THE EFFECTS OF *IN OVO* AND EARLY POST-HATCH DDT EXPOSURE ON AMERICAN ROBINS FROM THE OKANAGAN VALLEY, BRITISH COLUMBIA

by

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ABSTRACT

American robin (*Turdus migratorius*) eggs from orchard areas of the Okanagan Valley, British Columbia contain high levels of dichlorodiphenyltrichloroethane (DDT) and its metabolites. These contaminants are present in the soil as a result of heavy historical use. DDTs accumulate in the earthworms that live in the soil and are passed to the robins through their preference for earthworms as a food source during the breeding season. These chemicals are then passed to the offspring via the egg yolk and in the diet. Despite the high residue levels found in these robins, no impairments in their reproductive success have been found. In order to assess more subtle and/or long term effects of DDTs on a variety of parameters, ten-day old nestlings were collected from the Okanagan, along with controls from the Lower Mainland of British Columbia, and raised and bred in captivity. Eggs were collected in order to measure contaminant levels. Total DDTs in the Okanagan eggs ranged from 5.7 to 277.6 μg/g (mean 49.3 μg/g), whereas in the Lower Mainland eggs they ranged from 0.4 to 3.4 μg/g (mean 1.5 μg/g). Eggs collected from the Lower Mainland and Okanagan were of similar weights, lengths, and widths. Okanagan chicks collected in 1997 had significantly shorter middle toes than the other birds, and appeared to lag in their tarsus growth. As adults, these birds laid smaller eggs than the Lower Mainland controls, but showed no differences in the timing of their reproductive activities, their laying, hatching, and fledging success, or their reproductive behaviors. They demonstrated an increased susceptibility to infectious disease, lower corticosterone levels during the early phases of a restraint test, and enlarged hearts, livers, and kidneys. Although robins in the Okanagan continue to thrive and reproduce despite their high levels of contamination, DDT likely has the potential to influence several aspects of their lives, as evidenced by the many significant correlations with *in ovo* exposure. However, it remains that genetic differences between the Lower Mainland and Okanagan birds may account for many of the effects seen.

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First they tell you you're wrong, and they can prove it.
Then they tell you you're right, but it's not important
Then they tell you it's important, but they've known it for years.
C.F. Kettering

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Chapter III

Robin eggs and nestlings were collected and measured by Laurie Wilson and team, from the Canadian Wildlife Service. Egg contaminant levels were analyzed by Michael Mulvihill at the National Wildlife Research Center in Hull, Quebec. Body weights and tarsus lengths were measured by Chris Gill, Christy Morrisey, Chris Coker, Laurie Wilson, Sandi Lee, Harpreet Gill, and Lori Smith. Birds were sexed by Brett Vanderkist at Simon Fraser University, Burnaby, British Columbia. Blood samples were collected by Karen Petit, Laurie Wilson, Sandi Lee, Gabby Kardosi, Harpreet Gill, and Christy Morrisey, with help from Lori Smith. Blood smears were prepared by Sandi Lee and Lori Smith, and white blood cell counts were conducted by Lori Smith. Thyroid hormone levels were analyzed by Tracy Marchant at the University of Saskatchewan, Saskatoon, Saskatchewan. The phytohemagglutinin skin test was performed by Lori Smith and Maria Fronteddu, with help from Laurie Wilson. Coccidiosis was diagnosed by Ted Leighton at the Canadian Cooperative Wildlife Health Centre at the University of Saskatchewan, Saskatoon, Saskatchewan. Birds were housed at Monika's Wildlife Shelter, Surrey, British Columbia prior to sexual maturity and cared for by Monika Tolgsdorf and her staff. Birds were housed at the University of British Columbia San Rafael Research Aviary, Surrey, British Columbia after sexual maturity. The San Rafael pens were built by Chris Coker, Chris Gill, Sandi Lee, Lori Smith, Laurie Wilson, Kim Cheng, Terry Sullivan, and others. Pens were maintained by Lori Smith and Terry Sullivan. Birds were cared for at San Rafael by Lori Smith. Birds were sacrificed and dissected by Kim Cheng and Lori Smith. All statistical analyses were conducted by Lori Smith with help from Kim Cheng.

Chapter IV

Robin eggs and nestlings were collected from the Okanagan and Lower Mainland and measured by Laurie Wilson and team, from the Canadian Wildlife Service. Egg contaminant levels were analyzed by Michael Mulvihill at the National Wildlife Research Center in Hull, Quebec. Thyroid hormone levels were analyzed by Tracy Marchant at the University of Saskatchewan, Saskatoon, Saskatchewan. Eggs and chicks from San Rafael were weighed and measured by Lori Smith. The breeding birds were sexed by Brett Vanderkist at Simon Fraser University, Burnaby, British Columbia. San Rafael chicks were sexed by Maria Fronteddu at the University of British Columbia, Vancouver, British Columbia. All behavioral observations were conducted by Lori Smith. Birds were housed at Monika's Wildlife Shelter, Surrey, British

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Chapter V

Robin eggs and nestlings were collected and measured by Laurie Wilson and team, from the Canadian Wildlife Service. Egg contaminant levels were analyzed by Michael Mulvihill at the National Wildlife Research Center in Hull, Quebec. Body weights and measures, blood samples, blood smears, hematocrits, stress response testing, phytohemagglutinin skin testing obtained and conducted by Laurie Wilson and others. Blood samples were collected by Laurie Wilson and others. White blood cell counts were conducted by Lori Smith. Thyroid hormone and corticosterone levels were analyzed by Tracy Marchant at the University of Saskatchewan, Saskatoon, Saskatchewan. Blood lead levels were analyzed by Ewa Neugebauer at the National Wildlife Research Center. Mycoplasma diagnosis conducted by the Animal Health Centre at the British Columbia Ministry of Agriculture, Food, and Fisheries. Birds were housed at Monika's Wildlife Shelter, Surrey, British Columbia prior to sexual maturity and cared for by Monika Tolgsdorf and her staff. Birds were sacrificed and dissected by Malcolm McAdie. All statistical analyses were conducted by Lori Smith with help from Kim Cheng.

Overview

Chapter I

This chapter represents a review of some of the available literature on DDT and provides background information on DDT and its detrimental effects especially on birds. Focus is given to effects on growth and survival, reproduction, behavior, and the stress response.

Chapter II

This chapter provides background information on the study area (the Okanagan Valley of British Columbia) and species (the American robin) utilized for this research. Included is information on soil, earthworm, and American robin DDT contamination.

Chapter III

This chapter examines the effects of early DDT exposure on the growth and survival of American robins from the Okanagan Valley, British Columbia. Egg contaminants, egg measurements, chick measurements, thyroid hormone levels, immune response, mortality, and tissue weights at sacrifice are included. The goal of this part of the study was to determine if Okanagan birds are at a disadvantage in terms of growth and survival as compared to control birds from the Lower Mainland.

Chapter IV

This chapter focuses on the effects of early DDT exposure on reproduction and behavior in American robins. Breeding pairs were observed for nest building, egg laying, egg hatching, and chick fledging. Reproductive behaviors including mating, nest building, and parental care were monitored, as well as vocal behaviours (e.g., singing, chirping), maintenance behaviors (e.g., eating, drinking, preening), and aggressive behaviors (e.g., charging, chasing, biting). In addition, a subset of breeding pairs were blood sampled on a regular basis in order to monitor thyroid hormone levels. The offspring of these birds were also weighed and measured, their survival monitored, and their tissues weighed upon sacrifice. The goal of this part of the study was to determine if birds exposed to DDT exposure early in life suffered from decreased reproductive success, as compared to Lower Mainland birds, and if they demonstrated any alterations in their behaviors which could influence their survival and reproductive success.

Chapter V

This chapter deals with a second set of eggs and birds collected from the Okanagan and Lower Mainland in order to look more closely at the effects of early DDT exposure on immune response and other parameters that can influence immune response. Egg contaminants were determined, eggs and chicks were weighed and measured, thyroid hormones, white blood cell ratios, hematocrits, and T-cell mediated immune responses were evaluated, as well as corticosterone levels during a restraint stress test. The goal of this study was to determine if early exposure to DDT and its effects on immunity, thyroid hormones, and corticosterone release played a role in the coccidiosis infections and subsequent deaths of a number of juvenile robins (Chapter III).

Chapter VI

This chapter serves as an overview of the results found in the previous chapters, as well as problems encountered during the course of the study, things that could have been done differently, other factors that may have played a role in the results, implications of these findings, and avenues for future research.

Chapter I
Introduction

1.1. What Is DDT?

Dichlorodiphenyltrichloroethane, better known as DDT, is an organochlorine pesticide that was first produced in 1873. It was rediscovered in 1939, and at this point its insecticidal properties were revealed. This discovery was hailed as "the most revolutionary development in the history of pest control" and earned Paul Müller the Nobel Prize for Medicine and Physiology in 1948 (p. 1, Mellanby, 1992). DDT was touted as the ideal pesticide. It was highly toxic to insects, safe for plants and warm-blooded animals, non-irritating and odorless, widely applicable, cheap and easy to produce, and long-lasting. None of the pesticides in use at the time fulfilled all of these criteria (Mellanby, 1992). It comes in a variety of forms, with the para, para' (p,p') and ortho, para' (o,p') isomers being the most common. Commercial or technical grade DDT is composed of primarily p,p'-DDT (65 - 90%), with 10 - 30% o,p'-DDT and a variety of metabolites and other products (Jefferies, 1975; Mellanby, 1992; WHO, 1989). There are two major routes of metabolism of DDT (Figure 1-1), with the initial change being either to DDE (dichlorodiphenyldichloroethylene) or DDD (dichlorodiphenyldichloroethane, also known as TDE). The primary DDT metabolite in living tissues is DDE (Jefferies, 1975; Stickel, 1973). Under anaerobic conditions and post-mortem, DDT is metabolized primarily to DDD (Stickel, 1973).

DDT kills insects by working as a nerve poison. It may be ingested or absorbed through an insect's integument (Mellanby, 1992). Although DDT's molecular target and mode of action have yet to be clearly defined, it likely exerts its effects on the nervous system by blocking potassium efflux across the nerve axon membrane resulting in an increased negative after-potential. Consequently, it slows down the turning-off of sodium conductance across the membrane and inhibits the turning-on of potassium conductance, probably by interfering with the energy metabolism required for ion transport across the membranes (Bunyan & Stanley, 1982; Murphy, 1980; Younis et al., 2002).

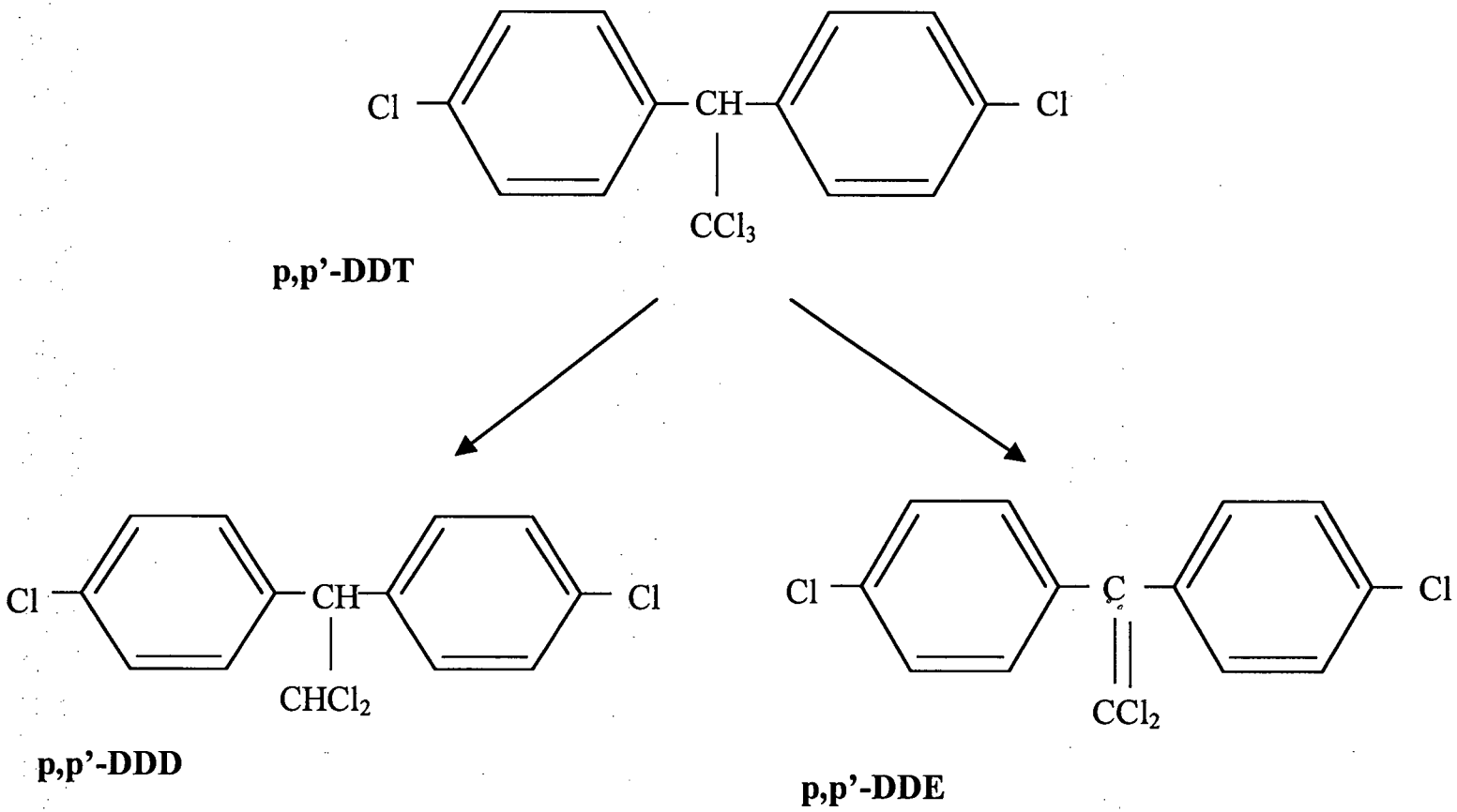

Figure 1-1: Schematic diagram of p,p'-DDT and its two primary metabolites, p,p'-DDD and p,p'-DDE.

DDT became commercially available in 1941, and was first available as dusts and wettable powders for plant and fabric protection and public hygiene. During World War II, DDT-containing products were used extensively to combat malaria-carrying mosquitoes, typhus-bearing lice, and bubonic plague-infested rat fleas. The number of lives saved worldwide by DDT are numbered in the millions, and the illnesses prevented in the hundreds of millions (Murphy, 1980). Following World War II, DDT was still used to combat disease-transmitting insects in areas of outbreak, but its use shifted to primarily plant protection. Fruit tree pests were among the first to be controlled by DDT products. DDT also found success against insects damaging everything from vegetables to forage crops, cotton to forests (Mellanby, 1992).

1.2. The Trouble with DDT

Many of the qualities that made DDT such an efficient pesticide also made it a cause for concern. Although not highly volatile or water soluble, DDTs can adsorb to particles and be carried by wind and water to all ecosystems of the world (Repetto & Baliga, 1996). They can remain in soils for more than 50 years (Colborn et al., 1993), where they may be consumed by a variety of organisms. Because DDTs are very lipophillic, they can bioaccumulate in lipid-rich tissues and biomagnify in food webs. Therefore, they can be passed on to offspring and predators far removed from the original exposure (Murphy, 1980; Repetto & Baliga, 1996). DDT is non-selective and, therefore, damaging not only to detrimental insects, but also beneficial ones (Carson, 1962; Murphy, 1980). As DDT was non-irritating, widely applicable, cheap, and easy to produce, it tended to be over-used, and often proper care was not taken to avoid over-spraying, to limit dosages, and to prevent contamination (Carson, 1962). It is this over-use of DDT that likely contributed to the rapid development of resistance in a number of insects (Mellanby, 1992). The metabolites, by-products, different isomers, and contaminants of DDT may also have different effects than the parent compound (Murphy, 1980), and as DDTs are rarely found alone, pesticide mixtures may have additive or synergistic effects (Fossi, 1998; McArthur et al., 1983; Tyler et al., 1998).

Although much less toxic than many of its predecessors (and successors), DDT has been linked to a number of animal deaths. Non-target species may be acutely or chronically poisoned, although there is great variability in species sensitivity to the toxic effects of DDT (Banerjee et al., 1996; Carson, 1962; Murphy, 1980). Fish, for example, are highly susceptible to DDT poisoning, with fish such as the dwarf perch (*Micrometrus minimus*) demonstrating LC_{50} levels

as low as, and even lower than, 0.3 μg/liter of water. Smaller fish tend to be more at risk than larger fish of the same species (WHO, 1989). Mammals and birds do appear to be relatively tolerant to the toxic effects of DDTs, but will succumb to high dosages and some species are more sensitive than others are. The LD_{50} for American brown bats (*Eptesicus fuscus*), for instance, may be as low as 25 mg DDT/kg in a single dose (WHO, 1989). The LD_{50} (oral) for male lab rats (*Rattus norvegicus*), on the other hand, is 217 mg/kg technical grade DDT, and 880 mg/kg DDE (Murphy, 1980). While the difference in susceptibility to DDT poisoning between insects and other organisms may simply be due to differences in scale (Mellanby, 1992), there are also likely to be differences in their respective nervous systems. For example, mammals do not appear to have a particular protein that is part of the sodium/potassium pump in nerve membranes (Younis et al., 2002).

Aside from its potential toxicity, DDT can have other detrimental effects. It has been suggested that DDTs are teratogenic, disrupting the organization of the brain and body of developing embryos and leading to altered cognition and behavior, as well as physical deformities (Colborn et al., 1996). DDT and DDE have been shown to induce nerve cell death and suppress the differentiation of these cells in rats and have been linked to the deterioration of neurobehavioral functions in DDT-exposed workers (Shinomiya & Shinomiya, 2003). There is also evidence that DDT disrupts sodium conductance in nerve cells, similar to that seen in insects. Sodium channels are held open in DDT poisoned animals leading to tremor, hyper-excitability, and changes in the levels of some neurotransmitters (Hong et al., 1986). The carcinogenicity of DDT is still under debate, but it has been shown to cause hepatic tumors in rodents and has been linked to both breast and testicular cancers (Murphy, 1980; Repetto & Baliga, 1996). DDTs have been shown to disrupt the endocrine system by mimicking, agonizing, or antagonizing the functions of endogenous hormones (Colborn et al., 1996). As well, they may have detrimental effects on normal immune system functioning (Banerjee et al., 1996; Barnett & Rodgers, 1994; Repetto & Baliga, 1996; Street, 1981; Voccia et al., 1999). Because of its potential as an environmental contaminant and health risk, the use of DDT was banned in Canada, the United States, Great Britain, and other industrialized countries in the early 1970's (Mellanby, 1992).

1.3. Effects of DDT on Birds

Some of the first public debates about DDT arose as a result of this chemical's effects on birds (Carson, 1962). "It was not until the American public became aware of the possible

extinction of the American Robin, because DDT causes paralysis of its central nervous system, that something was finally done to save it and other species....it was the American Robin that became the symbol of the fight to stop the use of this deadly chemical." (p. 80, Wauer, 1999). Birds may be more sensitive to DDTs and other chemicals than mammals because of their relatively smaller liver, rapid rates of food intake associated with maintenance of high body temperature, and the potential reabsorption of urinary metabolites from the cloaca (Rattner et al., 1984). They tend to respond very quickly to pesticide use, but their ability to absorb and metabolize chemicals may vary dramatically. Birds that eat other birds or fish usually have higher residues than those that eat seeds, vegetation, or mammals (Stickel, 1973). Insectivores also accumulate higher DDT burdens than granivores (Klemens et al., 2000). The half-life of DDE was found to be between 200 and 300 days for herring gulls (*Larus argentatus)* (Braune & Norstrom, 1989), 229 days for common grackles (*Quiscalus quiscalus)*, 250 days for pigeons (*Columbia livia*) (Stickel et al., 1984), and 129 days for Japanese quail (*Coturnix japonica*) (Braune & Norstrom, 1989; Norstrom et al., 1986). It can take up to 988 days for 95% of a bird's body burden of DDTs to be depleted (Stickel, 1973).

