in which zebra finches (*T. guttata*) that were co-exposed to *p,p'*-DDE and azinphos-methyl (OP pesticide) exhibited immunostimulation (Gill et al., 2005).

Smaller migratory thrushes may be more sensitive to the effects of persistent *p,p'*-DDE residues in orchards. For example, eastern bluebird nestlings from apple

orchards in Southern Ontario had high levels of *p,p'*-DDE (up to 95.4 ug/g, wet weight) and had lower corticosterone levels, which might have been attributed to the steroidogenic inhibition or an upregulation of the mixed-function oxidase (MFO) system, thereby increasing the metabolic clearance rate of corticosterone via cytochrome-P450 enzymes (Mayne et al., 2004). Corticosterone facilitates fat deposition in birds and therefore low blood corticosterone levels could compromise fledging and postnatal dispersal (Duffy & Belthoff, 1997; Mayne et al., 2004). These findings are of particular interest since the *p,p'*-DDE levels in these bluebirds were comparable to the concentrations in robin eggs from this study. American robins and bluebirds are both ground foragers in the thrush family (*Turdidae*) and are known to frequent orchards throughout the breeding season (Vanderhoff et al., 2020). These birds could therefore accumulate greater concentrations of DDE residues from the soil (Bishop et al., 2000), which may have important consequences for energy mobilization, growth, development, reproduction, digestion and adult survival (Bishop et al., 1998; Bishop et al., 2000; Mayne et al., 2004).

The effects of DDT on the survival and reproductive success in American robins has been studied under a range of field and laboratory conditions. Johnson et al. (1976) surveyed several fruit orchards during a period of DDT use from 1966-1968 and revealed that robins nesting in these orchards had a smaller clutch size and a smaller proportion of hatched chicks that fledged. Unfortunately, Johnson et al. (1976) claimed that many robins were predated by house wrens (*T. aedon*), blue jays (*Cyanocitta cristata*) and common grackles (*Quiscalus quiscula*), and therefore accurate comparisons of reproductive success between orchard and non-orchard nests was not possible. Johnson et al. (1976) also observed robins foraging outside their respective orchards, and many individuals were reportedly foraging in woodlots and adjacent lands where DDT concentrations were below the detection limit (Johnson et al., 1976), thus lending uncertainty between robin and earthworm contamination. Song thrushes (*Turdus philomelos*) found dead in a British apple orchard between 1971-1972 contained 19.6-84.1 ug/g of DDE in eggs and had mean DDE residues of 81 ug/g in breast muscle and 191.9 ug/g in liver, all of which were significantly higher compared to birds at control sites (Bailey et al., 1974). Common blackbirds (*Turdus merula*) that were also found dead in the same apple orchard exhibited more variation in DDT-r, with DDE levels ranging from 13.2-72.1 ug/g in eggs, 128.2 ug/g in breast muscle and 180.4 ug/g in liver

(Bailey et al., 1974). Stickel et al. (1984) investigated lethal *p,p'*-DDE residues in common grackles (*Q. quiscula*), red-winged blackbirds (*A. phoeniceus*), brown-headed cowbirds (*Molothrus ater*) and European starlings (*S. vulgaris*), and found that in birds that died, *p,p'*-DDE concentrations ranged from 305-694 ug/g (wet weight), which were well below the levels detected in this study. Fluetsch & Sparling (1993) demonstrated that American robins nesting in conventional orchards with extensive pesticide use from 1990-1991 had significantly reduced hatching success and lower daily survival rates in certain years compared to organic orchards. Bishop et al. (2000) also reported a significant increase in the occurrence of unhatched eggs in eastern bluebirds that had high *p,p'*-DDE concentrations in their eggs. These concentrations were comparable to those in robin eggs from this study, suggesting that DDE residues may affect egg survival in migratory thrushes nesting in orchards.

Although American robins continue to be exposed to high levels of *p,p'*-DDE and other OCs, reproductive success does not appear to be adversely affected as previously hypothesized. Elliott et al. (1994) surveyed American robins nesting in various conventional orchards in the 1990s and found no significant differences in the clutch size, nest success, hatching success or fledging success compared to robins at a non-orchard site. In a follow-up study by Gill et al. (2003), orchard-nesting robins (n = 186 nests) had consistently higher reproductive success via an increased clutch size and brood size, compared to robins nesting in non-orchard areas. Robin eggs collected from these orchards also contained up to 302 ug/g *p,p'*-DDE (wet weight), which was the highest value ever reported in a robin in the Okanagan and coincidentally, a value that was thought to be associated with mortality (Wurster et al., 1965). These studies suggest that robin populations in the Okanagan may have evolved some kind of tolerance to DDT as a result of long-term use. American robins could benefit by nesting in Okanagan orchards, which might provide inconspicuous nesting sites, better foraging opportunities, and lower predation risk compared to other areas (Gill et al., 2003). Elliott et al. (1994) ultimately questioned whether high DDE residues in Okanagan robins could pose a risk to local predators and birds of prey, such as Accipiter hawks and falcons, including peregrine falcons (*F. peregrinus*) and American kestrels (*F. sparverius*), who have been shown to experience eggshell thinning from *p,p'*-DDE concentrations as little as 3 ug/g and 2 ug/g, respectively (DeWeese et al., 1986; Lincer, 1975).

2.4.4. Stable isotopes, diet and trophic position

The use of carbon, nitrogen and other stable isotopes in the field of environmental toxicology has grown in recent years and has been widely used to characterize diet, and trophic position in birds (Currier et al., 2020; Elliott & Elliott, 2016; Elliott, 2005; Gagnon & Hobson, 2009; Hwang et al., 2007; Therrien et al., 2011; Brink et al., 2015; Williams et al., 2007). Nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$) generally increase by 2 to 3‰ per trophic level, with larger animals having higher ^{15}N ratios due to the preferential excretion of lighter amine groups (Elliott et al., 2008; Kelly, 2000). Stable isotopes of carbon during photosynthesis causes C_4 (higher $\delta^{13}\text{C}$ values) and C_3 (lower $\delta^{13}\text{C}$ values) plants to have distinct carbon-isotope ($^{13}\text{C}/^{12}\text{C}$) signatures, thereby potentially reflecting habitat use and feeding ecology within individuals (Morrissey et al., 2010; Williams et al., 2008). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes also provide more accurate and long-term estimates of diet assimilation in birds than traditional methods, such as foraging observations or stomach/fecal analyses, which can result in identification biases (i.e. fragments of tissue or disintegration of soft-bodied organisms), and only provides a snapshot of an organisms diet prior to sampling or collection (Kelly, 2000; Brink et al., 2015). $\delta^{13}\text{C}$ stable isotopes have also been useful in assessing chemical exposure in aquatic ecosystems, since different zones (i.e. benthic, littoral and limnetic) could have different patterns of stratification and/or mixing events influencing bioavailability (Elliott et al., 2008). In toxicology, one of the most important applications of nitrogen stable isotopes is its ability to infer biomagnification potential. Field studies investigating food chain exposure and biomagnification of organic contaminants in migratory birds have shown that concentrations of DDT (and other POPs) generally increase with lipid content and trophic position, resulting in increased food web $\delta^{15}\text{N}$ values (Currier et al., 2020; Fremlin et al., 2020; Huertas et al., 2016; Elliott, Bishop & Morrissey, 2011; Elliott et al., 2009; Elliott, 2005; Mora, 2008; Yordy et al., 2013).