1.3.1. Effects on Growth and Survival

1.3.1.1. Adult Survival

Although considered only moderately toxic to birds (WHO, 1989), in high doses, DDT can result in mortality. Extremely high mortality rates were seen in some species during the height of DDT use (Carson, 1962). An application of 5.6 kg of DDT per hectare resulted in immediate reductions in populations of songbirds and invertebrates in an upland hardwood forest in Pennsylvania (Blus, 1996). The use of DDD to control gnats in Clear Lake, California during the 1950's decimated the population of western grebes (*Aechmophorus occidentalis*). Not only did many of the adults die, the remaining ones failed to reproduce (Carson, 1962; Fry, 1995; Mellanby, 1992). Thirty μg/g of DDT plus DDD in the brain has been estimated as the lower lethal limit in birds (Blus, 1996). There is, however, a great deal of variation in the levels of DDT and its metabolites in the brains of different species that died from DDT. Brain levels of DDT, for instance, have been reported to range from 15 μg/g in American robins (*Turdus migratorius*) that died in tremors following spraying to control Dutch elm disease, to 40 μg/g in brown-headed cowbirds (*Molothrus ater*). Lethal DDD levels varied from 2 μg/g in northern bobwhite quail (*Colinus virginianus*) to 99 μg/g in brown-headed cowbirds. Brain residues of DDE varied from less than 1 μg/g in most species studied to highs of 57 μg/g in dead American

robins. This suggests that there may be species differences in the metabolism, storage, and excretion of DDT (Blus, 1996). Quail tend to build up higher concentrations before death than other species, and pigeons are unusually susceptible to DDE poisoning (Stickel, 1973).

1.3.1.2. Offspring Growth and Survival

DDTs accumulate in eggs in proportion to the amount received by the mother (Ohlendorf et al., 1978; Stickel, 1973), with the residue content of each egg reflecting the relative levels in the female at the time of yolk lipid deposition (Fox et al., 1978). Embryos are exposed not only to the parent compound, but also its metabolites. Concentrations of DDTs may or may not vary between eggs within a clutch as the mother's lipid stores are mobilized (Ohlendorf et al., 1985; Ottinger et al., 2001). Chickens (*Gallus*) may deposit up to 34% of their daily p,p'-DDT intake into their eggs, along with 42% of their p,p'-DDE and 3.5% o,p'-DDT (Cecil et al., 1972). As they cannot be excreted from the egg, DDTs are present during all critical periods of embryonic development (Jimènez, 1997). Effects of DDT on the embryo or hatchling may be manifested in an entirely different way, and with permanent consequences as compared to effects seen as a result of exposure only in adulthood. These effects may not become apparent until the offspring reach maturity or even middle age (Colborn et al., 1993), and the extent of potential developmental abnormalities cannot be predicted from chemical exposures in adults (Kelce et al., 1998). *In ovo* exposures may result in mortality, reduced hatchability, wasting syndrome, skeletal abnormalities, and impaired differentiation of the reproductive and nervous systems in offspring (Fry, 1995).

DDTs may increase embryo mortality, as seen in barn owls (*Tyto alba*), black ducks (*Anas rubripes*) (Blus, 1996), Bengalese finches (*Lonchura striata*) (Jefferies, 1971), California gulls (*Larus californicus*) (Fry & Toone, 1981), Japanese quail (Chang & Stokstad, 1975; Lillie et al., 1972), mallards (*Anas platyrhynchos)* (Lillie et al., 1972; WHO, 1989), and chickens (Britton et al., 1974; Lillie et al., 1972; Sauter & Steele, 1972). It is possible for eggs to carry enough DDT and metabolites to cause high mortality among chicks hatched from them without affecting their hatchability (Blus, 1996; Jefferies, 1971; Jones & Summers, 1968; Lillie et al., 1972; WHO, 1989). For example, black ducks that were fed 10 mg/kg DDE over two breeding seasons had reduced duckling survival to three weeks of age and this continued even two years after DDE dosing had ceased (WHO, 1989). Bengalese finches fed p,p'-DDT at both low (1-50 μg/day/bird) and high (51-250 μg/day/bird) doses for six weeks prior to breeding exhibited not only a decrease in hatching, but also chick survival. While 96.3% of the chicks hatched to

control pairs survived to fledging, only 75.8% of the chicks from low dosed birds and 48.3% of the chicks from high dosed birds survived that long. Most of the chicks from treated pairs died within a day of hatching (Jefferies, 1971). Japanese quail that consumed approximately 28 mg of p,p'-DDT during the first week of a study and 22 mg during the second week, showed no differences in their hatchability, but 79% of the chick deaths occurred within three days of hatching, most with clear symptoms of DDT poisoning (tremors, loss of balance, collapse) (Jones & Summers, 1968). As newly hatched chicks rely on stored yolk for nutrition for the first few days post-hatch, these early deaths could be attributed to the absorption of a large quantity of pesticide from the yolk (Britton et al., 1974; Jefferies, 1971; Jones & Summers, 1968). Other factors may include the stress of hatching, inadequate feeding or brooding by the parents, or reduced weight at hatching due to smaller egg sizes (Jefferies, 1971).

Three hundred mg/kg of DDT has been suggested as the critical level in the diet of white leghorn hens for negative effects on progeny performance, as it results in increased chick mortality and decreased chick body weight. The offspring of hens fed 310 or 620 mg/kg technical grade DDT for 133 days had significantly lower body weights at two weeks of age compared to controls (Britton at al., 1974). Chickens, however, appear to be more resistant to the effects of DDT on growth during the rearing period than wild birds (Lillie et al., 1972). Impaired growth has been reported in the offspring of birds exposed to a variety of chemicals, including DDTs. Great Lakes herring gulls, and other fish eating birds, have been shown to exhibit growth retardation and deformities, associated with *in ovo* exposure to contaminants (Fox, 1992; Tyler et al,, 1998; Vos et al., 2000).

1.3.1.3. Hormonal Influences

The thyroid gland hormones, triiodothyronine and thryoxine, play an important role in physical growth and development (Singh et al., 1968), behavioral, intellectual, and neurological development (Hauser et al., 1998), and feather growth and molt (Singh et al., 1968; Wentworth & Ringer, 1986). These hormones not only act to increase oxygen consumption, glucose oxidation, heat production, and lipolysis, and regulate temperature (Jefferies, 1975), they also induce linear growth, protein synthesis, and skeletal maturation. In addition, they have permissive effects on growth hormone target cells, they mediate the secretion of growth hormone from the pituitary gland (Bolander, 1994; Nelson, 2000). The thyroid glands of embryonic chickens become functional and secrete thyroxine after ten to eleven days of incubation. The pituitary gland becomes sensitive to thyrotropin releasing hormone from the hypothalamus as

early as six to seven days, with the thyroid glands becoming sensitive to thyroid stimulating hormone at the same time. Thyroxine concentrations increase during embryo development, whereas triiodothyronine levels remain low. The concentrations of both hormones peak on the day of pipping and then decrease after hatch until adult levels are reached (Wentworth & Ringer, 1986).

Jefferies and French (1969) reported that the thyroid glands of feral pigeons fed 3 to 36 mg DDT/kg/day for six weeks were twice as heavy as those of controls. The thyroids of dosed birds had smaller follicles, less colloid, and hyperplastic epithelia, regardless of the dose level. Similar results were found in birds dosed with DDE (Jefferies, 1975). Female Japanese quail fed 150 mg/kg DDE for 120 days had significantly enlarged thyroid glands despite being on a clean diet for 85 days prior to sacrifice (Richert & Prahlad, 1972). In the same study, the thyroids of birds fed 100 mg/kg DDT or 200 mg/kg DDA (2,2-bis(4-chlorophenyl)-acetic acid) were not significantly heavier than those of controls. Unlike pigeons, DDT and DDE dosed quail had enlarged thyroid gland follicles. The DDE treated group also demonstrated a reduction in I^{125} uptake (Richert & Prahlad, 1972). Bobwhite quail fed 500 mg/kg technical grade DDT had enlarged thyroid glands after three months on treatment, and an increase in I^{131} uptake between one and three months of treatment (Hurst et al., 1974).

Because thyroid hormones are instrumental in the control of metabolic rate, they can influence functioning of the liver and the heart. Bobwhite quail fed 500 mg/kg DDT had enlarged livers after two months of treatment (Hurst et al., 1974). Homing pigeons fed 18, 36, or 72 mg/kg p,p'-DDT every second day for 42 days showed increasing liver weights with increasing dose. The thyroid gland weights of these birds also increased as the concentration of DDT in the liver increased. It has been suggested that this increase in liver size may have been due to increased hepatic activity and metabolism of circulating hormones (Jefferies & French, 1969). DDT is known to induce enzyme breakdown of hormones (Peakall, 1967). Liver hypertrophy and liver glycogen accumulation can be induced by hypothyroidism in chicks (Wentworth & Ringer, 1986). Thyroxine can accelerate heart rate and increase heart weight in domestic fowls (Jefferies, 1975) and similar effects were seen with DDT in pigeons. Birds fed a low dose of DDT exhibited increases in amplitude of the ventricular beat and heart weight, but birds fed a high dose showed heart beat amplitudes lower than that in controls, decreased heart weights, and thin, flaccid heart musculature. Low doses of DDT in pigeons produce hyperthyroidism and an increase in metabolic rate, whereas higher doses result in hypothyroidism and a decrease in metabolic rate. Bengalese finches, in contrast, do not appear

to develop hypothyroidism symptoms with increasing doses of DDT, only hyperthyroidism. Heart rate, beat amplitude, and weight continue to increase with increasing dose in this species (Jefferies, 1975). Treatment with thyroxine results in a similar trend in chick growth. In small doses, thyroxine improves growth, doses beyond physiological levels depress growth rate, and toxic doses accelerate catabolic processes and reduce body weight (Singh et al., 1968).

It has been suggested that organochlorine contaminants alter thyroid functioning by disrupting the transport of triiodothyronine and thyroxine by prealbumin (transthyretin) and thyroid binding globulin (mammals). However, only the DDT metabolite DDOH (2,2-bis(4'-chlorophenyl)ethanol) has been shown to bind transthyretin, albeit with low affinity, and o,p'-DDD and DDOH bind thyroid binding globulin with affinities 70 - 800 fold lower than thyroxine (Cheek et al., 1999). A number of researchers purport that DDTs decrease thyroid hormone levels by increasing hepatic enzyme activity (Cheek et al., 1999; McArthur et al., 1983; Ohlendorf et al., 1978). Para,para'-DDT and o,p'-DDT are known inducers of hepatic microsomal cytochrome P-450 monooxygenase which catalyzes the metabolism of numerous xenobiotics as well as endogenous steroids (Kupfer & Bulger, 1980; Robison et al., 1984). This, in turn, could result in enlarged livers (Bunyan & Stanley, 1982; Örberg & Lundberg, 1974). DDTs may also affect the thyroid glands indirectly via the hypothalamic-pituitary-thyroid axis. Dietary levels as low as 2 mg.kg DDE can dramatically reduce brain levels of dopamine and norepinephrine in ring doves (*Streptopelia risoria*). The normal feedback loops through the hypothalamus may then be partially blocked due to low levels of neurotransmitters that would influence the pituitary gland's release of thyroid stimulating hormone and eventually the release of hormones by the thyroid glands (McArthur et al., 1983).

Gonadal hormones may also affect growth. Chicks treated with androgens showed a reduction both in body weight and skeletal growth (Fennell & Scanes, 1992). However, *in ovo* exposure to the anti-androgen, Flutamide, has also been shown to suppress body weight in male chicks (Burke, 1996). Burke (1996) has suggested that embryonic androgens not only influence post-hatching growth in male chickens, they also play a role in the sexual asymmetry observed in chicken body weights. Growth of the neuromuscular system may also be enhanced by testosterone in both male and female birds (Schwabl, 1993). Estrogens are known to play a crucial role in bone development (Migliaccio et al., 1995; Oestreicher et al., 1971), as well as the growth and function of many organs including the brain, breast, liver, organs of the reproductive system, and the cardiovascular system (McLachlan & Arnold, 1996). Avian embryos are exposed not only to their own hormones, but also to hormones from their mothers. Both

estradiol and testosterone have been shown to be transferred into the egg (Schwabl, 1993; Williams, 1999). DDTs may influence the levels of gonadal hormones by inducing hepatic steroid hydroxylases which breakdown gonadal hormones (Stickel, 1973; Thomas, 1998). They may also inhibit gonadotropin secretion (Rattner et al., 1984), leading to a decrease in the release of gonadal steroids. Thus, there is the potential for DDTs to have significant effects on the levels of gonadal hormones and therefore growth and development. No relationships, however, were found between p,p'-DDT levels in tree swallow (*Tachycineta bicolor*) eggs from southern Ontario orchards and estradiol and testosterone levels in chicks (Bishop, van der Kraak, et al., 1998).

1.3.2. Effects on the Immune System

A variety of pesticides, including DDT, are known to cause impairment of the vertebrate immune system. They may target the function of any of the cellular, sub-cellular, or molecular components of the immune system (Banerjee, 1999; Repetto & Baliga, 1996). A chemical's effects on the immune system may be suppressive and lead to an increased susceptibility to infectious diseases (Banerjee, 1999; Grasman et al., 1996), or it can be immunostimulatory leading to hypersensitivity, auto-immunity, and allergy (Bishop, Boermans et al., 1998; Stiller-Winkler et al., 1999). Birds contaminated with DDTs may be more prone to succumb to infectious diseases and parasites. Chickens fed 10, 30, or 50 mg/kg DDT on alternate days for 8 to 38 days demonstrated an increased susceptibility to the parasite *Histomonas meleagridis*. Ducks fed 500 and 900 mg/kg DDT for 10 days had higher mortality rates than controls when exposed to a hepatitis virus (Banerjee et al., 1996). In contrast, chickens given feed containing 500 mg/kg DDT for varying periods showed an increase in resistance to Marek's disease and *Mycoplasma gallisepticum*. Turkeys on a similar diet were less susceptible to hemorrhagic enteritis virus (Colmano & Gross, 1971).

The number and proportion of the various leukocytes, or white blood cells, including lymphocytes, heterophils/neutrophils, monocytes, basophils, and eosinophils, reflect the health status of individuals. The typical response to infectious diseases in birds is an increase in the total leukocyte count, mainly because of increases in heterophils and lymphocytes (Dufva & Allander, 1995). Injecting 2 or 4 mg/kg p,p'-DDE into Japanese quail eggs resulted in birds with higher leukocyte numbers (Quinn et al., 2002). Caspian terns (*Sterna caspia*) from the Great Lakes showed increasing heterophil:lymphocyte ratios with increasing DDE, although herring gulls from the same areas did not exhibit this pattern (Grasman et al., 1996). A number of other

hematological parameters may also be influenced by DDT contamination. Fourie and Hattingh (1979) treated crowned guinea-fowl (*Numida meleagris*) with 75% pure DDT at 75 mg/kg body weight via esophageal canula for six days. Analyses of the plasma from these birds revealed increased carbon dioxide and cholesterol, decreased hematocrit and red blood cell numbers, reduced hemoglobin, potassium, blood sugar, and urea, as well as elevated activity of the enzymes alkaline phosphatase, lactic dehydrogenase, and creatine phosphokinase.

Both humoral and cell-mediated immunity may be altered by DDT exposure. Japanese quail from eggs injected with p,p'-DDE had significantly higher bursa of Fabricius weights than controls, although they showed no differences in humoral immune responses (Quinn et al., 2002). On the other hand, DDT administered to chickens at doses of up to 800 mg/kg from hatch to six weeks of age, failed to affect bursa weights and antibody response to bovine serum albumin, but reduced immunoglobulin levels (Glick, 1974). Antibody titers to *Salmonella pullorum* or bovine serum albumin antigen were significantly higher in birds receiving 50 or 125 mg/kg DDT for two weeks than in controls and birds receiving higher doses. DDT at doses of 100 or 500 mg/kg over two weeks, did not influence agglutinin titers to sheep red blood cell antigen in chickens, although immunoglobulins were reduced (Latimer & Siegel, 1974; Rishi & Garg, 1993). Grasman et al. (1996), however, found no evidence for contaminant associated suppression of total antibody and immunoglobulin G responses following inoculation with sheep red blood cells in either herring gulls or Caspian terns. T-lymphocytes mature in the thymus, regulate immune responses, and attack virus-infected and malignant cells (Grasman et al., 1996). The phytohemagglutinin skin test is often used to measure the proliferative potential of T-lymphocytes in response to a mitogen (Smits & Williams, 1999). Herring gulls and Caspian terns from the Great Lakes demonstrated a decrease in phytohemagglutinin response as DDE levels increased (Grasman et al., 1996). A decrease in DNA synthesis in lymphocytes exposed to phytohemagglutinin was also found in rabbits treated with p,p'-DDT (Kannan & Sharma, 1979) and humans contaminated with o,p'-DDT (Lee & Park, 1979). Loggerhead sea turtles (*Carreta carreta*) with high organochlorine levels, however, showed an increase in mitogen-induced lymphocyte proliferation (Keller et al., 2002).

1.3.3. Effects on Stress Response

Of all the endocrine tissues, chemically induced lesions are most frequent in the adrenal glands of birds exposed to environmental contaminants. The high lipid content of the adrenal cortex makes it particularly susceptible (Lorenzen et al., 1999), especially to the effects of lipid

soluble organochlorine compounds like DDT. Significant accumulations of DDTs have been found in the adrenal glands of both mammals and birds (Biesmann & von Faber, 1981; Latimer & Siegel, 1974). Some metabolites of DDT have been shown to be adrenotoxic in birds. Five week old chickens treated with as little as 5 mg/kg of technical grade DDT over several weeks exhibited a significant decrease in corticosterone concentrations (Latimer & Siegel, 1974; Lorenzen et al., 1999). DDD has been shown to cause atrophy in the adrenal cortex of mammals (Biesman & von Faber, 1981; Jönsson et al., 1994). Ortho,para'-DDD (mitotane) is so effective at decreasing levels of glucocorticoids it has even been used to treat patients suffering from Cushing's syndrome and adrenal carcinoma, and to perform chemical adrenalectomies in the treatment of breast and prostate cancers (Peakall, 1967; Thomas, 1998). In birds, however, DDT treatments are more likely to result in increased adrenal gland weights and a higher percentage of cortical cells (Biesmann & von Faber, 1981). Pigeons dosed with p,p'-DDE or p,p'-DDT for eight weeks exhibited a decrease in adrenal gland weight at 6 mg/kg/day, but then a significant increase with increasing dosage (Jefferies, 1975).

The net effect of the stress response is an increased availability of energy, increased oxygen intake, decreased blood flow to body areas not necessary for survival and movement, inhibition of digestion, reproduction, immune function, growth, and pain perception, and enhancement of memory and sensory function (Nelson, 2000). An increase in corticosterone is usually the first significant response to stress (Puvadolpirod & Thaxton, 2000). Plasma corticosterone levels in chickens restrained by hand, for example, began to increase within 45 seconds and were six-fold higher than basal concentrations within eight minutes of handling (Siegel, 1980). Thyroid hormones are also released in times of stress, as they are important regulators of metabolism (Nelson, 2000; Stickel, 1973). Enlarged adrenal glands have been found in birds and mammals with hypofunctioning thyroid glands, as well as increased levels of adrenocorticotropic (Jefferies, 1975). Administration of thyroxine may decrease the concentration of corticosterone binding globulins, thus influencing the activity and metabolism of this stress hormone (Harvey et al., 1986).

Chicken embryos are capable of secreting glucocorticoids by day six of incubation. They experience a surge in corticosterone production around day fifteen of incubation (Harvey et al., 1986). The hypothalamus-pituitary-adrenal axis of developing chick embryos is sensitive to stressors such as changes in environmental temperature (Lorenzen et al., 1999). Levels of corticosterone begin to rise at the time of hatching but decline post-hatch, and for a brief period following hatching, neonatal birds may be relatively insensitive to environmental stressors

(Harvey et al., 1986). Similar patterns are seen in rats where corticosterone is secreted by the fetus in response to stressors during the late fetal stage of gestation, but for the first two weeks after birth, rat pups have extremely low levels of adrenal steroids and are hyporesponsive to stress. This suppression of the stress response may be necessary for normal growth and development (Kawata, 1995), as increased corticosterone levels inhibit growth and skeletal development (Siegel, 1980).

Elevated levels of adrenocorticotropic or glucocorticoids have been shown to result in involution of the avian bursa and thymus (Harvey et al., 1986; Siegel, 1980), as a result of B- and T-cell apoptosis (Lechner et al., 2001). Several studies have reported depressed antibody production in stressed birds (Bolander, 1994; Harvey et al., 1986; Latimer & Siegel, 1974; Siegel, 1980). Glucocorticoids serve as anti-inflammatory agents, stabilizing lysosomes, lowering antibody levels, inhibiting leukocyte migration, and destroying lymphocytes (Bolander, 1994). While corticosterone lowers the number of circulating lymphocytes in avian blood, the populations of heterophils and other granulocytes are often elevated (Harvey et al., 1986; Siegel, 1980).

There are a variety of theories on how DDTs exert their effects on the adrenal glands and hormones. As DDT is an inducer of mixed function oxidase enzymes, it may diminish the biological activity of estrogens, androgens, and glucocorticoids by promoting their metabolism in the liver (Bunyan & Stanley, 1982; Kupfer & Bulger, 1976). DDT may influence the adrenal glands indirectly via effects on the thyroid or pituitary glands (Biesmann & von Faber, 1981; Jefferies, 1975). It has even been suggested that DDT may act as a corticosterone mimic and bind to corticosterone receptors, at least in the Senegal walking frog (*Kassina senegalensis*) and potentially other amphibians (Hayes et al., 1997).

DDT exposure itself may serve as a stressor. Sub-lethal doses of DDT, and other contaminants, have been shown to provoke stress responses in rats (Stickel, 1973). As well, the stress response may potentiate the toxic effects of DDT. For example, in mice, DDT-induced immune suppression may be due primarily to stress. Short-term, sub-lethal DDT exposure in conjunction with restraint stress yielded the same decrease in antibody titers as long term DDT exposure alone (Banerjee, 1999). DDT is stored in an animal's adipose tissue, which sequesters it away from the nervous system and vital organs. When these fat reserves are utilized, the DDT is mobilized. Any events that speed up this mobilization may kill apparently healthy animals long after exposure and at dosages that would not normally kill them (Stickel, 1973). In birds, stressors associated with molting, migration, and reproduction could mobilize lethal levels of

DDTs (Bunyan & Stanley, 1982; Gish & Chura, 1970; Ohlendorf et al., 1978; Stickel, 1973). Japanese quail partially starved prior to p,p'-DDT dosage were more susceptible to DDT intoxication than were those not hunger stressed. Mortality of American robins in DDT treated areas was highest during the breeding season (Gish & Chura, 1970; Ohlendorf et al., 1978; Stickel, 1973). The birds would have lost a considerable amount of weight during the spring migration, and thus mobilized any DDT that was stored in their bodies. Males were more susceptible than females, as they continued to lose weight while establishing and defending their breeding territories. Only two of 50 brown-headed cowbirds fed 40 mg/kg DDT for eight weeks died during treatment. Six birds, however, died after being put on a clean diet, as a result of the stress induced by workers entering the cage. No quail died during six months of dosing with 25 mg/kg DDT, but eight died when the birds were forced to molt (Stickel, 1973). It is likely that steroid homeostatic mechanisms can deal with most sub-lethal contaminant residues (Bunyan & Stanley, 1982), but when homeostasis is disrupted by stress, the resulting hormonal disturbances may be enough to have profound effects on a variety of systems. Exposure to toxicants is of particular concern during development because many of the feedback mechanisms needed to maintain homeostasis are not yet functional (Crisp et al., 1998).

1.3.4. Effects on Reproduction

DDT exposure has been linked to reduced fertility, suppression of egg formation, eggshell thinning, impaired incubation and chick rearing behavior, and other reproductive problems in birds (Fry, 1995). There is a great deal of species diversity in the effects of DDT and its metabolites on reproduction. Brown pelicans (*Pelecanus occidentalis*) are among the most sensitive, showing depressed reproduction when egg DDE levels reach 3 μg/g and total reproductive failure when levels exceed 3.7 μg/g (Blus, 1996). Domestic chickens, on the other hand, appear to be very tolerant of DDT exposure, although reports are conflicting. For example, one study reports that hens fed 25 mg/kg of p,p'-DDT, o,p'-DDT or p,p'-DDT for 28 weeks, followed by doses of 300 mg/kg for an additional 12 weeks showed no decrease in their fertility, whereas another study found decreased egg production after two months of feeding as little as 10 mg/kg technical grade DDT (Lillie et al., 1972). Fertility was reduced in Bengalese finches fed low (1 – 50 μg/day/bird) or high (51 – 250 μg/day/bird) doses of p,p'-DDT and p,p'-DDE for six weeks (Jefferies, 1971) and Japanese quail fed 400 mg/kg DDT for as little as three days (Lillie et al., 1972). Parental mortality in these quail was also increased (Lillie et al., 1972).

DDE contamination has long been associated, in a number of species, with reduced eggshell quality and thinning. Thin eggshells lead to an increased incidence of broken eggs and inevitably, lowered reproductive success (Cecil et al., 1971; Stickel, 1973; WHO, 1989). In wild bird populations, average eggshell thinning greater than or equal to 18%, over several years, has been linked to population declines (Blus & Henny, 1997). While DDE adversely affects eggshell quality, it does not seem to affect the size or shape of the eggs. The effects of DDE on eggshell thickness are species specific, with Galliforms and Larids showing little sensitivity (Blus et al, 1997).

DDTs may also induce changes in the timing of reproductive events. Mallards, ring doves, Japanese quail, and Bengalese finches have demonstrated delays in egg laying after dosing with DDE or DDT (Cecil et al., 1971; Jefferies, 1971; WHO, 1989). In the finch, this delay between pairing and ovulation was still present in the second generation (Jefferies, 1971). It is possible that the delay in the ring doves was due to longer retention of the egg in the oviduct in the absence of the proper laying stimulus, an adequate nest (WHO, 1989).

1.3.4.1 Hormonal Influences

Many of the detrimental effects of DDTs on reproduction are related to their endocrine disrupting capabilities. These substances can act by: 1) mimicking endogenous hormones, 2) antagonizing normal endogenous hormones, 3) altering the pattern of synthesis and metabolism of natural hormones, and 4) modifying hormone receptor levels (Jimènez, 1997; Kelce et al., 1998; Sonnenschein & Soto, 1998). The o,p' isomer of DDT is known to bind directly to estrogen receptors, as does o,p'-DDD (Gaido et al., 1997); the p,p' isomer of DDT does not (Robison et al., 1984). Ortho,para'-DDT can compete with estradiol for binding to the estrogen receptor and demonstrates estrogen-like activity *in vitro* and *in vivo* (Gaido et al., 1997). Para,para'-DDE is an androgen antagonist that binds directly to androgen receptors (Gaido et al., 1997; Kelce et al., 1995; 1998). The concentration of p,p'-DDE needed to inhibit androgen receptor transcriptional activity in cell culture is 63.6 parts per billion, considerably less than levels that accumulate in the environment (Kelce et al., 1995). Para,para'-DDE has been shown to have varying degrees of androgenic, anti-androgenic, estrogenic, and anit-estrogenic activity (Hutz, 1999; Sohoni & Sumpter, 1998; Sonnenschein & Soto, 1998). The androgen receptor is less specific than the estrogen receptor, so o,p'-DDT and endogenous estrogens can also have anti-androgenic capacities (Kelce et al., 1995; Sohoni & Sumpter, 1998). The presence of anti-androgens can create an estrogenic environment resulting in symptoms indicative of estrogen

exposure (Sohoni & Sumpter, 1998). DDTs may influence the levels of gonadal hormones by inducing hepatic steroid hydroxylases (Stickel, 1973; Thomas, 1998) and inhibiting gonadotropin secretion (Rattner et al., 1984). Other hormones, including thyroid hormones, also influence reproduction and may be altered by DDT exposure (Cooke, 1996; Hurst et al., 1974; Nelson, 2000; Wilson & Donham, 1988; Wentworth & Ringer, 1986).

1.3.4.2. Early Exposure

In ovo and early post-hatch exposure to DDTs may have profound effects on the development and sexual differentiation of the reproductive systems of birds. Male California gulls from eggs injected with as little as 2 mg/kg o,p'-DDT had feminized gonads. Five mg/kg or higher o,p'-DDT resulted in the development of both left and right oviducts in female gulls (Fry & Toone, 1981). In chickens, p,p'-DDT injected into the yolk prior to incubation altered gonadal morphology in both sexes (Stickel, 1973). Four week old white leghorn cockerels fed DDT 12.5 to 37.5 mg/kg body weight for 24 weeks, had atrophied testes, and exhibited a disruption and necrosis of spermatogonial cells. These effects persisted even after treatment was discontinued (Balasubramaniam & Sundararaj, 1993). Cockerels injected with increasing amounts of DDT from eight days after hatch for up to 89 days had considerably smaller testes than controls. The development of combs and wattles in these birds was also severely impaired, beginning 25 days after the start of treatment (Burlington & Lindeman, 1950).

1.3.5. Effects on Behavior

An organism's behavior represents the final integrated result of a variety of physiological and biochemical processes (Peakall, 1996; Spyker, 1975). As behavior patterns can be highly sensitive to changes in the steady state of an organism, changes in behavior can serve as early indicators of potential problems (Peakall, 1996; Spyker, 1975). Nervous tissue, especially the brain, is extremely sensitive to the effects of a variety of foreign substances, such as pollutants. Changes in behavior may indicate toxic actions of a chemical long before any classical symptoms of poisoning occur and in the absence of gross functional or structural defects. Toxic effects in the nervous system may manifest as deficits in sensory function, motor control, intellectual processes, or emotional responses (Spyker, 1975).

Organisms are more vulnerable to many of the adverse effects of exogenous chemicals during development, when the central nervous system is undergoing rapid changes and protective mechanisms have not yet developed (Parmigiani et al., 1998; Spyker, 1975). Some pollutants, at environmentally relevant levels, can lead to irreversible alterations in brain

development at exposure concentrations that might produce little effect in an adult (Parmigiani et al., 1998). The effects of these compounds may not become apparent until much later in an individual's life (Spyker, 1975). As behavior represents an integrated response of an organism, it may also be influenced by deficits in the function of systems other than the nervous system. Although not all behaviors may be affected, defects in those relating to survival and reproduction may prove to be extremely disadvantageous (Spyker, 1975). Subtle behavioral changes in wild populations could even cause serious effects at low levels of pollutants which do not have overt effects on mortality or reproduction (Peakall, 1996).

1.3.5.1. Reproductive Behaviors

A number of behaviors involved in reproduction may be detrimentally affected by DDT exposure. For example, during the 1970s, male gulls in highly contaminated areas of California and in the Great Lakes exhibited feminized reproductive systems. These birds showed reduced sexual behavior and many of them failed to even migrate to the breeding grounds. This resulted in skewed sex ratios and homosexual pairings between the females (Fox, 1992; Fry & Toone, 1981). Herring gulls exposed to a mixture of organochlorines showed a reduction in defense of their territories. As aggression is related to the concentration of circulating testosterone, endocrine disrupting chemicals like p,p'-DDE that act in an anti-androgenic manner could cause aggressive behavior to diminish (Crain & Guilette, 1997). Japanese quail treated with o,p'-DDT showed an attenuation of mating behaviors in both sexes (Bryan et al., 1989). Ring doves fed a mixture of organochlorines, including DDE displayed a series of behavioral abnormalities. Because the behaviors of the two sexes in this species are so closely linked, alterations in the behavior of one caused changes in the other. The birds in this study showed an increase in the courtship period due to the females' failure to respond to the males' petitions for copulation. This forced the males to increase and prolong the use of these solicitation behaviors (McArthur et al., 1983). These birds also showed a reduction or delay in the behaviorally induced increase in progesterone which lead to a delay in nest building, ovulation, and incubation (McArthur et al., 1983).

1.3.5.2. Parental Behaviors

Parental care behaviors appear to be most influenced by DDT contamination. Merlins (*Falco columbarius*) whose eggs contained high levels of organochlorine pollutants, especially p,p'-DDE, have been shown to desert their clutches more readily and defend their nests less actively than birds with less contaminated eggs. There was an inverse relationship between the

DDE burdens in the eggs and the intensity of nest site defense (Fox & Donald, 1980). Herring gulls exposed to a number of chemical contaminants have also demonstrated decreased nest attentiveness. They applied less heat to their eggs, were absent from the nest more frequently and for longer periods, and more readily left their nests than birds from less contaminated areas. The degree of nest inattentiveness was correlated with the female's organochlorine burdens (Fox et al., 1978; Rattner et al., 1984). Prolactin levels were affected by organochlorine diets in ring doves, decreasing the amount of time spent incubating the eggs and brooding the young. The squabs of contaminated parents were fed less and thus had decreased weights and survival rates (McArthur et al., 1983).

Hyper-aggressive parents injured some of the squabs in McArthur et al.'s (1983) study. Increased parental aggression was also observed in Bengalese finches dosed with p,p'-DDT and p,p'-DDE (Jefferies, 1971). Parent birds contaminated with DDTs and other compounds may destroy their own eggs by eating, breaking, or ejecting them (Stickel, 1973). Gray herons (*Ardea cinerea*) have been observed breaking their own eggs and dropping live young from the nest (Ohlendorf et al., 1978). This increase in aggressive behavior seems contrary to the anti-androgenic, aggression decreasing effects of DDE. However, gonadal hormones are not the only aspects of the nervous and endocrine systems influenced by DDT contamination. McArthur et al.'s (1983) pigeons also demonstrated elevated thyroid hormone levels and decreased brain levels of the biogenic amines, dopamine, and norepinephrine, which influence hypothalamic and pituitary hormones. High levels of corticosterone, can also redirect behaviors away from reproduction and parental care activities (Silverin, 1990). Thus, the increase in aggression in DDT contaminated parents may be related to one or more of these other factors. Overall, birds that exhibit abnormal courtship and nest construction, altered breeding synchrony, decreased incubation attentiveness and parental care may alter their reproductive success, increase energetic costs, and decrease reproductive fitness (Rattner et al., 1984).

1.3.5.3. Other Behaviors

Other, non-reproductive, behaviors may also be influenced by DDTs. For example, the ducklings of mallards fed DDE were hyper-responsive to maternal calls. They approached the source of the call more and stayed near it more than control ducklings. They also traveled shorter distances from frightening stimuli (Ohlendorf et al., 1978). Hyper-excitability and hypersensitivity to stimuli are commonly listed among the symptoms of DDT poisoning in mammals, and similar results are likely in birds (Jefferies, 1975). Hyperactivity has been

suggested as a possible reason for the nest inattentiveness of organochlorine fed ring doves (McArthur et al., 1983). Pesticide use may reduce the food supplies of some birds. This would result in an increase in time spent foraging (Blus & Henny, 1997; Fox & Donald, 1980). A lack of appetite has been noted in pigeons orally dosed with p,p'-DDE, p,p'-DDT, or o,p'-DDT (Jefferies, 1975; Jefferies & French 1971). The amount of cover may also be reduced, affecting nest building and nest predation (Blus & Henny, 1997).

Thus, DDTs have been shown to have a number of detrimental effects on birds. The following chapters will focus on a particular example of DDT exposure in a bird species and its effects on survival, growth, reproduction, behavior, immunity, thyroid hormones, and the stress response.

1.4 References

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Chapter II

DDT, American Robins, and the Okanagan Valley

Historically, orchard crops were among the most heavily treated with dichlorodiphenyltrichloroethane (DDT) (Stringer et al., 1974), receiving more DDT than tobacco, field, and vegetable crops (Harris et al., 2000). As the Okanagan Valley of southern British Columbia is a major fruit growing area, it was exposed to large quantities of this pesticide. Prior to the restriction of DDT use in the early 1970's, Okanagan orchards received two to four applications of about 13.5 kg technical grade DDT/ha/year (3.4 - 6.8 kg active ingredient/ha/year) (Elliott et al., 1994; Gill et al., 2003).

2.1. Soil

Harris et al. (2000) reported that soil in the Okanagan consists of a thin superficial layer of about 10 cm above a dense sand/gravel mixture. Within Okanagan orchards, this top 10 cm of soil contained 3.8% organic matter and a pH of 6.4. Despite the fact that DDT had not been applied to these orchards in nearly thirty years, they found 0.25 mg/kg DDD (o,p' plus p,p', dry weight), 4.9 mg/kg DDE, and 9.3 mg/kg DDT in the top layer of soil. In contrast, at an Okanagan non-orchard reference site DDD, DDE, and DDT levels were less than 0.05 mg/kg. Soil pH at this site ranged from 7.8 to 8.0 and the average organic matter was 4.7%. The ratio of DDE to DDT in the orchards was only 1.10 (Harris et al., 2000). It has been suggested that fifteen to twenty years after DDT use has stopped, this ratio should exceed 20:1 (Elliott et al., 1994). Thus, in the Okanagan, degradation of DDT to DDE appears to be impeded. DDT residues were only found in the soils of previously sprayed areas, which suggests that atmospheric deposition does not contribute significantly to the DDT levels found (Harris et al., 2000).

A number of factors influence the extent to which pesticides will remain in the soil, including: soil type, temperature, moisture, pH, degradability of the pesticide, microorganism content, cover crops, and cultivation (Murphy, 1980). When orchard trees are sprayed, more than 80% of the pesticide may end up on the ground (Stringer et al., 1974). DDT may be lost soon after application via volatilization (Blus et al., 1987; WHO, 1989), however, due to DDT's low vapor pressure, volatilization is limited (Nair et al., 1992). All soils show a strong adsorptive capacity for DDT (WHO, 1989), but highly organic soils tend to have the highest retention times (Johnson et al., 1976; Stringer et al., 1974; WHO, 1989). Even several years

after DDT application, the majority of DDT residues found in soil is located within the top 10 cm (Cooke & Stringer, 1982; Stringer et al., 1974). The low water solubility of DDT prevents significant percolation and leaching (Stringer et al., 1974), so downward movement of DDT occurs as litter accumulates at the soil surface (Dimond et al., 1970). It has been suggested that the breakdown of DDT to DDE is the only significant change this pesticide undergoes in soil, as adsorption to the top 10 cm of soil impedes volatilization and losses through leaching are negligible (Cooke & Stringer, 1982). Some microorganisms are capable of degrading DDT, but there is little metabolism in situ (WHO, 1989).

Because of the various factors that influence the loss and breakdown of DDT in soil, a wide range of half-life values have been proposed, from 16 days (WHO, 1989) to 57.5 years (Blus et al., 1987). Concentrated DDT breaks down more slowly (Harris et al., 2000), so the half-life of DDT increases with increasing concentrations (Blus et al., 1987). Thus, it is not surprising given the heavy applications of DDT Okanagan orchards received that DDT levels in this area remain high thirty years after DDT use was discontinued. The lack of regular soil disturbances in orchards minimizes losses from volatilization and erosion. The climate in the Okanagan may also contribute to the slow elimination of DDT. As the Okanagan is temperate, low temperatures during the winter months may be impeding DDT breakdown (Harris et al., 2000). DDT breaks down more readily in warmer, tropical climates (Mellanby, 1992) and is also subject to greater volatilization (Nair et al., 1992). Although Okanagan orchards are irrigated in the summer, low soil moisture levels in the winter reduce the breakdown of DDT accumulating organic matter and slow the microbial degradation of DDT (Harris et al., 2000).

2.2. Earthworms

Another factor that influences the retention of DDT in soils is the presence and species composition of earthworms. Earthworms play an important role in the maintenance of soil fertility by incorporating leaf litter, and improving water holding capacity, nutrient enrichment, aeration, drainage, root penetration, and microorganism populations (Cooke et al., 1980). Earthworms are also indicators of soil pollution by organochlorine pesticides (Senthilkumar et al., 2001). DDT may kill earthworms (Johnson et al., 1976; Tomlin, 1992) and worms forced to live in DDT contaminated orchard soil have been known to exhibit high mortality rates and weight loss (Johnson et al., 1976). However, most reports suggest that earthworms are relatively insensitive to the toxic effects of DDT (Gish & Hughes, 1982; WHO, 1989), and may even develop physiological, biochemical, or behavioral resistance to ecotoxicants (Tomlin, 1992).