Gagnon & Hobson (2009) detected seasonal shifts in blood and claw $\delta^{15}\text{N}$ values in American robins, northern orioles (*Icterus galbula*), gray catbirds (*Dumetella carolinensis*), least flycatchers (*Empidonax minimus*) and warbling vireos (*Vireo gilvus*), and argued that many omnivorous birds incorporate food items low in $\delta^{15}\text{N}$ during autumn migration and higher $\delta^{15}\text{N}$ food items during the breeding season. In fact, American robins had the highest blood and claw $\delta^{15}\text{N}$ levels out of all insectivorous and omnivorous species sampled in boreal and parkland regions in eastern Canada (Gagnon

& Hobson, 2009), suggesting that robins are at greater risk of accumulating DDT residues due to their diet and ground foraging behavior. This contrasts with another study by Fremlin et al. (2020), who reported an average $\delta^{15}\text{N}$ value of 5.30 in American robin eggs collected from several urban areas in B.C during the spring season. This $\delta^{15}\text{N}$ value was considerably lower compared to our study (8.42), which could have implied that robins were feeding on lower trophic level organisms or fruits (Currier et al., 2020; Wheelwright, 1986) than robins in the Okanagan, or feeding in habitats with anthropogenic food items, which tend to be depleted in $\delta^{15}\text{N}$ (Caron-Beaudoin et al., 2013). For example, Currier et al. (2020) observed lower $\delta^{15}\text{N}$ signatures in European starlings (*S. vulgaris*) foraging near an urban landfill compared to non-urban starlings and suggested that urban starlings could be consuming meat refuse from anthropogenic sources. However, other environmental factors, such as climate, topography and soil characteristics could be influencing food web $\delta^{15}\text{N}$ values at different landscapes or continental scales (Gagnon & Hobson, 2009; Wan et al., 2001; Wilson et al., 2002). The intensive use of fertilizer and other agrochemicals in orchards may have contributed to higher $\delta^{15}\text{N}$ values in cultivated soils through ammonification (Elliott et al., 1996; Szeto & Price, 1991; Amundson et al., 2003), resulting in higher $\delta^{15}\text{N}$ values in earthworms and American robin eggs in this study. Regardless, the $\delta^{15}\text{N}$ and $\delta^{15}\text{C}$ analyses in this study confirm that *Aporrectodea* and *Lumbricidae* earthworms formed the base of our food chain, and that these earthworms were a significant source of exposure of DDT-r to American robins during the breeding season.

2.4.5. DDT-r contamination in Okanagan orchards

The Okanagan Valley in B.C is an intensive fruit growing region known for its large-scale use of DDT and the high levels detected in earthworms, and American robin eggs in the present study confirm the ongoing persistence of DDT. Blus et al. (1987) speculated four hypotheses for the persistence of DDT-r compounds in migratory birds, including: 1) The acquisition of DDT-r compounds in overwintering areas where DDT and other OCs are used, 2) The acquisition of DDT-r burdens in local areas from illegal use, 3) The acquisition of DDT-r burdens in local areas as a result of high application rate and long-term environmental persistence, and 4) The acquisition of DDT-r burdens from structurally similar compounds, including meta-, ortho-, and para-para DDT isomers. For instance, Kelthane (or Dicofol) is a structurally similar compound to DDT

and prior to being phased out in Canada in 1999, was a common acaricide used to control mites and other insects (Elliott et al., 1994). However, its persistence in Okanagan orchards is unlikely based on local pesticide surveys (Elliott et al., 1994), its lack of bioaccumulation (i.e. $\log K_{ow}$ 4.3) and small application rate of 4.4 kg/ha/year (British Columbia Ministry of Agriculture and Fisheries 1990). DDE is also not a major metabolite of Dicolof in birds or in the environment (Hebert et al., 1994). The illegal use of DDT in certain areas is also highly unlikely given that DDT use in Canadian orchards was already decreasing significantly without regulation by 1965, largely owing to insect resistance (Harris & Sans, 1971). In any case, all pesticides containing DDT were discontinued in Canada by 1985, after which any use was prohibited at the federal level under the Pest Control Products Act (Environment Canada, 1995). The high DDE:DDT ratios reported in the present study, along with those from other migratory birds (Elliott et al., 1994) also suggests that DDE, the main metabolite and breakdown product of DDT, is persisting at a higher rate compared to freshly sprayed DDT, thereby disproving the hypothesis of illegal DDT use. Furthermore, concentrations of DDT in air samples collected in 2001 at 40 stations across North America showed that *p,p'*-DDT levels were an order of magnitude higher in Mexico compared to Canada (Shen et al., 2005), possibly due to the low volatility of DDT (i.e. Henry's Law constant; 8.32×10^{-6} atm m^3/mol) and therefore low long-range transport potential. However, *p,p'*-DDE is considered to be more volatile (Mora et al., 2016) and DDE residues could still pose a risk to robins, as well as other birds using orchard habitat in the Okanagan, such as the European starling (*S. vulgaris*), townsend's solitaire (*Myadestes townsendi*), bullock's oriole (*Icterus bullockii*), yellow-rumped warbler (*Setophaga coronata*), gray catbird (*D. carolinensis*), black-headed grosbeak (*Pheucticus melanocephalus*) and chipping sparrow (*Spizella passerina*) (R. Kesic, personal communication). The high levels of DDT-r in Okanagan orchards may also pose a risk to aerial insectivores, including bats (Buchweitz et al., 2018), and many of the bat species in the South Okanagan are currently listed as endangered or threatened (Okanagan Similkameen Conservation Alliance, 2020).

Okanagan orchards are expected to retain DDT-r compounds in soil than other agricultural areas for several reasons. The lack of winter moisture and dry/humid summers in the Okanagan could result in higher levels of organic matter content, promoting the adsorption of DDT-r compounds to soil particulates and therefore affecting

the bioavailability of DDT-r in soil and earthworms (Szeto & Price, 1991). Certain land management practices, such as a lack of tillage or reduced soil disturbance, could have also resulted in more grass cover preventing UV degradation, as well as less volatilization of DDT from soils, thereby allowing DDE metabolites to persist in the soil (Bailey et al., 1974). The number of degree days when the temperature moves below 0°C in a year in the Okanagan is also reported to be lower than other regions in Canada, which could have resulted in a shorter winter senescence period (Harris et al., 2000), consequently affecting the amount of DDT-r that earthworms took up annually. In addition, the use of heavy metals in fruit orchards prior to DDT, such as synthetic lead arsenates (PbHAsO₄) and copper (i.e. via fungicides), is thought to inhibit the degradation of DDT to DDE via changes in soil microbial activity (Elfving et al., 1994; Giller et al., 1998). For instance, Gaw et al. (2003) collected samples from various New Zealand orchards and found that high DDE content was significantly correlated with high concentrations of copper (234-490 ug/g) and lead (23-126 ug/g) in soils. The presence of heavy metals in soil could alter microbial community structure by either decreasing microbial biomass via alternate substrate utilization, decreasing microbial respiration rate (qCO₂), and/or decreasing microbial diversity and species richness via competitive exclusion (Giller et al., 1998), consequently affecting DDT-r degradation pathways (Giller et al., 1998). There is currently a study underway to examine heavy metal levels in soil, worms and robins from the same orchards as sampled in the present work.