The behavior of an earthworm may greatly affect its susceptibility to DDT. For example, the surface mating and feeding of *Lumbricus terrestris* makes it vulnerable to chemicals applied to vegetation or the soil surface. This species also has slow growth and reproduction rates. Its habit of burrowing vertically and casting on the surface allows it to translocate pollutants from the soil surface to lower layers and vice versa (Tomlin, 1992). Earthworm species that have permanent burrows take up DDT from the soil surface more readily than residues mixed into the soil, whereas species that tunnel haphazardly through the soil can take up more residues that have been mixed into the soil (Edwards & Jeffs, 1974). Thus, ploughing or rotovation after spraying incorporates DDT into the soil, decreasing the exposure of worms that feed at the surface but increasing exposure for subterranean species (Cooke et al., 1992).

The uptake of DDT by earthworms is related not only to its concentration in the soil, but also to the activity of the worms (WHO, 1989). DDT residues in earthworms tend to be cyclic, with higher levels between late spring and early autumn and lower levels from late autumn to early spring. Residue levels peak in May and are at their lowest levels in January. This coincides with seasonal differences in activity (Gish & Hughes, 1982; WHO, 1989). When the worms are more active they process more soil through the gut and retain more DDT (WHO, 1989). Gish and Hughes (1982) found that earthworms did not show maximum residues immediately after application, but rather two months later. DDT can be converted to both DDD and DDE in worms (Edwards & Jeffs, 1974; Gish & Hughes, 1982). Earthworms may, thus, contribute significantly to the initial degradation of DDT (Edwards & Jeffs, 1974).

Harris et al. (2000) found that earthworm communities in the Okanagan were dominated by *Aporrectodea turgida*, but *Eisenia rosea*, *Lumbricus rubellus*, and *Octolasion tyrtaeum* were also present. The total earthworm biomass in orchard areas was 16 g/m^2, whereas in the non-orchard reference site it was 87 g/m^2. Overall, earthworms in the Okanagan orchards contained 2.2 mg/kg DDD, 43.5 mg/kg DDE, 17.2 mg/kg DDT, with a DDE:DDT ratio of 2.56. DDD, DDE, and DDT levels in the worms from the Okanagan reference site were below 0.6 mg/kg. Earthworm DDE values increased with increasing soil organic matter (Harris et al., 2000). The majority of earthworms in the Okanagan live and feed near the soil surface, thus helping to maintain the high DDT levels in the top layer of soil. Other species that burrow deeply into the soil facilitate the dilution of pesticides throughout the soil profile (Elliott et al., 1994).

Because of the high DDT residues earthworms can tolerate, they pose a significant hazard to predators (WHO, 1989). Robertson and Alexander (1998) found that the bioavailability of DDT to flies and cockroaches decreased with time as the chemical adsorbed to the soil, however,

this likely does not apply to animals like earthworms that live in and consume soil. Earthworms in contaminated soils continue to accumulate DDTs nearly thirty years after its use was discontinued (Harris et al., 2000). DDT contamination of earthworms can adversely affect vertebrate predators either directly through secondary poisoning or indirectly by reducing food supplies (Cooke et al., 1992). Worms that do not die from DDT poisoning have longer to accumulate residues. Sub-lethal levels may also alter an earthworm's behaviour. Although any behaviours that result in the worms spending more time on the surface may increase predator vulnerability (Cooke et al., 1992), DDT may elicit an avoidance reaction in earthworms (Tomlin, 1992). Both surface casting and the turn over of leaf litter were reduced in DDT contaminated areas (Cooke et al., 1980; Tomlin, 1992). This avoidance of the soil surface may also limit their availability as a food source.

Bird predators may pick up significant levels of DDT from eating earthworms. It has been suggested that 8 mg/kg DDT is a hazardous threshold for birds and 30 to 40 mg/kg represents a lethal short-term hazard. Total DDT concentrations of 32 mg/kg in worms could pose a hazard to the reproduction of some bird species (Harris et al., 2000). Residues in earthworms from the Okanagan may exceed this level. Bird deaths have been attributed to the mobilization of DDT in fat reservoirs during times of reproductive stress, but the fact that food intake is also increased during the breeding season may play a role (Cooke et al., 1992).

2.3. American Robins

An important consumer of earthworms is the American robin. This ubiquitous songbird has adapted well to human activities and is quite at home in orchard habitats (Cannings et al., 1987). Robins are opportunistic omnivores who subsist primarily on fruit through the summer and fall. During the breeding season, however, they eat and feed their young primarily insects and earthworms (Ehrlich et al., 1988; Sallabanks & James, 1999; Wauer, 1999). Montgomerie and Weatherhead (1997) reported that earthworms may comprise up to 20% of a robin's diet during the breeding season, and capture rates as high as 20 worms per hour have been recorded. Because of their penchant for earthworms, robins are susceptible to secondary DDT poisoning. A robin that consumes 100 earthworms may ingest more than 3 mg of DDT (Cooke et al., 1992). For a 77 g bird (Sallabanks & James, 1999), this may be an acutely lethal dose. Even sub-lethal levels of DDT can be hazardous, as DDTs accumulate in the body. Turkeys fed DDT accumulated it in their fat at concentrations four to eight times the level in the diet (Poland et al., 1972). DDTs tend to be lost relatively slowly from the body. Kan et al. (1978) found that the

loss of total DDT in feces as a proportion of daily intake for chickens was only 7%. Metabolism and excretion of DDTs depends on species, diet (Clark et al., 1987), lipid pool sizes (Norstrom et al., 1989) and the particular isomer being measured. Para, para'-DDE is considered the final breakdown product of DDT in living birds (Poland et al., 1972). This metabolite tends to be lost quite slowly. Stickel et al. (1984) found that in grackles (*Quiscalus quiscula*), red-winged blackbirds *Agelaius phoeniceus*), starlings (*Sturnus vulgaris*), and brown-headed cowbirds (*Molothrus ater*), the loss rate of DDE was only 0.30% per day. Ortho, para'-DDT tends to be stored less readily and eliminated more quickly than p,p'-DDT (Stickel, 1973). Dimond et al. (1970) found that DDT concentrations in robins were one order of magnitude higher than those in worms. These authors also found traces of o,p'-DDT in the earthworms but none in the robins suggesting differential absorption and/or breakdown between predator and prey. Adult robins contained about 2.6 times higher residue levels than immatures, as well (Dimond et al., 1970). Elliott et al. (1994) examined organochlorine residues in the eggs of Okanagan orchard nesting California quail (*Callipepla californica*), tree swallows (*Tachycineta bicolor*), black-billed magpies (*Pica pica*), house wrens (*Troglodytes aedon*), and American robins. Robin eggs contained 18 to 3,500 times more DDT and 4 to 100 times more DDE than those of the other species tested. They also had the lowest mean DDE:DDT ratios (9.5:1). Three recent studies (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000) examined the levels of DDTs in robin eggs from the Okanagan orchards and various control sites. These data are summarized in Table 2-1. Residue levels in eggs reflect the amount of DDT in the laying female at the time of yolk lipid deposition (Fox et al., 1978).

The high levels of DDTs found in Okanagan robin eggs could be attributed to a variety of sources. Low DDE:DDT ratios suggest recent applications of DDT (Elliott et al., 1994; Mora, 1997). DDT has been found as a contaminant in recent use pesticides such as dicofol (Blus et al., 1987; Elliott et al., 1994; Risebrough et al., 1986) and kelthane (Bunck et al., 1987), and it may still be used illegally (Blus et al., 1987; Bunck et al., 1987; Elliott et al., 1994). Neither of these scenarios, however, is likely to result in the high residue levels seen in these birds (Elliott et al., 1994). DDT burdens may be acquired by migratory birds on wintering grounds in Latin America where DDT may still be used legally (Elliott et al., 1994; Klemens et al., 2000; Mora,

Table 2-1. Mean DDT levels (mg/kg, wet weight) in American robin eggs collected from orchards in the Okanagan Valley and non-orchard areas of the Okanagan and Lower Mainland of British Columbia.

Period		1990 – 1991*	1993 – 1995**	1993 – 1998***
Orchard				
	DDT	16.7	13.0	10.1
	DDE	83.3	85.1	54.8
	DDD	2.4	1.1	0.7
	DDT:DDE	5.0	17.2	5.2
Non-orchard				
	DDT	0.08	0.4	0.4
	DDE	1.5	8.2	8.6
	DDD	0.001	0.06	0.05
	DDT:DDE	18.1	18.9	102.8

* Elliott et al., 1994

** Harris et al., 2000; para,para' isomers only

*** Gill et al., 2003

1997). American robins in the Okanagan, however, do not usually migrate as far as Latin America but rather travel to Washington, Oregon, and California (Campbell et al., 1997). Many Okanagan robins (up to 1,000) may even over-winter in the orchards and vineyards of the Okanagan valley (Campbell et al., 1997; Cannings et al., 1987) because of the mild climate and abundance of food. The robins in Elliott et al.'s (1994) study had considerably higher DDT levels than birds that winter in Latin America (tree swallows, barn swallows (*Hirundo rustica*), and house wrens). Thus, robins in the Okanagan are most likely acquiring their DDT burdens from the earthworms they eat, which, in turn accumulate
their residues from historically applied, slowly degrading DDT in the soil (Elliott et al., 1994).

Despite their high DDT burdens, American robins continue to thrive, and field studies have found no decreases in their reproductive success (Elliott et al., 1994; Gill et al. 2003). It has been implied that aside from the mass die-offs seen during the peak of DDT use (Carson, 1962), robins are relatively unaffected by the high levels of DDT they are exposed to. It is possible that the residues they ingest are diluted when they eat "clean" fruit and other less contaminated insects (Johnson et al., 1976). Robins may also avoid foraging in highly contaminated areas (Dimond et al., 1970). The extremely high concentrations of DDTs found in these birds and their eggs may, therefore, only be found during the breeding season when they are consuming primarily earthworms and insects. Aged DDT, while still bioavailable to earthworms, may be much less toxic than when it was first applied (Robertson & Alexander, 1998). It is possible that after decades of exposure, robins have developed a tolerance or resistance to the detrimental effects of DDTs as a result of genetic selection. A variety of insects became resistant to DDT soon after its introduction (Mellanby, 1992). The crayfish, *Procambarus clarkii*, a variety of other aquatic invertebrates, mosquitofish (*Gambusia affinis*), and two species of cricket frog (*Acris crepitans and Acris gryllus*) have also demonstrated tolerances to DDTs after long-term exposure (WHO, 1989). Animals often develop tolerances to the reproduction suppressing effects of phytoestrogens (Crain & Guilette, 1997; Sonnenschein & Soto, 1998), and resistance to the endocrine-disrupting effects of DDTs may have evolved in a similar way in robins.

2.4. This Study

The objective of this study was to assess long-term, delayed, or subtle effects of DDT contamination on American robins from the Okanagan. The focus was on two main areas: 1) growth and survival (Chapter III) and 2) reproduction and behaviour (Chapter IV). Included in

growth and survival are egg measurements, chick measurements, immune response, thyroid hormone levels, mortality, and tissue weights. Reproduction and behaviour include the timing of reproductive events, egg laying, egg hatching, chick fledging, and thyroid hormone levels in breeding birds, along with frequencies of reproductive, maintenance, aggressive, and vocal behaviours. The work of Elliott et al. (1994) and Gill et al. (2003) suggest that there should be no overall detrimental effects of early DDT exposure on survival or reproduction. However, these studies only observed the robins until the young had fledged. It may be that detrimental effects in the young were not evident until after fledging (Colborn et al., 1993; Spyker, 1975), that the stable populations in the Okanagan are due to the recruitment of birds from other areas, not from the return (or residency) of successful breeding pairs and their progeny (Cooke et al., 1992), or that more subtle detriments may work at the individual rather than the population level. Other studies, on a variety of species, indicate that DDT can, in fact, have a number of deleterious effects (see Chapter I for review), which may simply not have been observed in the field.

In order to study these potential long-term effects of early DDT exposure, nestlings from Okanagan orchards, exposed *in ovo* and for ten days post-hatch to DDTs, were captured and raised in captivity. These birds, along with controls from the British Columbia Lower Mainland, were maintained in captivity for three years. Raising the birds in captivity ensured that any differences between the two groups of birds could be related to their early experiences and contaminant exposures. This eliminated any extraneous variables due to differences in habitat, diet, and climate. It was assumed that the captive environment would also reduce the number of predation losses.

Eggs were collected from the same nests as the 10-day old chicks in order to evaluate differences between the Lower Mainland and Okanagan birds in relation to the levels of DDT contamination. The Okanagan eggs and chicks were collected from orchards near Penticton and Naramata, whereas the Lower Mainland eggs and chicks were collected from park areas within Vancouver, Surrey, and Delta, British Columbia (Figure 2-1). A complete listing of the organochlorines and polychlorinated biphenyls analyzed in these eggs is available in Appendix I.

Because a number of robins died after fledging but prior to reproductive maturity, a second study was carried out in order to evaluate immune response in more detail. A second cohort of eggs and birds were collected (Chapter V) and immune response, thyroid hormone levels, and stress responses were examined.

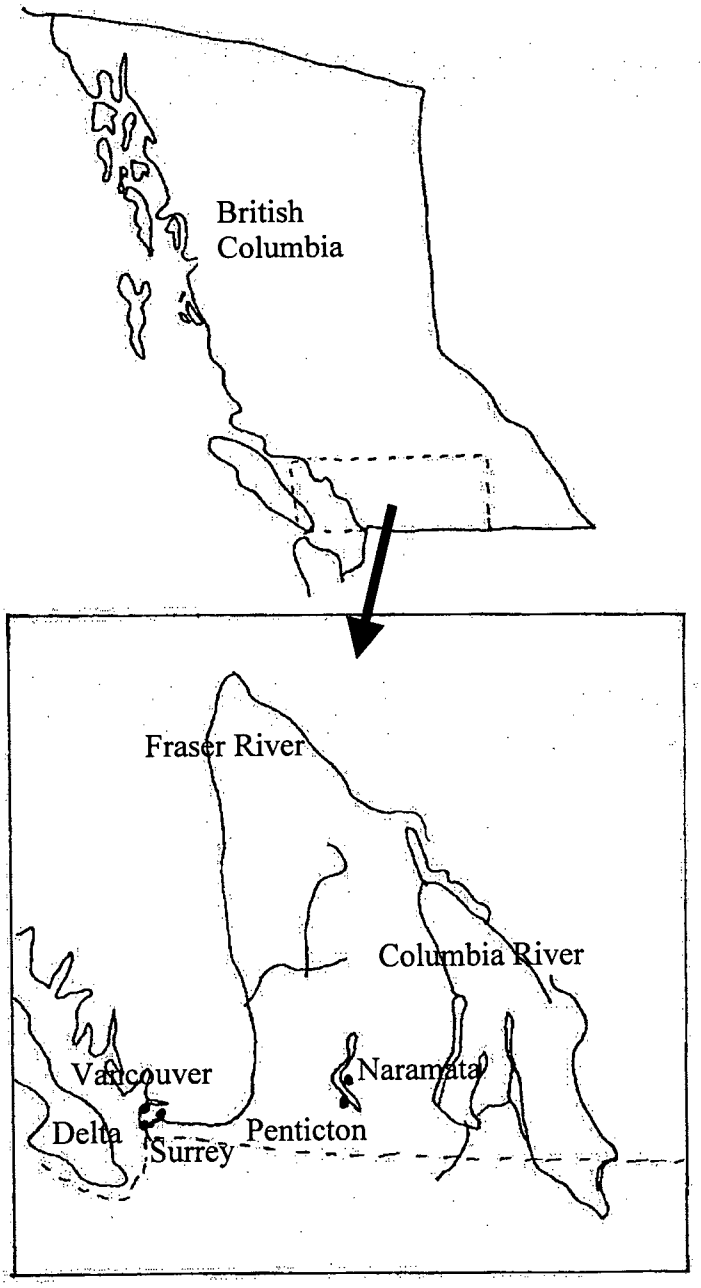

Figure 2-1: Collection sites of American robin eggs and nestlings. Lower mainland = Vancouver, Delta, and Surrey, British Columbia; Okanagan = Naramata and Pentiction, British Columbia

2.5 References

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Chapter III

Growth and Survival of DDT Contaminated American Robins

3.1. Introduction

Dichlorodiphenyltrichloroethane (DDT) and its metabolites, in high doses, can be toxic to birds, inducing reproductive disturbances and even mortality (Blus, 1996; Carson, 1962; Fry, 1995; WHO, 1989). Mother birds can pass these chemicals to their eggs during yolk formation (Brandt et al., 1978; Fox et al., 1978; Fry, 1995; Ohlendorf et al., 1978; Ottinger et al., 2001; Stickel, 1973). *In ovo* DDT exposure may result in reduced hatchability, embryo or hatchling mortality, wasting syndrome, skeletal abnormalities, retarded growth, and impaired differentiation of the reproductive and nervous systems (Blus, 1996; Britton et al., 1974; Chang & Stokstad, 1975; Fry, 1995; Fry & Toone, 1981; Jefferies, 1971; Jones & Summers, 1968; Lillie et al., 1972; Sauter & Steele, 1972; Tyler et al., 1998; Vos et al., 2000; WHO, 1989).

Fruit-growing areas, such as the Okanagan Valley of southern British Columbia, were historically treated with vast amounts of DDT (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000). This chemical and its primary metabolites, DDE and DDD[1] (Jefferies, 1975; Stickel, 1973; WHO, 1989), adsorb to soil particles (Colborn et al., 1993; Harris et al., 2000; WHO, 1989) and may be consumed by earthworms (Gish & Hughes, 1982; Harris et al., 2000; Johnson et al., 1976; Senthilkumar et al., 2001; Tomlin, 1992; WHO, 1989). The earthworms bioaccumulate these residues in their lipid rich tissues and then pass them on to their predators (Cooke et al., 1992; Harris et al., 2000). The American robin (*Turdus migratorius*) is a major earthworm predator (Ehrlich et al., 1988; Montgomerie & Weatherhead, 1997; Sallabanks & James, 1999; Wauer, 1999). Previous studies have found very high levels of DDT and DDE in Okanagan robin eggs (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000). Gill et al. (2003), for example, reported DDT levels as high as 60.7 mg/kg and DDE levels up to 302 mg/kg in robin eggs from Okanagan orchards, compared with DDT levels of 0.5 mg/kg and DDE levels of 12.0 mg/kg at relatively uncontaminated control sites. Although these studies only observed robins until the offspring had fledged, they reported no significant differences in survival between birds from the Okanagan and controls.

This study aimed to examine more long-term or delayed effects of early DDT exposure, with a focus on growth and survival. As many parameters are difficult to study in the wild, ten-

day old nestlings from orchards in the Okanagan and relatively clean areas of the British Columbia Lower Mainland were collected and raised in captivity. The objectives of the study were to:

1) Determine if *in ovo* and early post-hatch DDT exposure influenced growth and development as measured by tarsus length, body weight, and tissue weights. Thyroid hormone levels were also measured, as they play an important role in growth (Bolander, 1994; Hauser et al., 1998; Nelson, 2000; Singh et al., 1968). Correlations were conducted to discern if the level of DDT exposure resulted in differences within the Okanagan birds.
2) Determine if *in ovo* and early post-hatch DDT exposure influenced mortality. Birds were monitored from ten days of age until three years of age, and age and cause of death were discerned whenever possible. Immune response was also measured, as it is essential for survival (Banerjee, 1999; Grasman et al., 1996; Repetto & Baliga, 1996).

3.2. Methods

3.2.1. Egg Contaminants

Between June 2 and July 3, 1997, 70 eggs were collected from robin nests in orchard areas surrounding Penticton and Naramata in the Okanagan Valley of British Columbia. An additional 36 eggs were collected from control sites in Vancouver and Delta, in the Lower Mainland of British Columbia, between April 10 and June 18, 1997. Thirty-one of these Okanagan eggs and 16 of the Lower Mainland eggs were analyzed for moisture, lipid, organochlorine, and polychlorinated biphenyl content. The Okanagan eggs were analyzed individually, and the Lower Mainland eggs were pooled (n = 3; one pool of 6 eggs, one pool of 9 eggs, and one individual egg) in order to save costs and because the expected values would be low.

All of the eggs were analyzed at the National Wildlife Research Center (Hull, Quebec). Analyses were conducted using gas chromatography and mass spectrophotometry, following the methods outlined in Won et al. (2001). Results were expressed in μg/g and on a wet weight basis. All eggs were collected during times of no current-use pesticide application (L. Wilson, Canadian Wildlife Service, personal communication). Although it has been suggested that contaminant loads may vary between eggs within a clutch (Ohlendorf et al., 1985; Ottinger et al.,

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[1] DDE = dichlordiphenyldichloroethylene, DDD = dichlorodiphenyldichloroethane

2001), for the purposes of this study it was assumed, as in previous studies (Harris et al., 2000; Gill, 2003), that all eggs within a clutch contained similar contaminant loads.

3.2.2. Egg Measurements

A total of 106 eggs (36 Lower Mainland, 70 Okanagan) were weighed and measured. Where measurements were available for more than one egg per nest, nest means were used for the analyses. Egg length refers to the distance from end-to-end, while egg width refers to the measurement at the widest part of the egg.

3.2.3. Rearing Protocol

Between April 28 and July 16, 1997, 10-day-old robin nestlings were collected from the same nests as the eggs. Ninety-one chicks were collected from the Okanagan and 59 from the Lower Mainland. All nestlings were collected during periods of no current-use pesticide treatment (L. Wilson, Canadian Wildlife Service, personal communication). The birds were hand reared at Monika's Wildlife Shelter (Surrey, British Columbia) where they were fed a mixture of dry cat food, peanut butter, hard-boiled chicken eggs, commercial chick starter, and vitamins until they were self-feeding (M. Tolgsdorf, Monika's Wildlife Shelter, personal communication). At this time they were provided with dry cat food and clean water for drinking and bathing *ad libitum*. The birds were housed in communal outdoor pens measuring approximately 3.7 m long x 3.7 m wide x 3.7 m high and then transferred to 3.7 m x 2.4 m x 3.7 m pens with gravel floors when self-feeding. Each bird was outfitted with both a numbered metal leg band and plastic coloured leg bands for individual identification.

Upon reaching sexual maturity, the birds were transferred to breeding pens at the University of British Columbia's San Rafael Research Aviary (Surrey, British Columbia). One pair was housed per pen and three pen types were employed. Twelve pairs were housed in indoor pens constructed of wood frame and chicken wire, with tarpaulin used for visual isolation. These pens had cement floors and measured 3.8 m x 1.9 m x 3 m. Six windows provided natural lighting, and additional artificial lighting was timed to mimic the natural light/dark cycle. Sixteen pairs were kept in outdoor holding pens constructed of metal-pipe frame with nylon netting or chicken wire over chain-link fencing. Several centimeters of gravel covered the ground and tarpaulin was used for visual isolation. These pens measured approximately 3.0 m wide x 3.6 m long x 1.9 m high. Twelve pairs were housed in outdoor side-by-side pie-shaped pens constructed with metal pipes, wood fence posts, and chicken wire. Pens were visually isolated by tarpaulin, and netting was used as roofing. These pens measured approximately 1.5

m wide (at the widest end) x 4.8 m long x 2.1 m high. Dry cat food (Kirkland Signature, Burnaby, British Columbia) and water were provided *ad libitum* and supplemented with liquid calcium (Stanley Pharmaceuticals Ltd., North Vancouver, British Columbia), vitamins (8 in 1 Pet Products Inc., Hauppauge, New York), and mealworms (M. Tolgsdorf, Monika's Wildlife Shelter, personal communication). The birds, especially those housed outdoors, also had access to any insect and/or plant material they could procure themselves. Each pair was also provided with a covered feeder, two nesting platforms, paper bowls (to serve as a nest foundation), dishes of mud, and a variety of nesting materials, including: straw, shredded paper, string, strips of cloth, wool, and assorted grasses collected from around the aviary. Cedar branches and tarpaulin were hung over the nesting platforms for protection against inclement weather. All treatment and housing protocols were approved by the University of British Columbia Animal Care Committee (Certificate # A97-0043).

3.2.4. Chick Measurements

Blood samples were sent to the Centre for Wildlife Ecology, Simon Fraser University, Burnaby, British Columbia, for DNA sexing (by Brett Vanderkist), following the protocols outlined in Griffiths et al. (1988). Sex was later confirmed when the birds were dissected at sacrifice at the end of the study. Weights and measurements were available for 55 Lower Mainland (from 33 nests) and 91 Okanagan (from 41 nests) 10-day old chicks. Chicks were weighed and body measurements taken approximately ten days post-hatch, using an electronic scale and calipers. Tarsus length refers to the distance from the junction of the tibiotarsus and the tarsometatarsus to the junction with the middle toe. Wing length was measured from the bend of the folded wing to the tip of the longest primary. Toe length refers to the distance from the junction of the distal end of the tarsometatarsus to the proximal end of the claw of the middle toe (Aldrich & James, 1991). As more than one chick was collected from most nests, means per brood were used for the analyses to minimize psuedoreplication.

3.2.5. Growth Measurements

Body weights and tarsus lengths were recorded when the chicks were approximately 10 days (May-July, 1997), 2 months (July-September, 1997), 5 months (October-December, 1997), and 7-9 months (February, 1998) of age. Body weights were also measured following their first breeding season (August-September, 1998). Means per brood were used for the analyses.

3.2.6. Thyroid Hormones

Blood samples were collected from the birds in July, August, or September (Summer 1997), October, November, or December (Fall 1997), February 1998, and August 1998. Samples from 11 Lower Mainland and 11 Okanagan birds (all from different nests) were analyzed for plasma triiodothyronine and thyroxine levels. All blood samples were collected from the jugular vein using a 27 gauge heparinized needle and 1 ml syringe (L. Wilson, Canadian Wildlife Service, personal communication). Approximately one ml of whole blood was collected from each bird, centrifuged at 3300 rpm for 5 minutes, and the plasma separated from the blood cells. Total plasma triiodothyronine and thyroxine concentrations were analyzed by Tracy Marchant at the University of Saskatchewan, Saskatoon, Saskatchewan. Analyses were conducted using unextracted serum and a radioimmuno-assay following protocols outlined by Chopra (1972).

3.2.7. Immune Response

3.2.7.1. Differential White Blood Cell Counts

Blood smears were made when the birds were 10-days-old (99 Lower Mainland birds from 32 broods, and 159 Okanagan birds from 41 broods) and post-breeding adults in August 1998 (33 Lower Mainland birds from 24 broods, 45 Okanagan birds from 31 broods). Blood for the smears was collected from the jugular vein using a 27 gauge heparinized needle and 1 cc syringe. Smears were allowed to dry and then stained using a Hemacolor (EM Diagnostic Systems, Gibbstown, New Jersey) staining kit. Differential white blood cell counts were conducted using 1000x oil immersion microscopy. One hundred white blood cells were counted and ratios of heterophils + eosinophils to lymphocytes + monocytes were determined. Cell types were combined due to counting difficulties (Hodges, 1979), but as numbers of eosinophils and monocytes are normally low in birds (Dufva & Allander, 1995), these values primarily represent heterophil and lymphocyte numbers and the ratio can be treated as heterophil:lymphocyte ratio.

3.2.7.2. Phytohemagglutinin Skin Test

Twelve Lower Mainland and 13 Okanagan adult male robins were used for the phytohemagglutinin tests in May 2000. For each bird a small (approximately 1 cm) patch of skin on each wing web (patagium) was plucked or trimmed clean of feathers and down. This spot was then cleaned with alcohol and measured using a calibrated Mitutoyo digital pressure-sensitive micrometer (Dyer, Lancaster, Pennsylvania). The left wing (control) of each bird was then injected sub-cutaneously with 50 μl of sterile Dulbecco's phosphate buffered saline (Sigma,

St. Louis, Missouri) using a 27 gauge needle. Care was taken to ensure that the needle did not go through the patagium and that the saline did not leak out of the injection site. The right wings of the same birds were injected with 1 mg/ml phytohemagglutinin (Sigma, St. Louis, Missouri) dissolved in 50 μl saline. Twenty-four hours later, the injection sites on both wings were re-measured. Patagium thickness was measured three times both prior to and 24 hours after the injections and means were used for the analyses. A wing index was calculated for each bird by subtracting the difference in wing web thickness for the saline wing from that of the phytohemagglutinin wing ([post-phytohemagglutinin - pre-phytohemagglutinin] - [post-saline - pre-saline]) (Kean & Lamont, 1994; Smits et al., 1999; Smits & Williams, 1999).

3.2.8. Mortality

Age at time of death or disappearance and cause of death were recorded when known. Birds that died prior to fledging age (approximately 14 days post-hatch) were considered nestlings, whereas those that died between 14 and 30 days of age were labeled as fledglings. Robins that died after 30 days but prior to their first breeding season and the move to San Rafael, at approximately 7 to 10 months of age, were considered juveniles. Any birds that survived to February 1998 were considered adults and sexually mature. Cause of death was classed as undetermined, accidental, escape/disappearance, depredation, euthanasia (due to injury), or starvation/coccidiosis. Healthy birds that were sacrificed at the end of the study were not included here.

3.2.9. Tissue Weights and Body Measurements

Following completion of the study 23 Lower Mainland and 31 Okanagan birds were sacrificed by decapitation and dissected. Collected tissues (heart, spleen, liver, kidneys, gonads, thyroid glands, thymus, bursa, oviduct, and brain) were weighed when fresh and then frozen or stored in formalin for future analyses. The same body measurements were taken as at 10 days (tarsus, wing cord, middle toe, body weight). Tissue weights and body measurements for individual birds were analyzed.

3.2.10. Statistics

Differences between Lower Mainland and Okanagan samples in egg contaminants, egg measurements, chick measurements, thyroid hormone levels, and immune response were analyzed using one-way analyses of variance (ANOVAs) and nest means. Sex ratios were analyzed using nominal logistics (χ^2), as were age and cause of death. Tissue weights at time of sacrifice were corrected for body weight by dividing the tissue weight by the body weight minus

the tissue weight. Differences in tissue and body measurements at sacrifice were analyzed using one-way ANOVAs and data from individual birds. Data were common log transformed, where necessary, in order to increase normality. Mahalanobis outlier tests were used to identify outliers. Pair-wise correlations were conducted to determine the effects of egg p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, p,p'-DDE, and o,p'-DDE in the Okanagan birds only. Contaminant non-detects and zeros were replaced by 0.00005 (half the detection limit) for the correlations. P values less than or equal to 0.05 were considered significant. All statistics were conducted using JMP version 3.2.1 software (SAS Institute, Cary, North Carolina). Values presented represent means $\pm$ standard errors, and ranges.

3.3. Results

3.3.1. Egg Contaminants

Table 3-1 illustrates the means, standard errors, and ranges for moisture content, lipid content, p,p'-DDT, p,p'-DDD, p,p'-DDE, o,p'- DDT, o,p'-DDD, o,p'-DDE, and the ratios of DDE to DDT for the eggs collected in 1997. The complete listing of organochlorine and polychlorinated biphenyls analyzed is available in Appendix I. As expected, Okanagan eggs had significantly higher levels of p,p'-DDT ($F_{1, 32} = 66.0$, $p < 0.0001$), p,p'-DDD ($F_{1, 32} = 24.3$, $p < 0.0001$), and p,p'-DDE ($F_{1, 32} = 41.8$, $p < 0.0001$) than Lower Mainland eggs. While p,p' isomers were found in all the samples, o,p' isomers were only found in the Okanagan eggs. O,p'-DDT was detected in 74.2%, o,p'-DDD in 48.4%, and o,p'-DDE in 45.2% of the Okanagan eggs. The DDE:DDT ratio was not significantly different between the two groups, although there was a trend ($p = 0.06$) towards higher ratios occurring in the Lower Mainland eggs than the Okanagan eggs. Lower Mainland eggs contained a significantly higher percentage of lipid than Okanagan eggs ($F_{1,32} = 5.7$, $p = 0.02$), but there were no differences in moisture content. Lipid content was negatively correlated with o,p'-DDD ($r = - 0.4$, $n = 31$, $p = 0.02$) in the Okanagan eggs, but there were no other significant correlations.

3.3.2. Egg Measurements

Eggs collected from the Lower Mainland averaged 6.7 grams ($\pm$ 0.1, range = 5.6 – 8.3 grams) in weight, as did eggs collected from the Okanagan ($\pm$ 0.1, range = 5.1 – 8.4). Egg lengths ranged from 27.5 to 32.2 mm (29.7 $\pm$ 0.2 mm) in the Lower Mainland samples and 26.3 to 32.6 mm (29.2 $\pm$ 0.2 mm) in the Okanagan samples. Egg widths were very similar between

Table 3-1: Means (± se) and ranges of DDTs, moisture content, and lipid content (μg/g) in American robin eggs collected from the Okanagan and Lower Mainland.

	Lower Mainland n = 3	Okanagan n = 31
% Moisture	82.0 (± 0.2) 81.6 - 82.3	82.8 (± 0.3) 80.2 - 85.3
% Lipid	5.7 (± 0.2) 5.4 - 6.1	4.3 (± 0.2)* 1.5 - 5.8
p,p'-DDT	0.1 (± 0.01) 0.1 - 0.2	12.1 (± 1.6)* 0.9 - 30.5
o,p'-DDT	ND	0.07 (± 0.01) ND - 0.3
p,p'-DDD	0.009 (± 0.004) 0.003 - 0.02	1.0 (± 0.3)* 0.06 - 8.7
o,p'-DDD	ND	0.005 (± 0.001) ND - 0.02
p,p'-DDE	1.9 (± 0.7) 0.9 - 3.2	51.7 (± 8.7)* 10.0 – 245.0
o,p'-DDE	ND	0.005 (± 0.0009) ND - 0.01
DDE:DDT	12.8 (± 3.7) 8.0 - 20.1	6.8 (± 2.0) 0.7 - 63.1

ND = not detected, *$p < 0.05$

the two groups (Lower Mainland 21.0 ± 0.1, range 19.3 – 22.6 mm; Okanagan 21.0 ± 0.1, range 19.3 – 23.0 mm). There were no significant differences in weight, length, or width of the Lower Mainland and Okanagan eggs. The Okanagan pair-wise correlations between egg measurements and DDT contaminant levels were also not significant.

3.3.3. Chick Measurements

Chick measurements are listed in Table 3-2. Lower Mainland chicks had significantly longer middle toes than their Okanagan counterparts ($F_{1,68} = 475.9$, $p < 0.0001$). There were no significant type differences in the other body measurements. For the Okanagan chicks, body weight was positively correlated with p,p'-DDE ($r = 0.5$, $n = 31$, $p = 0.009$), but there were no other significant correlations. Sex was determined for 104 birds. There were 22 female and 21 male chicks collected from the Lower Mainland and 27 females and 34 males collected from the Okanagan. There were no significant differences in sex ratio between the two groups.

3.3.4. Growth Measurements

Figure 3-1 demonstrates body weights for the birds at the various ages measured, and Figure 3-2 illustrates changes in tarsus length with time. There were no significant differences in weight at any of the ages tested. However, when outliers were removed from the analyses, Okanagan birds were shown to weigh more than Lower Mainland birds at 2 months of age, but Lower Mainland birds weighed more at 7 - 9 months of age. Within the Okanagan birds, only 10- day weight was positively correlated with p,p'-DDE ($r = 0.4$, $n = 25$, $p = 0.04$). Tarsus lengths were significantly larger in the Lower Mainland than in the Okanagan broods at 2 months ($F_{1,65} = 15.8$, $p = 0.0002$), 5 months ($F_{1,62} = 6.2$. $p = 0.02$), and 7 to 9 months ($F_{1,61} = 5.8$, $p = 0.02$) of age. The difference in tarsus lengths at 7 to 9 months, however, was non-significant when an outlier was removed. There were no significant correlations between egg contaminant levels and tarsus length.

3.3.5. Thyroid Hormones

There were no significant differences between Lower Mainland and Okanagan birds in triiodothyronine or thyroxine levels, at any of the ages tested (Figure 3-3). No significant correlations between thyroid hormone levels and egg DDT residues were found.

Table 3-2: Means (± se) and ranges of body weight, and tarsus, wing cord, and middle toe lengths of ten day old American robin nestlings collected from the Okanagan and Lower Mainland.

	Weight (g)	Tarsus (mm)	Wing (mm)	Toe (mm)
Lower Mainland	58.4 (± 1.5) 34.0 - 76.0	37.3 (± 0.3) 32.8 - 41.5	63.4 (± 1.7) 46.0 - 79.0	21.6 (± 0.3) 19.0 - 26.0
Okanagan	60.4 (± 0.8) 50.1 - 75.1	36.8 (± 0.2) 33.9 - 40.6	64.6 (± 0.9) 50.7 – 74.3	10.9 (± 0.3)* 6.7 – 20.0

*$p < 0.05$

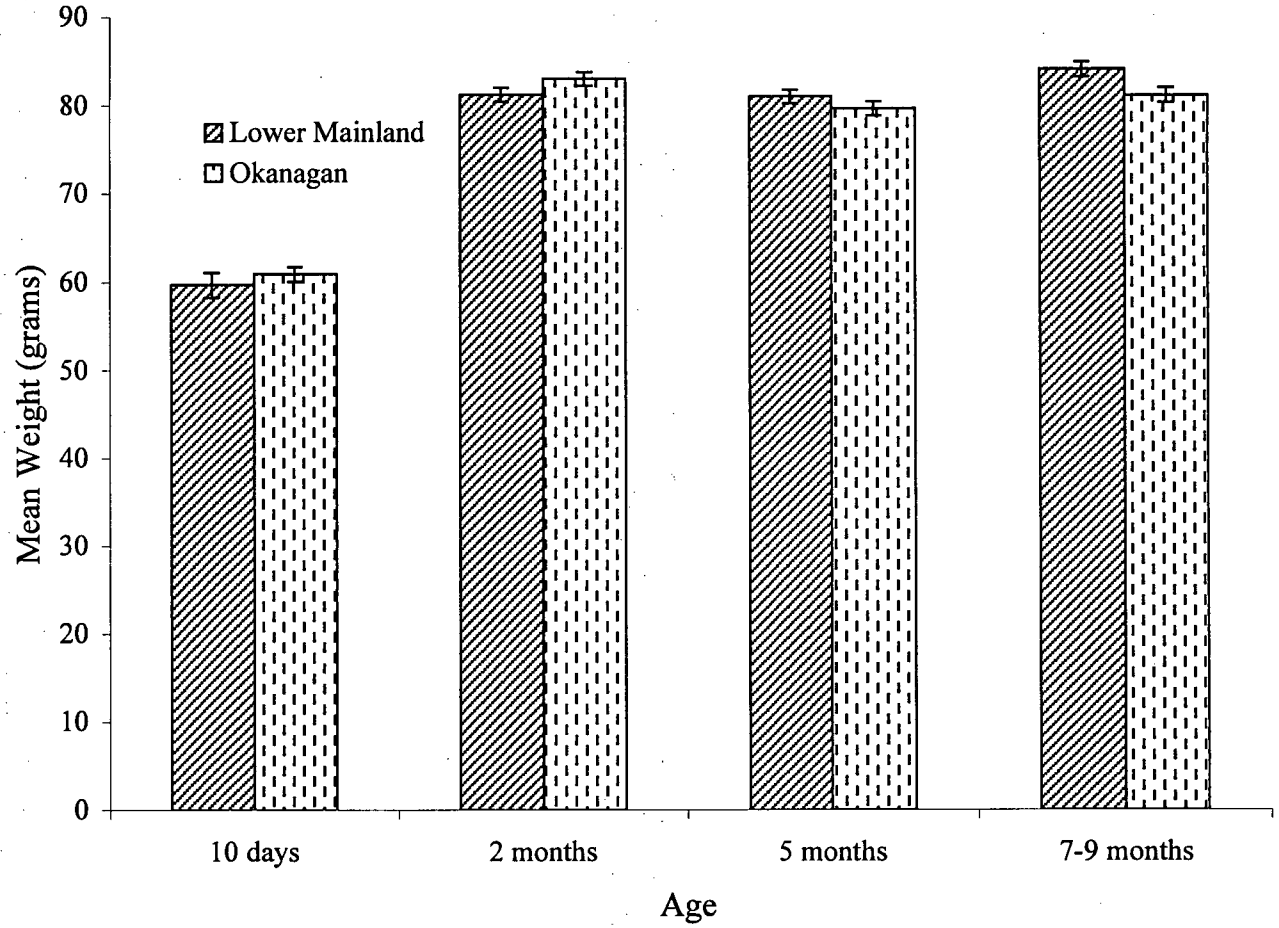

Figure 3-1: Mean (± se) body weights (grams) of Lower Mainland and Okanagan American robins at various ages.