Above all, DDT application rates appear to be the most important factor influencing DDT-r contamination in Okanagan orchards. During the 1990s, the BC Ministry of Agriculture and Food (1969) was recommending that up to 7 kg of active ingredient DDT/ha be applied up to four times per year, and based on available spray information, would have meant that up to 27 kg of DDT/ha/year could have been sprayed on different crops throughout the growing season (Harris et al., 2000). Different areas within the Okanagan could have also received different application rates of DDT. For example, soft fruits, such as cherries, apricots and peaches are usually planted in the South Okanagan Valley, whereas apples and pears tend to be planted in and around the Penticton and Naramata regions (Kuo et al., 2012). On the other hand, the Ontario Department of Agriculture (1968) recommended that up to 3.4 kg/g of active ingredient/ha be applied two to three times per year, which would have meant that up to 10 kg of DDT/ha/year could have been sprayed in Ontario orchards during the same

time period. These differences in spray concentrations and frequencies, combined with different pest outbreaks in orchards throughout the year, meant that Okanagan growers could have potentially applied almost three times as more DDT in some years, compared to Ontario growers (Harris et al., 2000). These findings are further corroborated by temporal and latitudinal analyses which show that DDE concentrations in the upper mid-latitudes, particularly in the Pacific Northwest, remain higher than other North American regions (Mora et al., 2016; Pozo et al., 2009; Shen et al., 2005).

2.4.6. Other halogenated chemicals

Although chlorinated legacy POPs continue to persist in the environment, local food chains are frequently being contaminated with emergent legacy pollutants that were only recently phased out, including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Elliott et al., 2005). PCBs, and their subsequent PBDE replacements, were used in the 1960s in a variety of commercial and household products, including electrical equipment, construction materials, coatings, textiles and furniture padding (Grimm et al., 2015). Due to their toxic properties, environmental persistence and long-range transport potential, these chemicals were globally restricted and banned in the 2000s by the Stockholm Convention on Persistent Organic Pollutants. Environmental exposure to PCBs and PBDEs has been linked to various adverse effects in marine mammals, including whales and sharks, as well as non-cetacean species, such as seals, otters and minks (Jepson et al., 2016). PCBs and PBDEs are also known to bioaccumulate and biomagnify in avian tissue, particularly in urban raptors (Currier et al., 2020; Elliott et al., 2005; Fremlin et al., 2020), with long-term exposure resulting in reduced hatching and fledging success, delayed clutch initiation, reduced incubation and eggshell thinning (Elliott et al., 2005).

The sum of all PCBs in robin eggs in this study averaged 0.0044 ug/g (wet weight), which was much lower than the sum of PCBs in robin eggs collected from the 1990s at similar sites (Harris et al., 2000). Concentrations of 2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) were noticeably higher in robin eggs in this study and averaged 5.22 ug/g (wet weight). European starlings (*S. vulgaris*) orally exposed to BDE-99 showed no significant changes to the volumes of different brain regions part of the song-control system, including the HVC and RA (Eng et al., 2014). However, zebra finch (*T. guttata*) nestlings that were orally exposed to BDE-99 concentrations of up to

50.7 ng/g body weight had significantly smaller HVC volumes and females appeared to be more sensitive (Eng et al., 2012a), suggesting that exposure to BDE-99 in some passerines might be sex-specific. Similar studies have also shown that early exposure to BDE-99 can decrease the motivation of zebra finch males to mate, particularly in high dose groups, which arguably could be caused by a reduction in sex steroid hormones (Eng et al., 2012b). In field studies, European starlings (*S. vulgaris*) breeding near urban landfills have been shown to have higher BDE-99 levels in their eggs, as well as other PBDE congeners, as evidenced by the high biota-soil accumulation factors (BSAFs) and biomagnification factors (BMFs) calculated from foraged food items, compared to reference birds. Exposure to BDE-99 and other PBDEs might therefore pose a toxicological risk to robins and other passerines foraging near landfills, waste disposal sites, and/or other urbanized areas.

2.4.7. Conclusion

DDT is a legacy organochlorine that has been used since the late 1940s, causing extensive contamination of fruit orchards in the Pacific Northwest, as well as high concentrations of the main metabolite, *p,p'*-DDE, in soil invertebrates and birds. The primary objective of this study was to determine whether DDT-r compounds are persisting in fruit orchards after historical agricultural use. This was done by: 1) collecting soil, earthworms and American robin eggs from various orchards and reference sites in the Okanagan Valley, 2) comparing previous and current contaminant DDT burdens in orchard-nesting robins, and 3) analyzing earthworm-robin biomagnification factors (BMFs) for different DDT-r compounds. Overall, 100% of robin eggs contained detectable levels of *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD, and the highest concentration of *p,p'*-DDE was 107 ug/g (wet weight), confirming that DDT contamination is still present at similar and high levels as in the 1990s. Concentrations of *p,p'*-DDE and *p,p'*-DDT were significantly higher in American robins compared to *Aporrectodea* and *Lumbricidae* earthworms and earthworm-robin regressions for *p,p'*-DDE showed a significant positive relationship, suggesting that robins were acquiring DDT burdens locally during the breeding season. BMFs calculated for robins and earthworms were generally > 1 and were significantly higher for *p,p'*-DDE, confirming the ongoing persistence, and trophic magnification of DDT compounds in American robin fruit orchard food chains.

The relatively high concentrations of DDT-r in American robins in this study indicate that the source of contamination is still local and likely the result of past application rates and slow degradation rates determined by soil, and earthworm activity in orchards. The use of synthetic lead arsenates (PbHAsO_4) in Okanagan orchards prior to DDT may be inhibiting the microbial degradation of certain DDT metabolites. Further studies are planned to look at the relationship between lead arsenates and DDT-r compounds in soil, earthworms and robin eggs in Okanagan orchards. Despite the high DDT-r concentrations detected in this study, the reproductive success of American robins does not appear to be affected, as concentrations in this study were considerably lower than those reported in the 1990s and in other studies where DDT levels had no significant effect on robin nest productivity. However, American robins exposed to environmental levels of *p,p'*-DDE *in-ovo* and during early post-hatching development in Okanagan orchards could have smaller brain regions, as well as reductions in song nuclei, which could potentially disrupt normal sexual behaviour. Concentrations of DDT-r in American robins and other ground-foraging birds in Okanagan orchards could pose a health risk to local predators and birds of prey, such as Accipiter hawks and falcons, who generally feed at higher trophic levels and are known to be more sensitive to eggshell quality effects, and population declines from DDT exposure.

2.5. Tables

Table 1. DDT-r concentrations (ug/g wet weight) in earthworm species collected from orchards in the Okanagan Valley

Species ^a	Location	n ^b	Total ^c	Biomass ^d	% Moisture	% Lipid	<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p'</i> -DDT	DDE:DDT
<i>Lumbricus terrestris</i>	Orchard 1	3	4	25.43	90.4	0.59	0.0251	0.001	0.0074	3.39
<i>Lumbricus rubellus</i>	Orchard 1	3	28	18.74	86.3	1.19	0.23	0.0057	0.0809	2.84
<i>Lumbricus rubellus</i>	Orchard 2	3	60	38.79	84.9	0.77	2.53	0.0712	0.638	3.97
<i>Aporrectodea</i> spp.	Orchard 2	3	4	10.58	86.3	0.26	0.711	0.02	0.231	3.08
<i>Lumbricus rubellus</i>	Orchard 3	3	73	56.17	86.2	1.12	1.79	0.0624	0.502	3.57
<i>Aporrectodea</i> spp.	Orchard 3	3	13	9.16	86.5	0.59	1.82	0.0628	0.509	3.58
<i>Lumbricus rubellus</i>	Orchard 4	3	8	6.14	85.1	0.39	0.967	0.0103	0.0603	16
<i>Lumbricus rubellus</i>	Orchard 5	3	25	17.82	76.3	0.00 ^e	1.54	0.0247	0.364	4.23
<i>Aporrectodea</i> spp.	Orchard 5	3	15	5.32	81.8	0.79	1.66	0.0247	0.347	4.78
<i>Lumbricus terrestris</i>	Orchard 6	3	6	19.79	86.0	0.32	0.885	0.0215	0.222	3.99
<i>Lumbricus rubellus</i>	Orchard 6	3	47	30.37	86.6	0.94	0.566	0.0236	0.0894	6.33
<i>Lumbricus terrestris</i>	Orchard 7	3	6	25.9	86.0	1.11	5.06	0.325	0.444	11.4
<i>Lumbricus terrestris</i>	Orchard 8	3	9	50.05	87.0	1.38	1.63	0.0545	0.153	10.7
<i>Lumbricus rubellus</i>	Orchard 8	3	41	22.37	80.7	1.64	2.41	0.239	0.144	16.7
<i>Lumbricus terrestris</i>	Orchard 9	3	14	33.8	94.7	1.23	1.52	0.0508	0.283	5.37
<i>Lumbricus rubellus</i>	Orchard 9	3	40	18.94	85.1	1.42	1.11	0.0283	0.233	4.76
<i>Lumbricus terrestris</i>	Orchard 10	3	3	11.17	91.9	1.22	2.08	0.0593	0.496	4.19
<i>Lumbricus rubellus</i>	Orchard 10	3	18	9.61	85.4	1.56	1.75	0.035	0.405	4.32
<i>Aporrectodea</i> spp.	Orchard 10	3	23	7.97	84.7	1.32	2.18	0.0462	0.486	4.49
Species average			23	22.01	85.89	0.939	1.603	0.0614	0.2997	6.19

^a All earthworm samples include adults and juveniles

^b n = number of sites sampled within each orchard

^c Total count is defined as the total number of individual earthworms collected within a 60 cm² quadrat across all sites

^d Biomass is the total weight of earthworm species collected from all sites within an orchard and is measured in grams

^e % lipid could not be calculated for this sample due to an unexpected lab issue

Table 2. DDT-r concentrations (ug/g wet weight) in American robin eggs collected from orchard and reference sites in the Okanagan Valley

Location	% Moisture	% Lipid	<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	<i>p,p'</i> -DDD	DDE:DDT
Orchard 1	80.60	5.50	12.80	0.0205	0.0025	624.4
Orchard 1	80.20	6.02	14.0	0.0404	0.0043	346.5
Orchard 2	82.40	5.18	26.50	1.05	0.11	25.2
Orchard 2	83.60	4.17	70.0	2.06	0.31	34
Orchard 2	82.50	4.52	32.30	1.14	0.18	28.3
Orchard 3	82.60	5.80	36.90	1.77	0.21	20.8
Orchard 4	81.80	5.84	27.70	1.24	0.09	22.3
Orchard 5	82.90	5.58	11.20	0.29	0.03	38.5
Orchard 5	83.80	5.31	14.30	0.54	0.04	26.7
Orchard 6	82.40	4.91	3.28	0.18	0.02	17.8
Orchard 7	82.30	4.76	107.0	2.03	0.46	52.7
Orchard 7	81.60	5.54	39.0	1.45	0.16	26.9
Orchard 7	83.70	4.29	35.2	1.30	0.24	27.1
Orchard 8	83.20	4.35	39.2	1.30	0.09	30.2
Orchard 8	83.90	3.55	22.7	0.83	0.14	27.4
Orchard 8	83.0	4.74	42.6	1.10	0.20	38.7
Orchard 9	81.90	5.63	40.8	1.52	0.18	26.8
Orchard 10	83.30	5.86	79.3	1.94	0.20	40.9
Orchard 10	84.60	5.04	50.5	1.97	0.33	25.6
Orchard 10	84.0	5.06	26.0	1.08	0.11	24.1
Orchard AVG	82.72	5.08	36.56	1.14	0.155	75.24
Ref 1	82.40	5.65	0.848	0.0397	0.0026	21.4
Ref 2	82.70	5.95	0.141	0.0117	0.0006	12.1
Ref AVG	82.55	5.80	0.495	0.0257	0.0016	16.75

Table 3. Biomagnification factors (BMFs) from earthworms to American robins in Okanagan orchard food chains^a

Location	<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	<i>p,p'</i> -DDD
Orchard 1	24.32	0.16	0.22
Orchard 2	5.78	0.68	0.95
Orchard 3	4.04	0.69	0.67
Orchard 4	4.15	2.98	1.23
Orchard 5	1.13	0.16	0.18
Orchard 6	0.77	0.19	0.18
Orchard 7	3.17	0.95	0.24
Orchard 8	6.80	2.82	0.41
Orchard 9	7.62	1.46	1.12
Orchard 10	7.47	1.05	1.33
Average ^b	6.53 ± 2.12	1.12 ± 0.33	0.65 ± 0.15

^a BMFs were calculated using organic carbon-lipid normalized concentrations (ug/g OC-lipid equiv) based on pooled earthworm and robin DDT-r data from each orchard; BMFs > 1 are in bold

^b Average BMF across all sites; expressed as mean ± standard error

Table 4. Presence of other organochlorines (OCs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in robin eggs collected from Okanagan orchards, 2019. Values represent means \pm standard error (ug/g wet weight). % = % of robin eggs with detectable levels based on the MDL reported for each compound.

Compound	Concentration	%
Hexachlorobenzene	0.000626	52
Heptachlor Epoxide	0.000365	22
Oxychlorane	0.001091	52
cis-Nonachlor	0.0004	26
trans-Nonachlor	0.00341	61
Dieldrin	0.0115	78
Sum PCBs ^a	0.0045	42
BDE-47	3.41 \pm 0.53	96
BDE-49	0.0244	17
BDE-85	0.0546	22
BDE-99	5.22 \pm 1.0	100
BDE-100	2.25 \pm 0.47	100
BDE-138	0.0237	9
BDE-153	0.979 \pm 0.24	96
BDE-154/153	0.4312 \pm 0.09	91
BDE-183	0.690 \pm 0.61	22

^a Sum of 35 congeners (17/18, 28/31, 33, 44, 49, 52, 70, 74, 87, 96, 99, 101, 105, 110, 118, 128, 138, 149, 151, 153, 156, 158, 170, 171, 177, 180, 183, 187, 194, 195, 199, 205, 206, 208, 209).