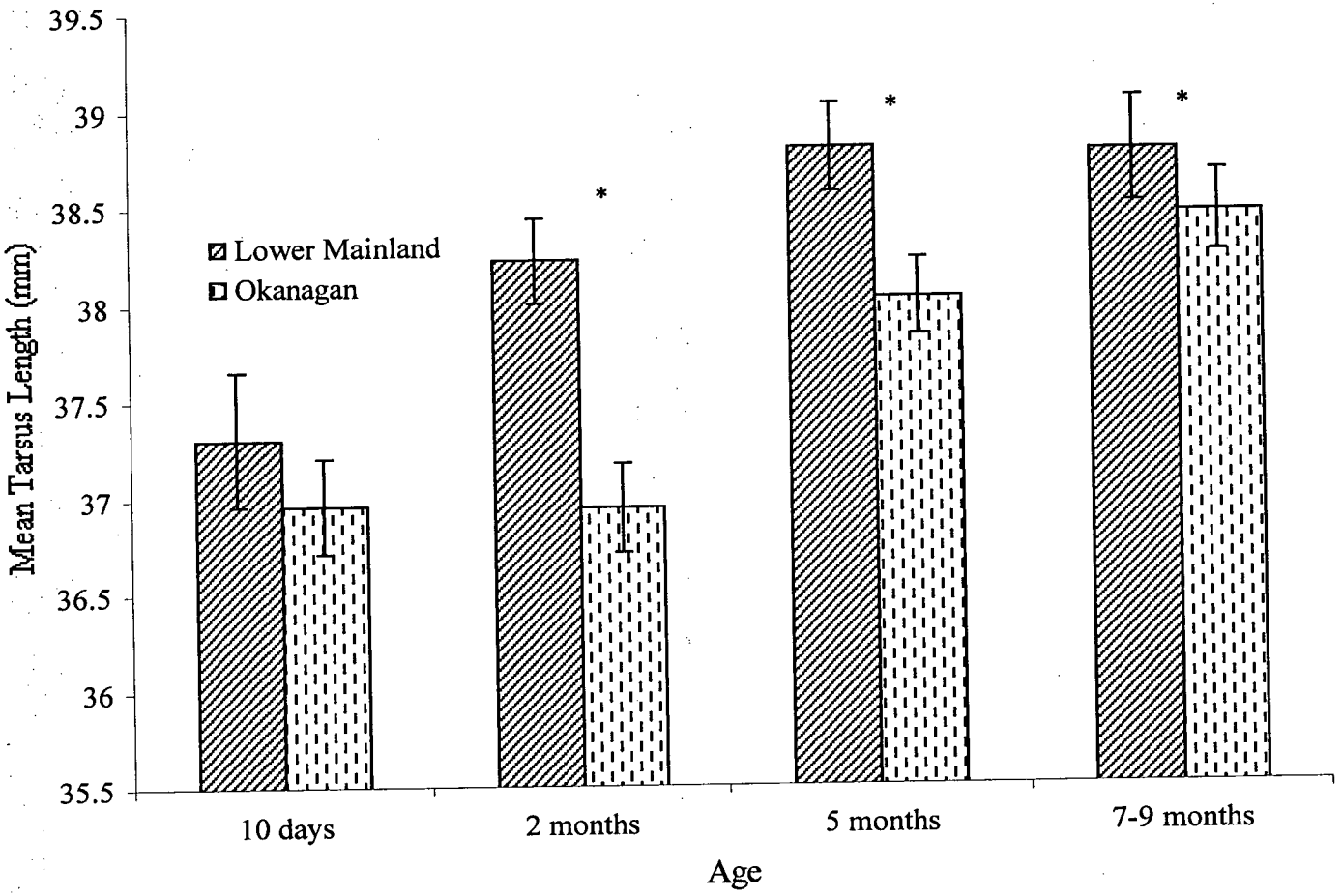

Figure 3-2: Mean (± se) tarsus lengths (mm) of Lower Mainland and Okanagan American robins at various ages. * $p < 0.05$

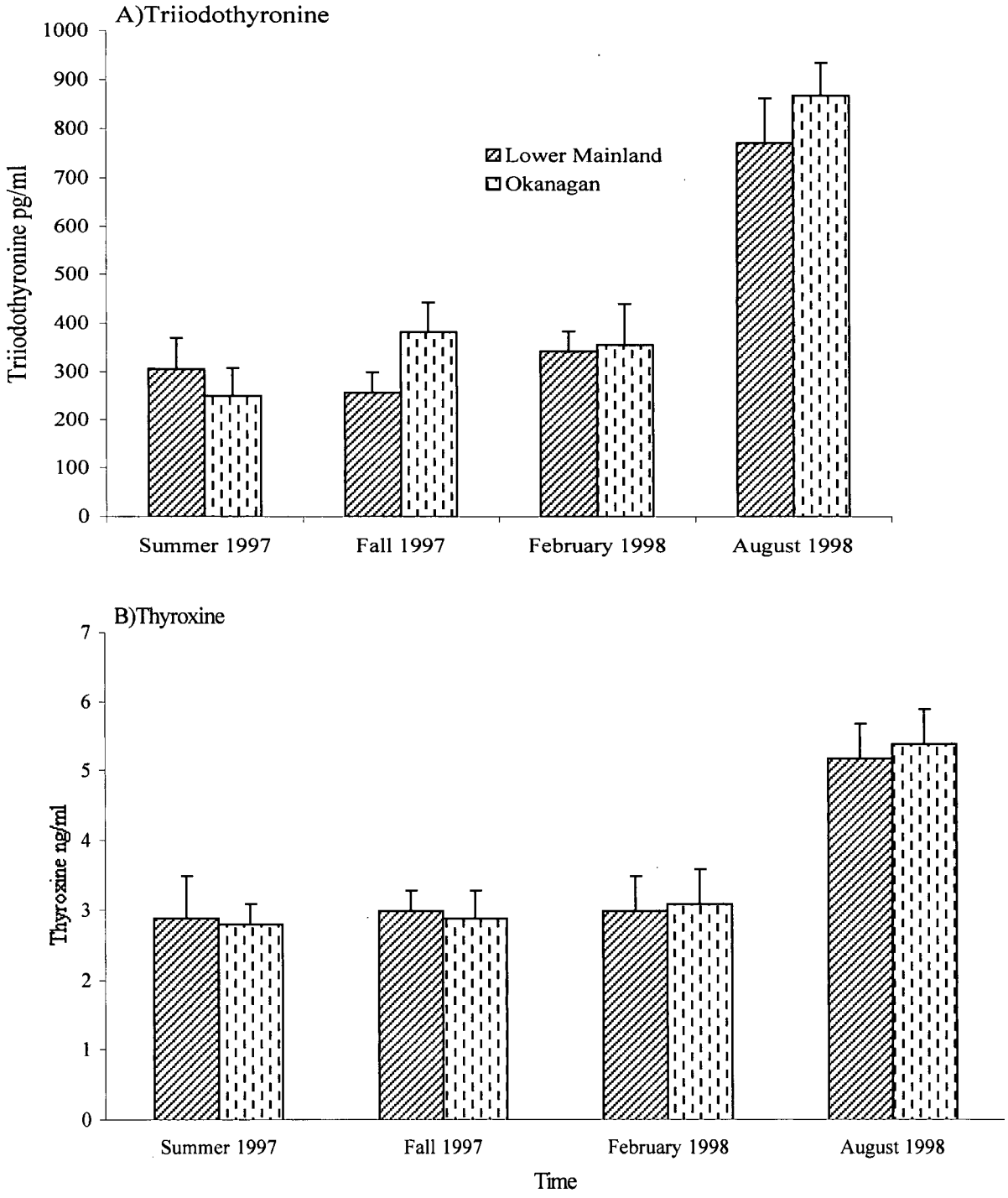

Figure 3-3: Plasma levels of A)triiodothyronine (pg/ml) and B)thyroxine (ng/ml) in Okanagan and Lower Mainland robins at various ages.

3.3.6. Immune Response

3.3.6.1. White Blood Cell Ratios

Lower Mainland birds had significantly higher heterophil to lymphocyte ratios than Okanagan birds at ten days of age ($F_{1,71} = 11.1$, $p = 0.001$; Figure 3-4), but there were no significant differences between the groups when they were post-breeding adults. White blood cell ratios in the Okanagan birds, however, were significantly correlated with p,p'-DDT when they were post-breeding adults ($r = 0.4$, $n = 25$, $p = 0.03$). Ratios were considerably higher when the birds were 10-day-old nestlings as compared to post-breeding adults ($F_{1,126} = 244.0$, $p < 0.0001$; Figure 3-4).

3.3.6.2. Phytohemagglutinin Skin Test

There were no significant differences between Lower Mainland and Okanagan males in their response to the phytohemagglutinin skin test, nor were there any significant correlations with egg DDT levels for the Okanagan birds. Wing web indices in the Lower Mainland males ranged from – 0.04 to 1.0 (0.5 ± 0.1), while Okanagan males' ranged from 0.1 to 1.1 (0.4 ± 0.08).

3.3.7. Mortality

Table 3-3 illustrates cause of death for the robins in this study. In a number of cases, cause of death was not determined. Five of the Okanagan birds were diagnosed as having intestinal coccidiosis (parasites *Eimeria* and *Isospora*), and it was suspected that all 13 of the Okanagan birds that appeared to starve to death (wasting) were infected. Two of these birds were also suffering from leucocytozoonosis (protozoan blood parasite, *Leucocytozoon*). None of the Lower Mainland birds housed under the same conditions were affected. Seven birds were euthanized following debilitating injuries. Accidental deaths included those incurred during blood sampling, drowning (in water dishes), and a variety of other incidents. Birds that disappeared or escaped were presumed dead and were likely depredated. Several birds died following trauma as a result of house cat (*Felis domesticus*) and hawk attack attempts from outside the cages, but exact cause of death was not determined. Rats (*Rattus norvegicus*) were common in the breeding pens and were considered responsible for the predation deaths. There was a significant difference between the Lower Mainland and Okanagan birds in cause of death ($\lambda^2 = 14.2$, $p = 0.01$), most likely due to the starvation deaths in the Okanagan birds.

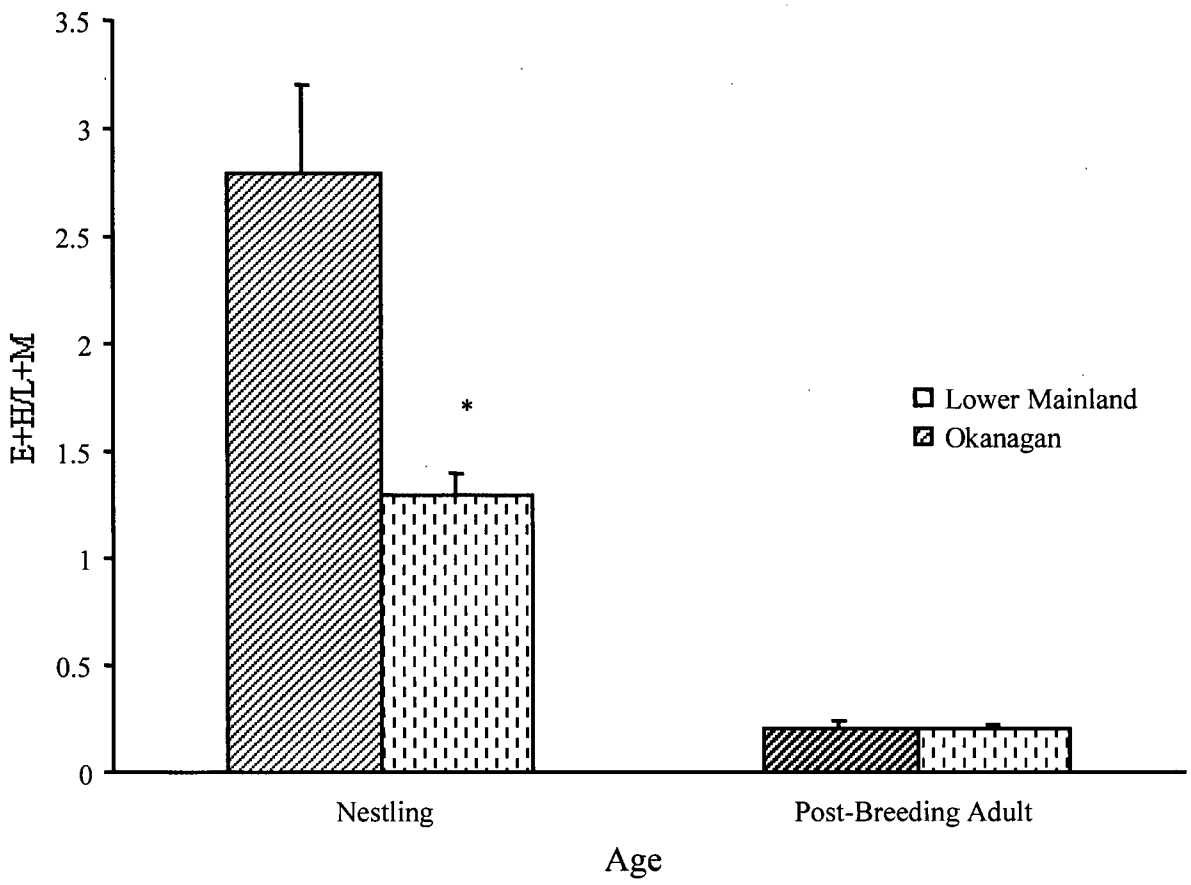

Figure 3-4: Differences (+ se) in white blood cell ratios (eosinophils + heterophils / lymphocytes + monocytes) for Lower Mainland and Okanagan American robins as 10 day old nestlings and post-breeding adults. *$p < 0.05$

Table 3-3: Causes of death for American robins collected from the Okanagan and Lower Mainland.

	Lower Mainland n = 33	Okanagan n = 60	Total n = 93
undetermined	19 57.6%	22 36.7%	41 44.1%
accidental	3 9.1%	10 16.7%	13 14.0%
disappeared/ escaped	5 15.2%	5 8.3%	10 10.8%
depredated	3 9.1%	6 10.0%	9 9.7%
euthanized	3 9.1%	4 6.7%	7 7.5%
starved/coccidiosis	0	13 21.7%	13 14.0%

Contaminant levels in the egg did not significantly influence the cause of death for Okanagan birds. The Okanagan birds that died as juveniles from starvation/coccidiosis, for instance, came from nests with a wide range of contaminant levels. Total egg DDT levels for these birds ranged from 13.5 to 104.3 μg/g. Although, most of the birds survived to adulthood (107/147, 72.8%), 4 (2.7%) birds died when still in the nestling stage (≤ 14 days of age), and 5 (3.4%) chicks died during what would be considered the fledgling (> 14 days but < 30 days) stage. An additional 31 (21.1%) birds died prior to sexual maturity and transfer to the breeding cages (February 1998). There was a significant difference between the Lower Mainland and Okanagan birds in time of death ($\lambda^2 = 14.6$, $p = 0.002$; Table 3-4). Although the majority of both Lower Mainland and Okanagan died as adults, a larger number of Okanagan birds died as juveniles, likely as a result of coccidiosis infections.

3.3.8. Tissue Weights and Body Measurements

Tissue weights and body measurements at sacrifice are listed in Table 3-5. The only significant differences in organ weights (corrected for body weight) between the Lower Mainland and Okanagan birds were in liver weight ($F_{1,36} = 4.3$, $p = 0.04$), and heart weight ($F_{1,36} = 9.6$, $p = 0.004$). Both of these tissues were heavier in Okanagan than Lower Mainland birds. The removal of an outlier rendered the difference in liver weight non-significant. Differences in brain, thyroid glands, thymus, spleen, bursa, gonad, and oviduct weights were not significant, nor were the differences in body measurements. Within the Okanagan birds, significant correlations were found between gonad weight and p,p'-DDE ($r = -0.5$, $n = 18$, $p = 0.04$; Figure 3-5), and oviduct weight and o,p'-DDT ($r = 0.8$, $n = 9$, $p = 0.009$; Figure 3-6). Egg DDT levels did not significantly correlate with the other tissue weights or with the body measurements taken at sacrifice.

3.4. Discussion

The objective of this study was to examine whether early exposure to DDT has any delayed or long-term effects on the growth and survival of American robins. This was done by: 1) comparing contaminated Okanagan birds with uncontaminated Lower Mainland controls, and 2) correlating contamination levels of Okanagan eggs with the growth parameters of their clutch mate(s). The design of the study was based on three assumptions: a) that the Okanagan and Lower Mainland birds were of similar genetic background, b) the level of contamination in all

Table 3-4: Ages at time of death for American robins collected from the Okanagan and Lower Mainland.

	Lower Mainland n = 33	Okanagan n = 60	Total n = 93
adult	24 72.7%	29 48.3%	53 57.0%
juvenile	4 12.1%	27 45.0%	31 33.3%
fledgling	4 12.1%	1 1.7%	5 5.4%
nestling	1 3.0%	3 5.0%	4 4.3%

Table 3-5: Means (± se) and ranges of body measurements and tissue weights (corrected for body weight) of Okanagan and Lower Mainland robins at sacrifice.