2.6. Figures

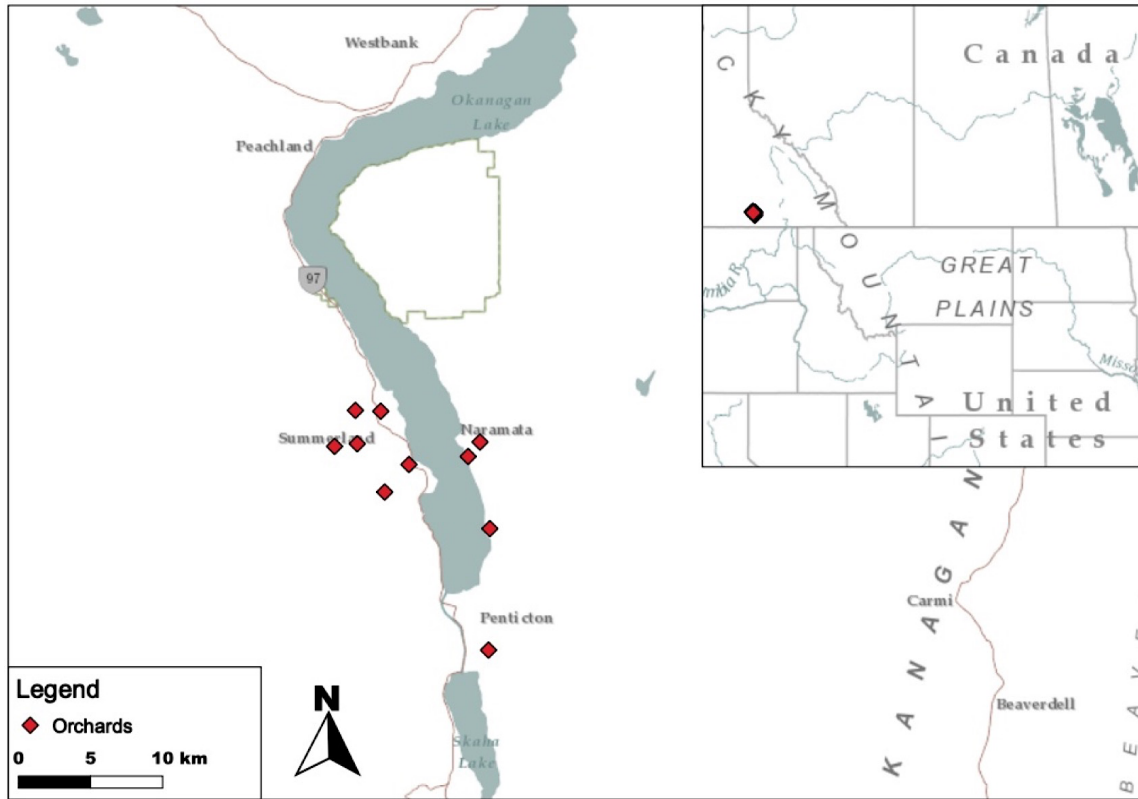


Figure 1. Location of fruit orchards sampled in the Okanagan Valley, British Columbia.

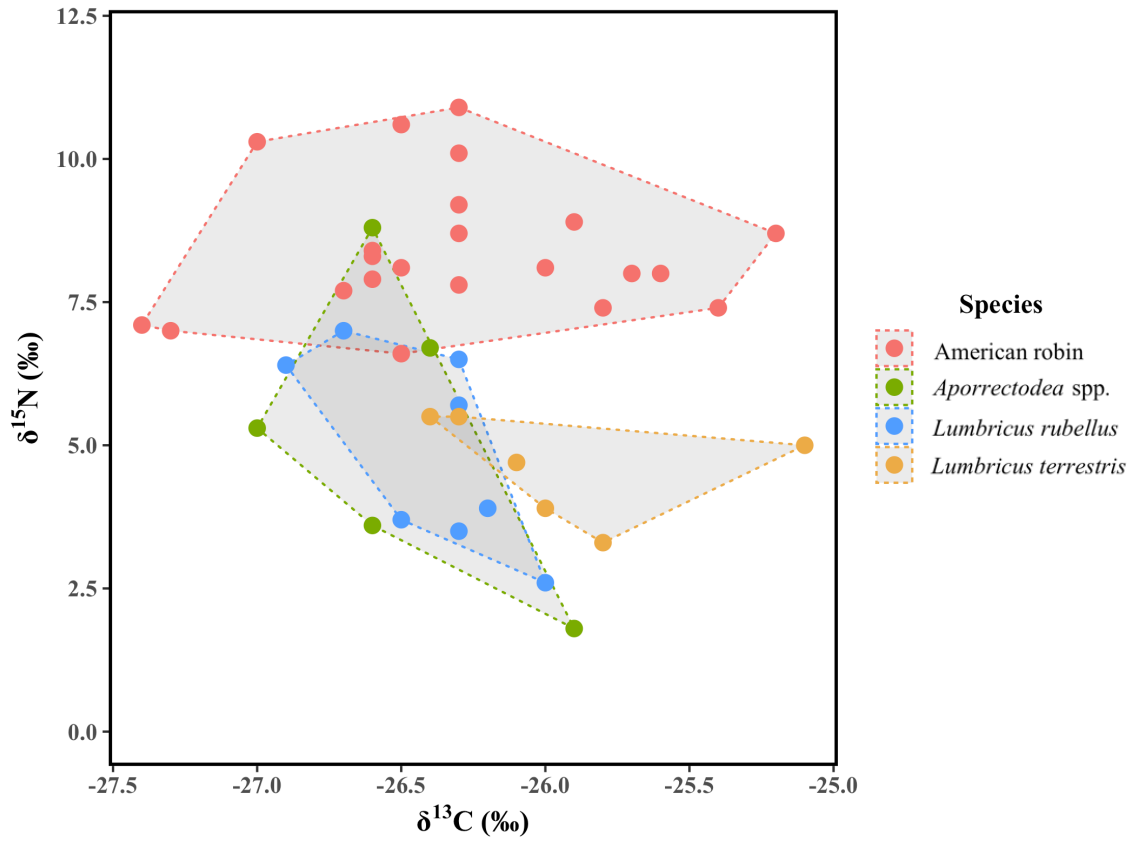


Figure 2. Geometric spread of stable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures of American robin eggs, and earthworm samples collected from Okanagan fruit orchards.