	Lower Mainland n = 15	Okanagan n = 23
body weight (grams)	79.9 (± 1.1) 72.1 – 85.4	81.4 (± 1.0) 66.6 – 86.6
wing (cm)	12.8 (± 0.2) 11.5 – 14.1	12.9 (± 0.1) 11.5 – 13.3
toe (cm)	1.7 (± 0.3) 1.5 – 1.9	1.7 (± 0.3) 1.5 – 1.9
tarsus (cm)	3.7 (± 0.3) 3.4 – 3.9	3.7 (± 0.3) 3.5 – 4.0
brain (g)	0.021 (± 0.00043) 0.018 – 0.023	0.020 (± 0.00040) 0.018 – 0.024
thyroids (g)	0.00025 (± 0.000018) 0.00013 – 0.00038	0.00031 (± 0.000025) 0.00013 – 0.00062
thymus (g)	0.0013 (± 0.00015) 0.00039 – 0.0023	0.0011 (± 0.00011) 0.00036 – 0.0025
heart (g)	0.012 (± 0.00025) 0.011 – 0.014	0.013 (± 0.00029)* 0.011 – 0.016
spleen (g)	0.002 (± 0.00043) 0.00054 – 0.0074	0.0023 (± 0.00019) 0.0010 – 0.0041
gonads (g)	0.00052 (± 0.000047) 0.00021 – 0.00079	0.00041 (± 0.000039) 0.00016 – 0.00079
bursa (g)	0.00086 (± 0.000082) 0.00042 – 0.0014	0.00092 (± 0.000072) 0.00052 – 0.0018
kidneys (g)	0.011 (± 0.00053) 0.0082 – 0.015	0.011 (± 0.00023) 0.0090 – 0.014
oviduct (g)	0.00073 (± 0.000072) 0.00044 – 0.0012	0.00070 (± 0.000096) 0.00031 – 0.0015
liver (g)	0.024 (± 0.00093) 0.018 – 0.031	0.026 (± 0.00060)* 0.022 – 0.035

*$p < 0.05$

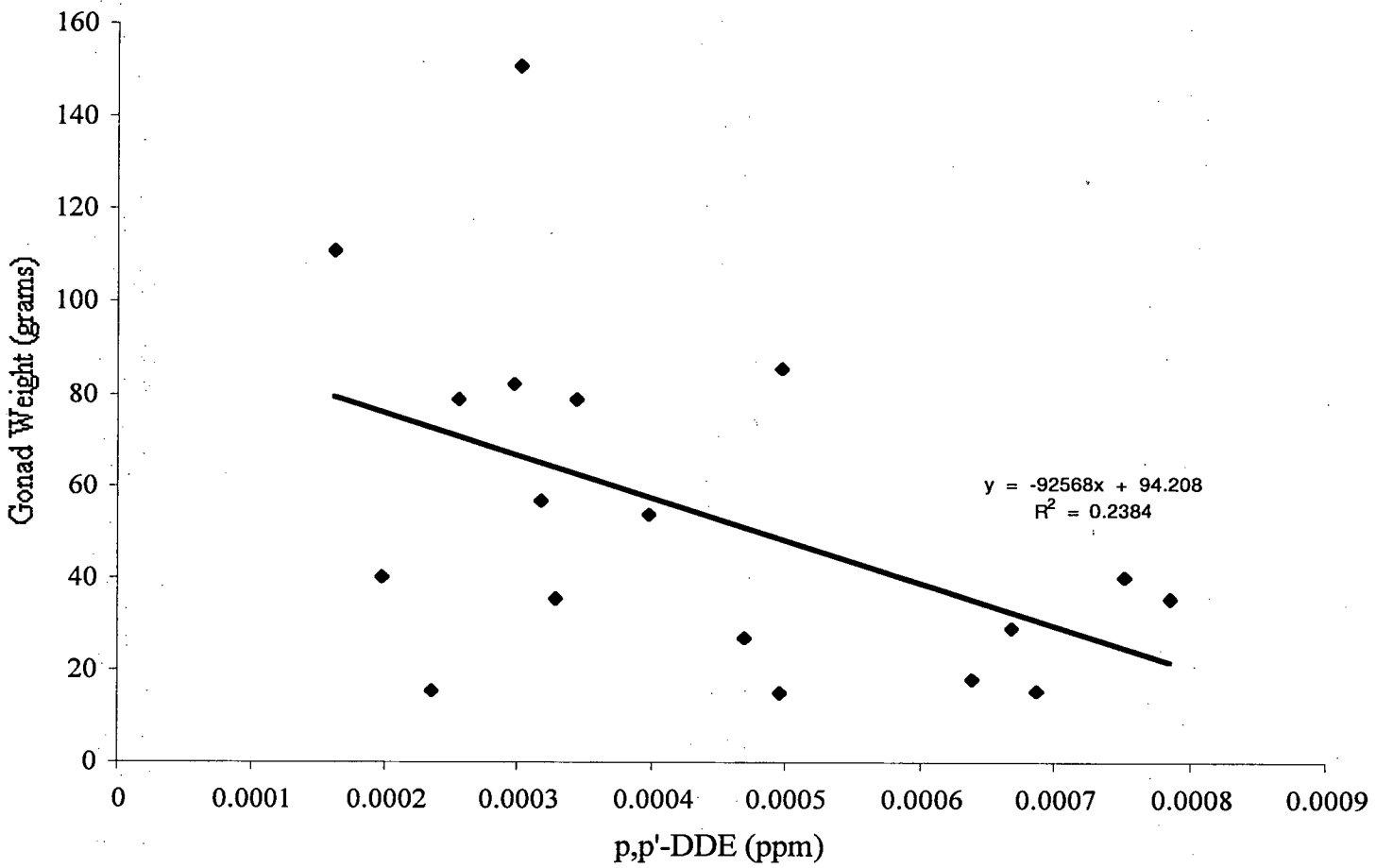

Figure 3-5: Relationship between gonad weight at sacrifice (grams, corrected for body weight) and egg p,p'-DDE (μg/g) levels.

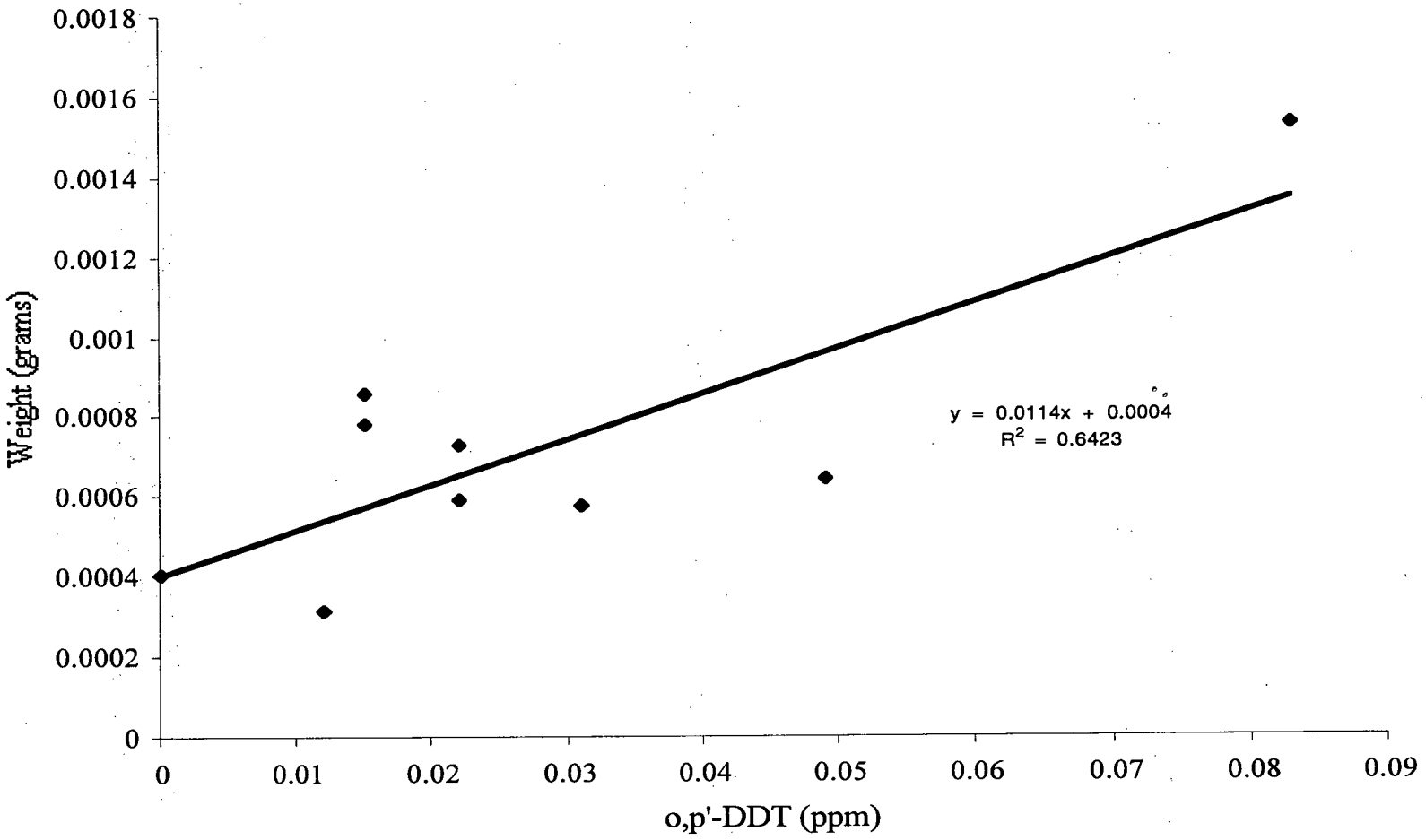

Figure 3-6: Relationships between egg o,p'-DDT (μg/g) and female Okanagan American robin oviduct weights (grams) at time of sacrifice.

the eggs in a clutch was similar, and c) among Okanagan birds, parental care and post-hatch DDT exposure (from hatching to about ten-days of age) was not a significant variable.

3.4.1. Growth and Development

As expected, Okanagan eggs contained considerably higher levels of DDTs than Lower Mainland eggs (See Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000). It was also found that o,p' isomers were only present in Okanagan eggs and not Lower Mainland eggs. Okanagan eggs also had higher lipid contents. Chicks collected from the Okanagan had shorter middle toes and at certain points in development also had shorter tarsi. After reaching adulthood, their hearts and livers (adjusted for body weight) were heavier than those of Lower Mainland birds. Of the parameters examined, only body weight at ten days of age was correlated with p,p'-DDE levels in the eggs. Chicks from more contaminated nests were heavier at ten-days than those from nests with lower DDE levels. These results suggest that *in ovo* contamination may have influenced weight gain in these chicks shortly after hatching. The majority of studies suggest that DDTs suppress rather than promote growth (Britton et al., 1974; Fox, 1997; Tyler et al., 1998; Vos et al., 2000). In Bengalese finches (*Lonchura striata*), for instance, both egg weight and chick weight decreased with increasing p,p'-DDT and p,p'-DDE levels (Jefferies, 1971). As parental care measures and immediate post-hatch DDT exposure are not known for the robins in this study, they could serve as confounding factors.

Okanagan birds had shorter middle toes than Lower Mainland chicks, and they had shorter tarsi than Lower Mainland birds at two, five, and seven to nine months of age. Lower Mainland birds showed the most dramatic increase in tarsus length between ten days and two months of age. The Okanagan birds, in contrast, did not exhibit this growth spurt until between two and five months of age. Tarsus growth is often used as an indicator of body growth (Aldrich & James, 1991). These results suggest that growth in Okanagan birds was delayed relative to the Lower Mainland controls. This is an effect which may be attributed to *in ovo* DDT exposure (Britton et al., 1974; Fox, 1997; Tyler et al., 1998; Vos et al., 2000). While Okanagan chicks weighed more than Lower Mainland chicks at two months of age, by the time they were seven to nine months old they weighed less.

DDTs could potentially influence body weight and weight gain through their effects on the thyroid glands, as thyroid hormones influence both body and organ growth (Jefferies, 1975; King & McLelland, 1984). The steroid hormone effects of DDTs may also play a role in body weight. The anti-androgen, flutamide, depressed the body weights of three and seven week old

broiler chicks, suggesting that interference with the actions of endogenous androgens during embryonic life can suppress post-hatching growth (Burke, 1996). As p,p'-DDE is an anti-androgen (Gaido et al., 1997; Kelce et al., 1995, 1998), it may have similar effects, although this cannot be clearly demonstrated in this study. There were no significant differences between Lower Mainland and Okanagan birds in either plasma triiodothyronine or thyroxine levels at any of the ages tested and there were no significant correlations between the DDTs measured in the eggs and thyroid hormones at any age.

Both livers and hearts were heavier in Okanagan than Lower Mainland birds. Previous studies have linked both enlarged hearts and livers to thyroid hormone levels. For example, liver hypertrophy and liver glycogen accumulation can be induced by hypothyroidism in chicks (Wentworth & Ringer, 1986), and thyroxine can accelerate heart rate and increase heart weight in domestic fowls (Jefferies, 1975). Similar effects have been seen with DDT. Bobwhite quail (*Colinus virginianus*; Hurst et al., 1974) and pigeons (*Columba livia;* Jefferies & French, 1969) showed increasing liver weights with increasing DDT dose. The increase in liver size may have been due to increased hepatic activity and metabolism of circulating hormones, as DDT is known to induce enzyme breakdown of hormones (Peakall, 1967). Pigeons fed a low dose of DDT exhibited increases in amplitude of the ventricular beat and heart weight, but birds fed a high dose showed heart beat amplitudes lower than that in controls, decreased heart weights, and thin, flaccid heart musculature. Heart rate, beat amplitude, and weight continued to increase with increasing dose, however, in Bengalese finch (Jefferies, 1975). Although the robins in this study did not demonstrate any significant differences in thyroid hormone levels, little is known about the effects of *in ovo* exposure to DDT on these organs.

While there was no significant difference in gonad and oviduct weights between Okanagan and Lower Mainland birds, gonad weights in the Okanagan birds were negatively correlated with p,p'-DDE. Para,para'-DDE has been shown to act as an androgen antagonist (Gaido et al., 1997; Kelce et al., 1995, 1998), thus its effects on testes growth and development in particular could be profound. Oviduct weights were positively correlated with o,p'-DDT exposure. This form of DDT is a known estrogen agonist that has been shown to increase oviduct weight in other species (Stickel, 1973). This study demonstrates that early exposures to these DDT isomers may also have long term effects on these reproductive organs. It is therefore of interest to examine the reproductive performance and behavior of these Okanagan birds (See Chapter IV).

3.4.2. Immunity and Survival

At ten-days of age, Lower Mainland chicks had higher percentages of heterophils and eosinophils than Okanagan chicks, whereas Okanagan birds had higher levels of lymphocytes and monocytes. There are several possible explanations for this. Normal stress response and corticosterone release increase heterophil levels and decrease lymphocyte levels (Dufva & Allander, 1995; Grasman et al., 1996; Siegel, 1980; Smits & Williams, 1999), and the heterophil to lymphocyte ratio has been used extensively as a reliable indicator of physiological and social stress (Gross & Siegel, 1983; Ehrich & Gross, 1986; Gross, 1990; de Jong et al, 2002). While DDT may inhibit the stress response in Okanagan chicks (see Chapter V) thus influencing the white blood cell ratios (Biesmann & von Faber, 1981; Latimer & Siegel, 1974), it has been shown in chickens that heterophil/lymphocyte ratios can take up to two days to reflect the effects of a stressor (Puvadolpirod & Thaxton, 2000). As adults these birds would likely be less prone to stress-related effects on white blood cell ratios, as they had been captured and handled on numerous occasions and were likely somewhat habituated to it. Thus, stress of handling likely did not play much of a role in the different white blood cell ratios seen in the robin chicks, as blood samples were obtained immediately upon capture. Glucocorticoid levels and white blood cell numbers fluctuate throughout the day, with variations as high as 50% (Crisp et al., 1998). Thus, simply sampling the birds at different times of the day may influence heterophil/lymphocyte ratios. As lymphocyte levels tend to rise in response to viruses, and heterophil levels tend to rise in response to bacteria (Siegel, 1980), it is also possible that one or both groups were battling low levels of infection. Alternatively, these differet white blood cell ratios may normally be different between these two groups due to genetic differences (see below). DDT exposure may have triggered immune responses in the Okanagan birds or made them more susceptible to infection. However, the cell ratios were not significantly correlated with *in ovo* DDT exposure.

Regardless of the the reason behind the difference in white blood cell ratios in nestlings, as adults, Lower Mainland and Okanagan birds did not show differences. As adults, the birds would have been less prone to stress induced changes as they were more used to being handled and the adrenal response to adrenicorticotropic hormone is lower in adults than in young birds, making them less responsive to stressful stimuli (Harvey et al., 1986). Differences in white blood cell ratios at various life stages may simply reflect normal age and/or seasonal changes (Sturkie & Griminger, 1986). Smits and Williams (1999) found an increase in lymphocytes and a decrease in heterophils and eosinophils between 11 and 21 days in zebra finches (*Taeniopygia*

guttata), but Dufva and Allander (1995) found no differences in white blood cell counts between different age classes of Great tits (*Parus major*). Seasonal changes, even within a period of a few weeks were associated with changes in the immune systems of tree swallows (*Tachycineta bicolor)* (Bishop et al., 1998). However, within the Okanagan birds, p,p'-DDT was positively correlated with white blood cell ratios. Thus, birds from more contaminated nests had higher heterophil levels as adults.

It is not known for sure how many chicks died prior to ten-days of age when they were collected. Gill et al. (2003) reported no significant differences in hatch rate, brood size, or fledge rate between the Lower Mainland and Okanagan nests from which the birds were collected. Under captivity, however, the Okanagan birds appeared to be more susceptible to infectious disease than Lower Mainland birds. Thirteen (21.7%) of the Okanagan chicks died of starvation, likely due to coccidiosis, whereas no Lower Mainland birds appeared to be affected. Significantly higher juvenile mortality occurred in Okanagan birds due to predation, accidents, and disease. These three factors are probably related as sick birds are more easily depredated and are more prone to accidents. As birds from the most contaminated nests were no more or less likely to succumb to coccidiosis than those from less contaminated nests, DDT exposure may not be the factor involved. Although Grasman et al., (1996) found a decrease in phytohemagglutinin response with increasing DDE levels in gulls and terns, the robins in this study showed no differences in response related to their levels of *in ovo* DDE exposure. Roe et al. (2002) also did not find that bald eagles (*Haliaeetus leucocephalus*) from contaminated coastal sites on the Great Lakes were more susceptible to blood borne parasites than birds from cleaner interior sites. Parasite exposure can suppress and/or stimulate immune responses depending on the life stage of the parasite and the host (Bishop et al., 1998).

It is possible that there are inherent differences between the Lower Mainland and Okanagan birds which affect their susceptibility to disease. Birds from the Lower Mainland may be genetically predisposed to have greater resistance to the particular coccidia parasites found in these birds. The Lower Mainland and Okanagan birds may even represent different subspecies (Aldrich & James, 1991). Robins from the Lower Mainland likely belong to the subspecies *Turdus migratorius caurinus*, whereas Okanagan robins are probably *T. migratorius propinquus* (Aldrich & James, 1991; Cannings, 1998). If the birds had been raised in the Okanagan, as opposed to the Lower Mainland, Lower Mainland birds may have been more prone to infection. Genetic differences between the birds may also be exacerbated by early DDT exposure.

3.5. Conclusions

This study demonstrated that *in ovo* DDT exposure influenced growth and organ weights in American robins from the Okanagan Valley. It appears that most of the detrimental effects of *in ovo* contaminant exposure in robins occur relatively early in the birds' lives. Whether the difference in susceptibility to coccidiosis infection between the Okanagan and Lower Mainland birds was DDT related cannot be established, and requires further examination (see Chapter V.). It is possible that genetic differences between the two groups of birds may have influenced the results.

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Chapter IV

Reproduction and Behavior in American Robins Exposed *In Ovo* and Early Post-Hatch to DDT and its Metabolites

4.1. Introduction

Dichlorodiphenyltrichloroethane (DDT), its primary metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), and a variety of other organochlorine chemicals have long been linked to reproductive abnormalities and failure in birds (Murphy, 1980). As these chemicals are deposited into eggs during yolk formation (Brandt et al., 1978; Fry, 1995; Ottinger et al., 2001), they can influence the reproduction not only of the exposed generation, but also their offspring. *In ovo* and early post-hatch exposure to DDTs may have profound effects on the development and sexual differentiation of the reproductive systems of birds (Balasubramaniam & Sundararaj, 1993; Burlington & Lindeman, 1950; Fry & Toone, 1981; Stickel, 1973). For example, male California gulls (*Larus californicus)* from eggs injected with as little as two mg/kg o,p'-DDT had feminized gonads, while five mg/kg or higher o,p'-DDT resulted in the development of both left and right oviducts in female gulls (Fry & Toone, 1981).

DDT contamination can also lead to alterations in parental and other behaviours. For example, merlins (*Falco columbarius*) whose eggs contained high levels of p,p'-DDE, and other organochlorines, have been shown to desert their clutches more readily and defend their nests less actively than birds with less contaminated eggs (Fox & Donald, 1980). Herring gulls (*Larus argentatus*) exposed to a number of chemical contaminants have also demonstrated decreased nest attentiveness (Fox et al., 1978; Rattner et al., 1984).

Hyper-aggressive parents that injured their offspring were found in dosing studies of ring doves (*Streptopelia risoria*) (McArthur et al., 1983) and Bengalese finches (*Lonchura striata*) (Jefferies, 1971). Parent birds contaminated with DDTs and other compounds may destroy their own eggs by eating, breaking, or ejecting them (Ohlendorf et al., 1978; Stickel, 1973). Hyperexcitability and hypersensitivity to stimuli are commonly listed among the symptoms of DDT poisoning in mammals, and similar results are likely in birds (Jefferies, 1975). Hyperactivity has been suggested as a possible reason for the nest inattentiveness of organochlorine fed ring doves (McArthur et al., 1983).

American robins (*Turdus migratorius*) from the Okanagan Valley of British Columbia are known to be highly contaminated with DDT and DDE which they acquire from eating earthworms that have consumed contaminated soil (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000). Despite the very high levels of DDT found in these birds, reproduction does not appear to be adversely affected. A study of robins nesting in organic (non-pesticide sprayed) and conventionally (pesticide sprayed) managed orchards in the Okanagan revealed no significant differences in clutch size, nest success, hatching success, or fledging success between the two orchard types (Elliott et al., 1994). Gill et al. (2003) also found no significant differences in hatch rate and fledge rate between robins from Okanagan orchards and controls from park areas within the British Columbia Lower Mainland. These authors, however, reported that clutch size and brood size were significantly higher in orchard than non-orchard control sites.