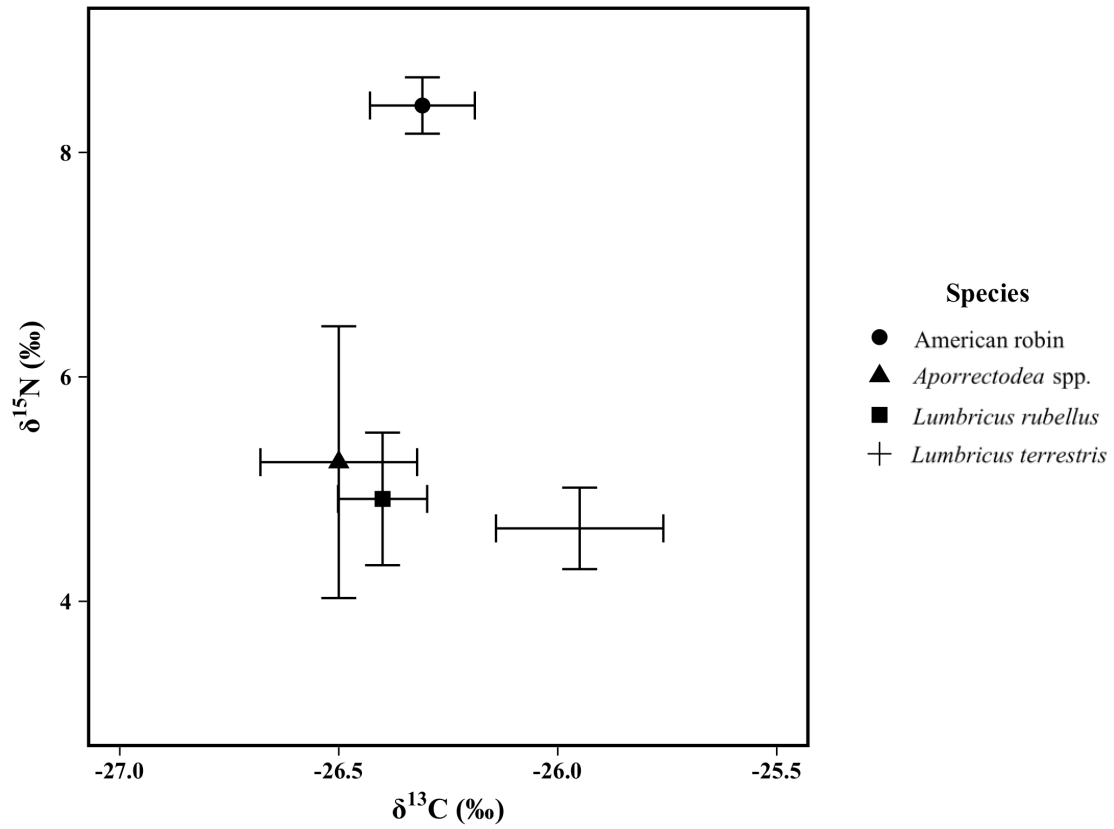


Figure 3. Mean stable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope signatures of American robin eggs, and different earthworm species collected from Okanagan fruit orchards. Error bars represent \pm 95% confidence limits.

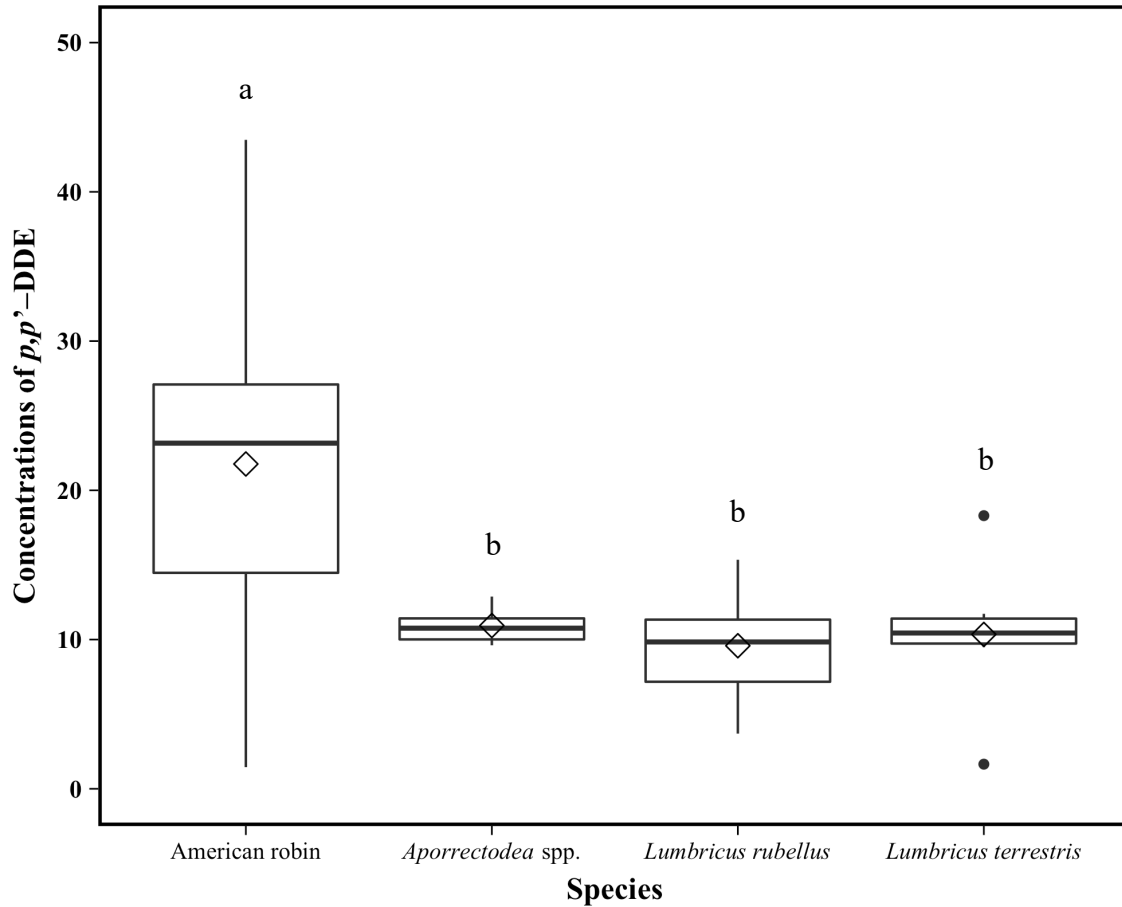


Figure 4. Concentrations of p,p' -DDE (ug/g organic carbon-lipid equivalent) in American robin eggs and earthworms. Concentrations of p,p' -DDE are square-root transformed. Lower whisker tips represent the minimum concentration and the higher whisker tips represents the maximum concentration. Black horizontal lines represent the median. Clear diamonds represent the mean. Black circles represent outliers. Letters above plots denote significance in concentrations across species.

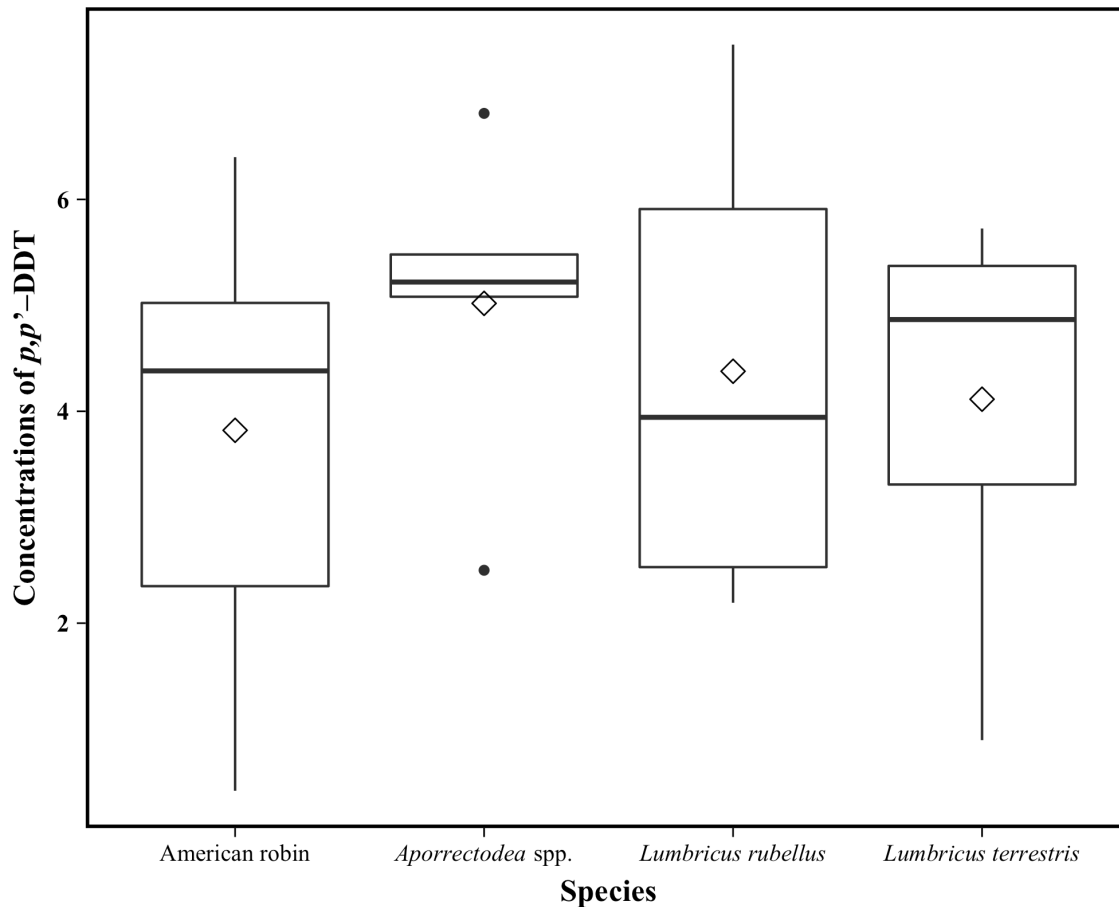


Figure 5. Concentrations of p,p' -DDT (ug/g organic carbon-lipid equivalent) in American robin eggs and earthworms. Concentrations of p,p' -DDT are square-root transformed. Lower whisker tips represent the minimum concentration and the higher whisker tips represents the maximum concentration. Black horizontal lines represent the median. Clear diamonds represent the mean. Black circles represent outliers.

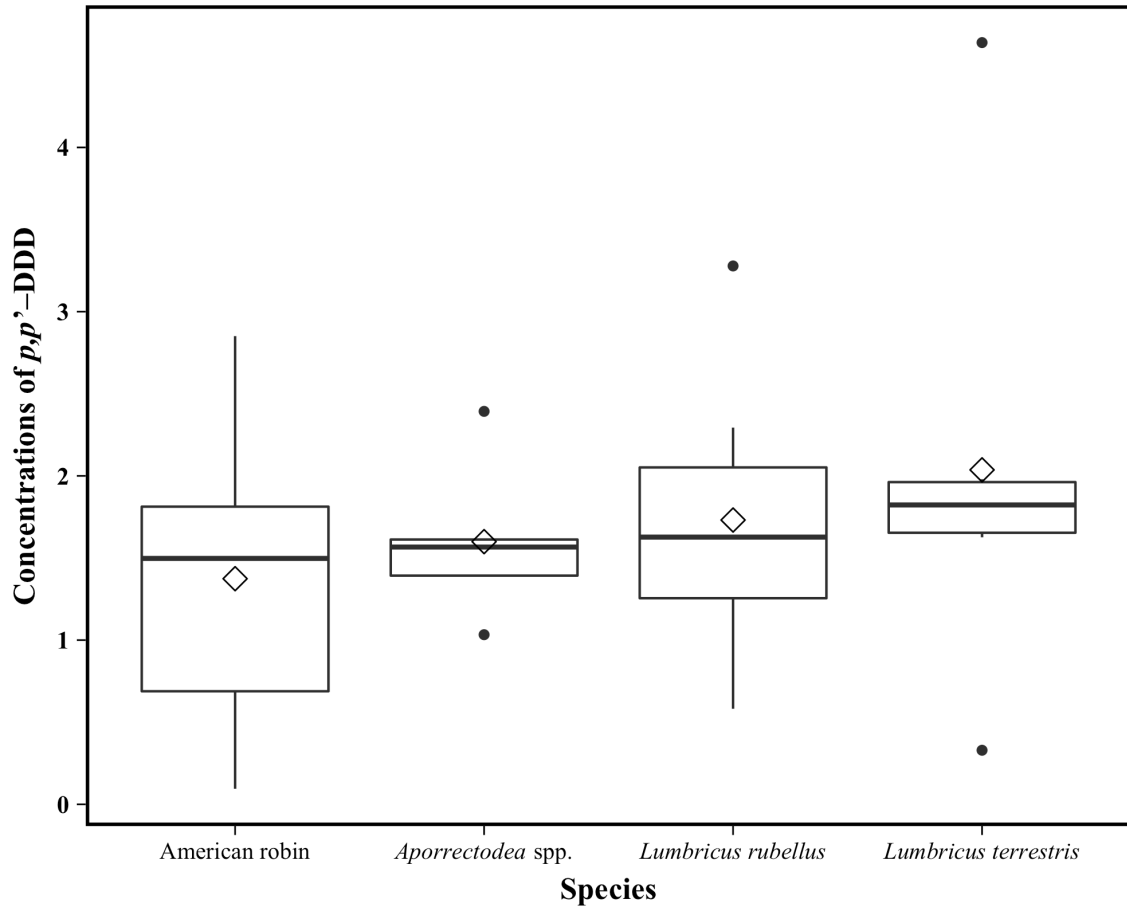


Figure 6. Concentrations of *p,p'*-DDD (ug/g organic carbon-lipid equivalent) in American robin eggs and earthworms. Concentrations of *p,p'*-DDD are square-root transformed. Lower whisker tips represent the minimum concentration and the higher whisker tips represents the maximum concentration. Black horizontal lines represent the median. Clear diamonds represent the mean. Black circles represent outliers.

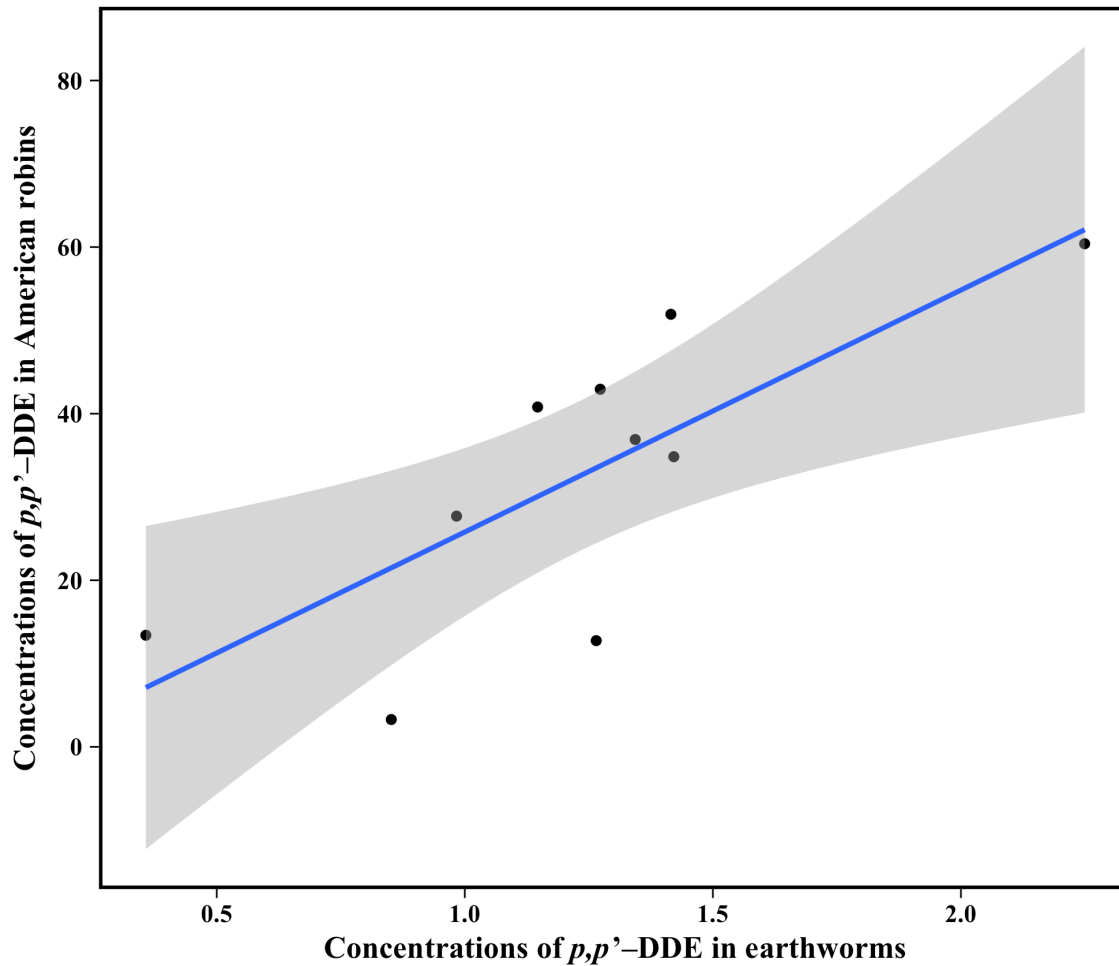


Figure 7. Linear regression between p,p' -DDE concentrations (wet weight) in American robin eggs and earthworms with proportional relationship (blue line). Concentrations for earthworms are square-root transformed. Grey bands represent the $\pm 95\%$ confidence intervals. Data points represent pooled robin and earthworm data from each orchard ($DDE_{\text{robin}} = (29.05 \times DDE_{\text{earthworm}}) - 3.25$; $F_{1,8} = 11.62$; $P = 0.0092$; $R^2 = 0.60$).

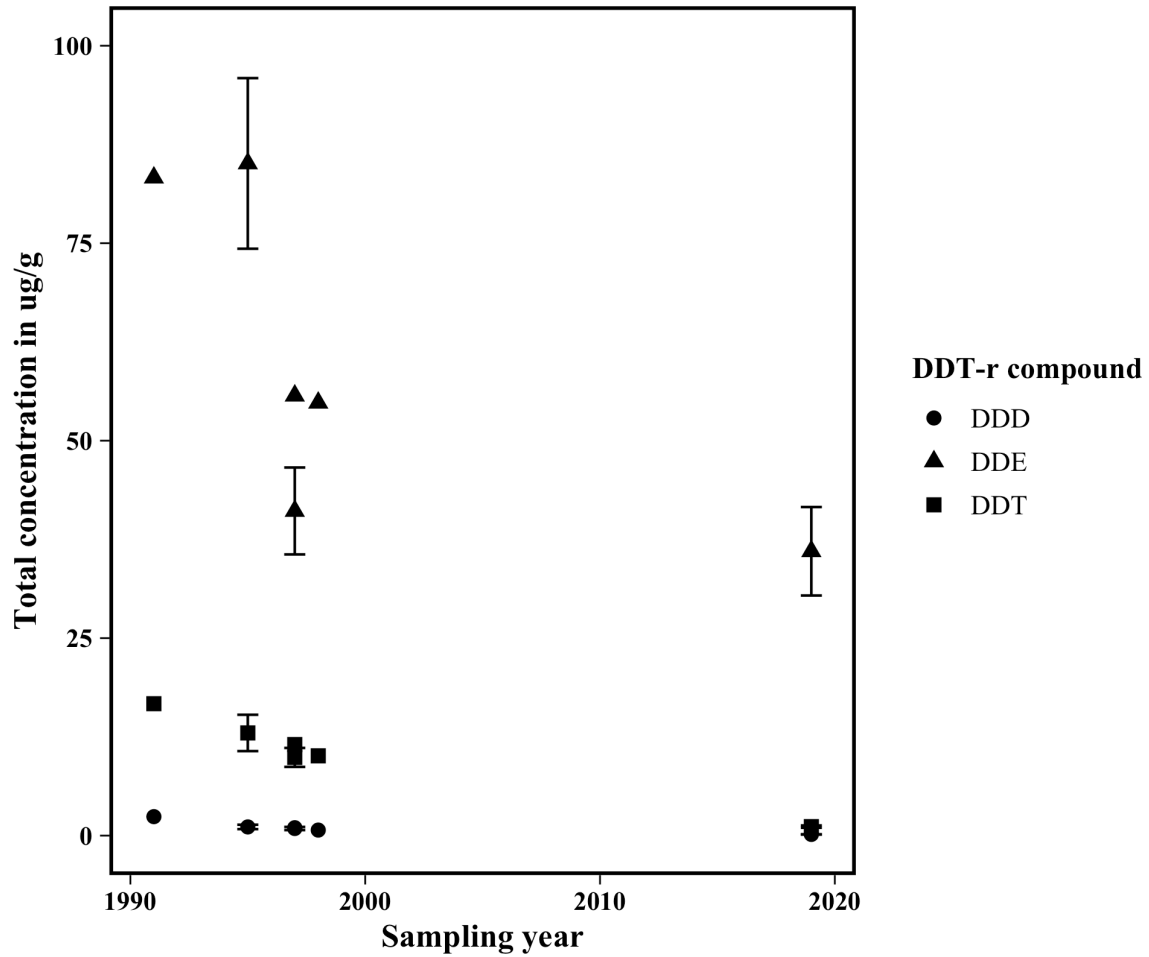


Figure 8. Time series plot showing DDT-r concentrations (ug/g wet weight) from 1990 to 2019 (present study) in American robin eggs from Okanagan fruit orchards. Data points represent mean concentrations in eggs reported by Elliott et al. (1994), Gill et al. (2003), Harris et al. (2000), Iwaniuk et al. (2006) and Smith (2004). Error bars represent \pm 95% confidence limits.

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