The purpose of this study was to examine latent effects of *in ovo* and early post-hatch DDT exposure on American robin reproduction. To do this, ten-day-old nestlings from Okanagan orchards, along with Lower Mainland controls were raised in captivity and then transferred as pairs to breeding pens where they were monitored over two seasons. Nesting success was monitored, eggs and chicks weighed and measured, and tissues collected at the time of sacrifice. Thyroid hormone levels were also measured in a subset of breeding pairs, every two weeks during one breeding season, as these hormones can play a significant role in reproduction (Cheek et al., 1999; McArthur et al., 1983; Nelson, 2000; Wentworth & Ringer, 1986; Wilson & Donham, 1988). A number of behaviours were observed, including aggressive, vocalization, maintenance, and reproductive behaviours. As no significant differences were found in the reproduction of British Columbia robins in the wild, it was expected that captive birds from the Okanagan and Lower Mainland would demonstrate similar levels of reproductive success. However, it is possible that early DDT exposure has more subtle, long-term effects on the reproduction of these birds that have heretofore gone unnoticed. Thus, the goals of this study were to:

1) Determine if early DDT exposure had a detrimental effect on the reproductive success of Okanagan robins, as measured by egg laying, egg hatching, and chick fledging, as well as egg and chick measurements.
2) Determine if early DDT exposure affected reproductive behaviours (e.g., nest building, mating, chick feeding, nest cleaning) in Okanagan robins. In addition, aggressive (e.g., charging, chasing, and fighting), vocalization (e.g., singing, chirping, laughing), and maintenance (e.g., eating, drinking, preening) behaviours were also examined. Aggressive

and vocalization behaviours play an important role in reproduction in terms of territory and mate acquisition and defense (Silver et al., 1979).

4.2. Methods

4.2.1. Eggs and Chicks

Ten-day old American robin nestlings were collected from nests in the Okanagan Valley and the Lower Mainland of British Columbia. Chicks were marked with numbered metal leg bands and colour plastic leg bands for individual identification. Eggs were also collected for contaminant analyses. See Chapter III for details on eggs, nestlings, housing, and contaminant analyses protocols. Birds were sexed by external morphology and later confirmed by DNA sexing by Brett Vanderkist at Simon Fraser University, Burnaby, British Columbia (Griffiths et al., 1998). In 1998, 40 breeding pairs were established in both indoor and outdoor pens, one pair per pen at the University of British Columbia San Rafael research aviary (Surrey, British Columbia). The birds were over-wintered at Monika's Wildlife Shelter (Surrey, British Columbia), and in 1999, 56 birds were brought back to San Rafael and housed as breeding pairs in the outdoor pens only. Both same type (Okanagan x Okanagan and Lower Mainland x Lower Mainland) and different type (Okanagan x Lower Mainland and Lower Mainland x Okanagan) pairs were included. A total of 15 Lower Mainland x Lower Mainland (male x female), 18 Lower Mainland x Okanagan, 22 Okanagan x Lower Mainland, and 23 Okanagan x Okanagan pairs were studied. Care was taken to ensure that siblings and close neighbors were not paired. Individuals that died or escaped were replaced with another bird of the same type (Lower Mainland or Okanagan) whenever possible. Thus, in 1998, 46 females (Lower Mainland = 22, Okanagan = 24) were introduced into the breeding pens, and in 1999 32 females (Lower Mainland = 15, Okanagan = 17) were studied.

4.2.2. Reproduction

4.2.2.1. Nesting Success

The pairs were monitored daily for evidence of nest building, egg laying, egg hatching, and chick fledging from the end of February until September. The observer was blind to the type and level of contamination of all birds. As incubation usually begins after the last egg is laid (Howell, 1942; Kemper, 1971, but see Sallabanks & James, 1999; Wauer, 1999), incubation period was defined as the time between the laying of the last egg and the hatching of the last chick. The nestling period encompassed the time between the first egg hatching and the last

chick fledging. Hatching success refers to the number of females with eggs in nests that hatched at least one chick. Fledging success refers to the number of females with eggs in nests that fledged at least one chick. Clutch size and number of clutches laid were also noted. Only eggs that were in the nests were included in clutch size and number calculations, as females sometimes laid eggs outside of the nest and eggs could be removed from the nest by the birds themselves and/or predators.

4.2.2.2. Egg Measurements

The first two eggs from each clutch were weighed and measured after the second egg was laid, using an electronic scale and calipers. A number of abandoned eggs were also measured. Egg length refers to the distance from end-to-end, while egg width refers to the measurement at the widest part of the egg.

4.2.2.3. Chick Measurements

Progeny were weighed and body measurements taken five- and ten-days post-hatch, using an electronic scale and calipers. Tarsus, wing, and middle toe lengths were measured as described in Aldrich and James (1991) (see Chapter III). DNA sexing of the chicks was conducted at the University of British Columbia (Fronteddu, 2001) using a different set of primers (Griffiths et al. 1998) than the sexing of the adults. These new primers produced more consistent and observable results.

4.2.2.4. Chick Mortality and Tissue Weights at Sacrifice

Age of chick death or disappearance as well as cause of death, when known, were noted. At the end of the study (August, 2000), the birds were sacrificed by decapitation and tissues immediately dissected out and weighed. Tarsus, toe, and wing measurements were also recorded. Tissues collected included: heart, brain, kidneys, liver, spleen, thyroid glands, bursa, thymus, oviduct, and gonads. Tissue weights were adjusted for body weight by dividing the tissue weight from the total body weight minus the tissue weight.

4.2.3. Thyroid Hormones

Ten pairs were bled every two weeks during the 1999 breeding season (12 testing periods, February to July). All blood samples were collected from the jugular vein using a 27 gauge heparinized needle and 1 cc syringe. Approximately one millilitre of whole blood was collected from each bird, centrifuged at 3300 rpm for five minutes, and the plasma separated from the blood cells. Total plasma triiodothyronine and thyroxine concentrations were analyzed

by Tracy Marchant at the University of Saskatchewan. Analyses were conducted using unextracted serum and a radioimmuno assay following protocols outlined by Chopra (1972).

4.2.4. Behavioral Observations

A sub-sample of twelve pairs, encompassing both same and different type pairs with various degrees of contamination, were observed for thirty minutes a day (per pair), three times a week, from early March until early August (1998 and 1999) to examine aggressive, reproductive, vocalization, and maintenance behaviors, throughout the breeding season. In 1998 six of the observed pairs were in the indoor pens and six in the outdoor pie-shaped pens. All 12 observed pairs were in the outdoor pie-shaped pens in 1999.

4.2.4.1. Reproductive and Parental Care Behaviors

Reproductive behaviors (Howell, 1942; Sallabanks & James, 1999; Young, 1955; Wauer, 1999) included:

collecting – bird picks up and carries nesting material (mud, hay, string, etc.)

building - bird engages in characteristic body movements in the nest bowl or on nest platform, bird usually crouches low, with wings spread, and stamps and back-pedals feet to move nest material around, movements may also be seen when bird is on perch or bathing in water dish, but these were not recorded

mounting - male mounts or attempts to mount female or inanimate object, not known if intromission and/or ejaculation achieved

sitting - female sits on nest incubating eggs or brooding chicks, female sleeping in nest bowl when no chicks or eggs present was not recorded

feeding - bird carries food to nest and places in chick's mouth, may remove food from one chick's mouth and place in another

cleaning - bird removes fecal sacs and other wastes from nest, may be carried away from nest and discarded or eaten

'other' parental care - bird is active at nest but observer unable to discern behavior, may include feeding of chicks, cleaning of nest, and moving of eggs or chicks

parental care - all behaviors concerning eggs and/or chicks in the nest, includes feeding, cleaning, sitting, and other behaviors.

Mounting was recorded only for males and sitting was recorded only for females. As males rarely engaged in nest material collecting and nest building, only the females' collecting and building were included here. All the behaviors except mounting were recorded as

proportions (the number of minutes, out of 30, in which the behaviour was observed at least once). Mounting was recorded as frequency per 30-minute observation period.

4.2.4.2. Aggressive Behaviors

Aggressive behaviors were performed by both males and females, and were directed towards the mates, neighbors, chicks, or other birds that may land on or near the pens. Instances of biting and fighting were rare and so were not included in the analyses. All aggressive behaviors were recorded as frequencies. The behaviors are defined (Howell, 1942; Sallabanks & James, 1999; Young, 1955; Wauer, 1999) as:

charging - bird rushes towards a conspecific, may be flying or running

chasing - one bird chases its mate, may be running or flying

snapping - sound made by quickly bringing mandibles together, considered a threat display

gaping - bird holds its mouth open while facing other bird, may be considered a threat display, or signify intention to bite

overall aggression - frequency of all aggressive behaviors performed by a bird, sum of charging, chasing, snapping, and gaping

4.2.4.3. Vocalizations

Vocalizations were defined (Howell, 1942; Sallabanks & James, 1999; Wauer, 1999) as:

chirping - usually only one note, significance not known, used in a variety of contexts

chukking - series of notes similar to "clucking" of chickens, also described as "cuck", often slower and softer version of laughing, significance not known

laughing - series of often loud and fast notes; described as "ha-ha-hi-hi-hi-ha-ha", often used following aggressive behavior but may be used in other contexts, has been described as being associated with sociability and a sense of well being

singing - characteristic song or parts thereof, often described as "cheerily-cheer up", may be used for mate attraction and/or territorial display

singing proportion - the proportion of minutes during the observation that the birds engaged in at least one singing bout, out of 30 minutes

'other' vocalizations - any other vocalizations that do not fit into the previous categories, may include song-like vocalizations performed by females and combinations of other categories of vocalizations

overall vocalizations - sum of chirping, chukking, laughing, and 'other' vocalizations

Vocalizations, other than singing proportion, were recorded as frequencies. Both males and females engaged in chirping, chukking, laughing, and other vocalizations. Although some females were witnessed singing, it was relatively rare, so singing and singing proportion were only recorded for males.

4.2.4.4. Maintenance Behaviors

Maintenance behaviors included those activities related to daily routines and were not directly associated with reproduction or aggression. Both males and females performed all of these behaviors. With the exception of eating, drinking, and flying, which were recorded as frequencies, these behaviors were recorded as proportions. These behaviors were defined (Sallabanks & James, 1999; Wauer, 1999) as:

bathing - bird sits or stands in water dish and proceeds to dunk and splash to move water over its feathers

pecking - bird pecks at various objects in the pen (weeds, cedar branches, insects, etc.), bird may be eating things it pecks at

preening - bird uses bill to clean and smooth feathers

eating - bird picks up and swallows cat food or mealworm from feeder or other dishes

drinking - bird drinks from water or mud dish

flying - bird flies from one position (perch, feeder, ground, etc.) to another

flying proportion- the proportion of time spent flying, out of 30 minutes

4.2.5. Statistics

All statistical analyses were conducted using JMP version 3.2.1 (SAS Institute, Cary, North Carolina) software. P values less than or equal to 0.05 were considered significant. Values presented represent means $\pm$ standard errors, and ranges. Data were common log transformed to increase normality where necessary. Four females were studied in both breeding seasons, but as they were mated with different males and housed in different pens in the two years, they were treated as different samples. Outliers were determined using Mahalanobis outlier tests. Nominal logistic (χ^2) tests were used to determine differences in whether or not a female built a nest, laid eggs, hatched chicks, fledged chicks, incubated eggs, or dumped eggs. Nestling periods, incubation periods, number of eggs per clutch, and number of clutches were tested using standard least squares analyses of variance (ANOVA). Analyses were conducted using the model:

$$Y_{ijklm} = \mu + M_k + F_l + (MF)_{kl} + E_{ijklm.}$$

The dependent variable being measured is represented by Y, M is male type (Lower Mainland vs. Okanagan), F is female type (Lower Mainland vs. Okanagan), MF is the two-way interaction between male type and female type, and E is the error term. Non-significant variables were removed from the model and the data were re-analyzed. The number of eggs laid per female, egg weights and measurements, the numbers of chicks hatched and fledged, chick weights and measurements, and chick sex ratios were also analyzed using the same standard least squares ANOVA. Means per female per year were used to analyze egg and chick measurements in order to avoid pseudo-replication. Female type and age at chick death were analyzed using χ^2 tests. Pair-wise correlations were conducted to determine the effects of the Okanagan mothers' *in ovo* p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, p,p'-DDE, and o,p'-DDE (see Chapter III and Appendix I for egg contaminant levels) exposure on the morphological and physiological measurements of their eggs and chicks. Contaminant non-detects and zeros were replaced by 0.00005 (half the detection limit) for the correlations. Age and cause of death, as well as the presence of bruises and wounds were analyzed using nominal logistics. Body measurements and tissue weights at time of sacrifice were also analyzed using the same ANOVA model as above. Here data for individual birds were used, as at the time of sacrifice all of the birds hatched at San Rafael had been full-grown and independent for at least a year. Differences between Lower Mainland and Okanagan birds in thyroid hormone levels were analyzed using one-way ANOVAs and log-transformed data, as were sex differences.

Behavior data were common log transformed to increase normality and differences between Lower Mainland and Okanagan birds were analyzed using one-way ANOVAs. Pair-wise correlations were conducted to determine the effects of *in ovo* p,p'-DDT, p,p'-DDD, p,p'-DDE, o,p'-DDT, o,p'-DDD, and o,p'-DDE (Chapter III and Appendix I) exposure on the behaviors of Okanagan birds. The untransformed data were used for the correlations. Although not the focus of this study, other factors were also investigated. The effects of year (1998 vs. 1999), sex (male vs. female), pen type (indoor vs. outdoor), and mate type (same vs. different) on behaviour were analyzed using one-way ANOVAs.

4.3. Results

4.3.1. Egg Contaminants

See Chapter III for details on the differences between Lower Mainland and Okanagan eggs in terms of contaminant levels.

4.3.2. Reproduction

4.3.2.1. Nesting Success

In 1998, the first egg was laid on April 7, and the last chick was fledged on July 24, thus defining the nesting period. In 1999 the nesting period was from April 10 to August 5. Usually one egg was laid per day during the laying period, however several females skipped a day between eggs in a clutch, and occasionally more than one day would be skipped (Young, 1955). Incubation periods ranged from 8 to 14 days (median = 13 days). Chicks within a brood took 1 to 3 days to hatch. Nestling periods ranged from 13 to 17 days (median = 15 days), with all chicks leaving the nest within a day or two of each other and usually on the same day. Neither incubation period nor nestling period was significantly influenced by the male type or female type.

Overall, 21 of 67 (31.3%) pairs nested during the two-year study. Thirty-three (49.3%) pairs laid at least one egg, 15 (22.4%) hatched at least one egg, and 11 (16.4%) fledged one or more chicks. Table 4-1 illustrates the numbers of Lower Mainland and Okanagan females that successfully nested, laid eggs, hatched eggs, and fledged young. Okanagan females were no more or less likely to build a nest, lay eggs, hatch chicks, or fledge chicks than Lower Mainland females. Lower Mainland males, however, fledged proportionally more chicks than Okanagan males ($\chi^2 = 4.9$, $p = 0.03$). In total, 19 Lower Mainland females laid 150 eggs and 14 Okanagan females laid 62 eggs. Of the females that laid eggs, Lower Mainland females laid significantly ($F_{1,31} = 5.1$, $p = 0.03$) more eggs (7.9 ± 1.1) than their Okanagan (4.4 ± 0.8) counterparts. However, one Lower Mainland female laid 16 eggs in both years and removal of this bird from the analyses resulted in non-significance. Of the eggs laid by Lower Mainland females, 53 (35.3%) hatched, 53 (35.3%) broke, 19 (12.7%) were missing or their fate was unknown, and 25 (16.7%) were unhatched. Okanagan females had 28 (45.2%) eggs that hatched, 24 (38.7%) broke, 5 (8.1%) had unknown fates, and 5 (8.1%) were unhatched. Seven of the 11 (63.6%) Lower Mainland females with nests produced at least one fledgling, whereas 4/10 (40.0%) of the Okanagan females with nests were successful. Of the 20 females that laid eggs in a nest, nine (45.0%) laid one clutch, five (25.0%) laid two clutches, three (15.0%) laid three clutches, and three (15.0%) laid four clutches. There were no significant male type effects on the number of clutches laid by a female, nor on the number of eggs laid per clutch. Lower Mainland females laid significantly ($F_{1,28} = 4.3$, $p = 0.05$) more clutches (2.3 ± 0.3) than Okanagan females (1.6 ± 0.2). However, the removal of one highly productive female from the analysis rendered the

Table 4-1: Numbers of Lower Mainland and Okanagan American robin females nesting, laying eggs, hatching eggs, and fledging young in 1998 and 1999.

	Nested	Laid	Hatched	Fledged
1998:				
Lower Mainland	5/19 26.4%	11/19 57.9%	3/5 60.0%	2/5 40.0%
Okanagan	6/19 31.4%	9/19 47.4%	4/6 66.7%	1/6 16.7%
1999:				
Lower Mainland	6/15 40.0%	8/15 53.3%	5/5 100%	5/5 100%
Okanagan	4/14 28.6%	5/14 35.7%	3/4 75.0%	3/4 75.0%
Total:				
Lower Mainland	11/34 32.4%	19/34 55.9%	8/11 72.7%	7/11 63.6%
Okanagan	10/33 30.3%	14/33 42.4%	7/10 70.0%	4/10 40.0%

result non-significant. Forty clutches were laid in total by these birds: 12 (30.0%) were of three eggs, 27 (67.5%) were of four eggs, and one (2.5%) was of five eggs. There were no differences between Lower Mainland and Okanagan females in the number of eggs laid per clutch. Clutches were not determined when eggs were found outside of a nest. These eggs may have been laid outside of the nest or removed from the nest by the birds or predators.

Over the two seasons, 81 chicks were hatched by 15 females (Okanagan = 7, Lower Mainland = 8). Lower Mainland females that laid eggs hatched a mean of 2.7 $\pm$ 0.9 chicks, and fledged 0.9 $\pm$ 0.3 of them. Okanagan females hatched a mean of 2.1 $\pm$ 0.7 chicks and fledged a mean of 0.9 $\pm$ 0.4 chicks. These differences were not statistically significant.

4.3.2.2. Egg Measurements

Weights and measurements were obtained for 102 eggs, laid by 14 Lower Mainland females and 11 Okanagan females. Eggs laid by Lower Mainland females averaged 7.0 g ($\pm$ 0.07, range = 5.5 – 8.5 g) in weight, 28.8 mm ($\pm$ 0.2 mm, range = 25.1 – 33.5 mm) in length, and 21.2 mm ($\pm$ 0.2 mm, range = 19.1 – 30.2 mm) in width. Eggs from Okanagan females averaged 6.5 g ($\pm$ 0.08, range = 5.8 – 7.3 g) in weight, 28.96 ($\pm$ 0.2, range = 27.1 – 30.9 mm) in length, and 20.3 mm ($\pm$ 0.1, range = 19.1 – 21.5 mm) in width. Lower Mainland females laid significantly heavier ($F_{1,23}$ = 7.6, p = 0.01) and wider ($F_{1,23}$ = 12.1, p = 0.002) eggs than Okanagan females. Egg weight, length, and width were not significantly influenced by male type nor were there significant male by female interactions. Mean egg weight was positively correlated with the Okanagan females' *in ovo* p,p'-DDT (r = 0.7, n = 9, p = 0.03; Figure 4-1) exposure, egg length was positively correlated with p,p'-DDD (r = 0.7, n = 9, p = 0.03), and p,p'-DDE (r = 0.9, n = 9, p = 0.0009) levels., and egg width was positively correlated with p,p'-DDT (r = 0.7, n = 9, p = 0.04) and o,p'-DDT (r = 0.8, n = 9, p = 0.02) burdens.

4.3.2.3. Chick Measurements

Weights and morphometric measurements were obtained for the offspring of eight Lower Mainland and six Okanagan females. Fifty-eight chicks were measured at five days of age and 40 chicks at ten days of age. The means ($\pm$ se) and ranges are summarized in Table 4-2.

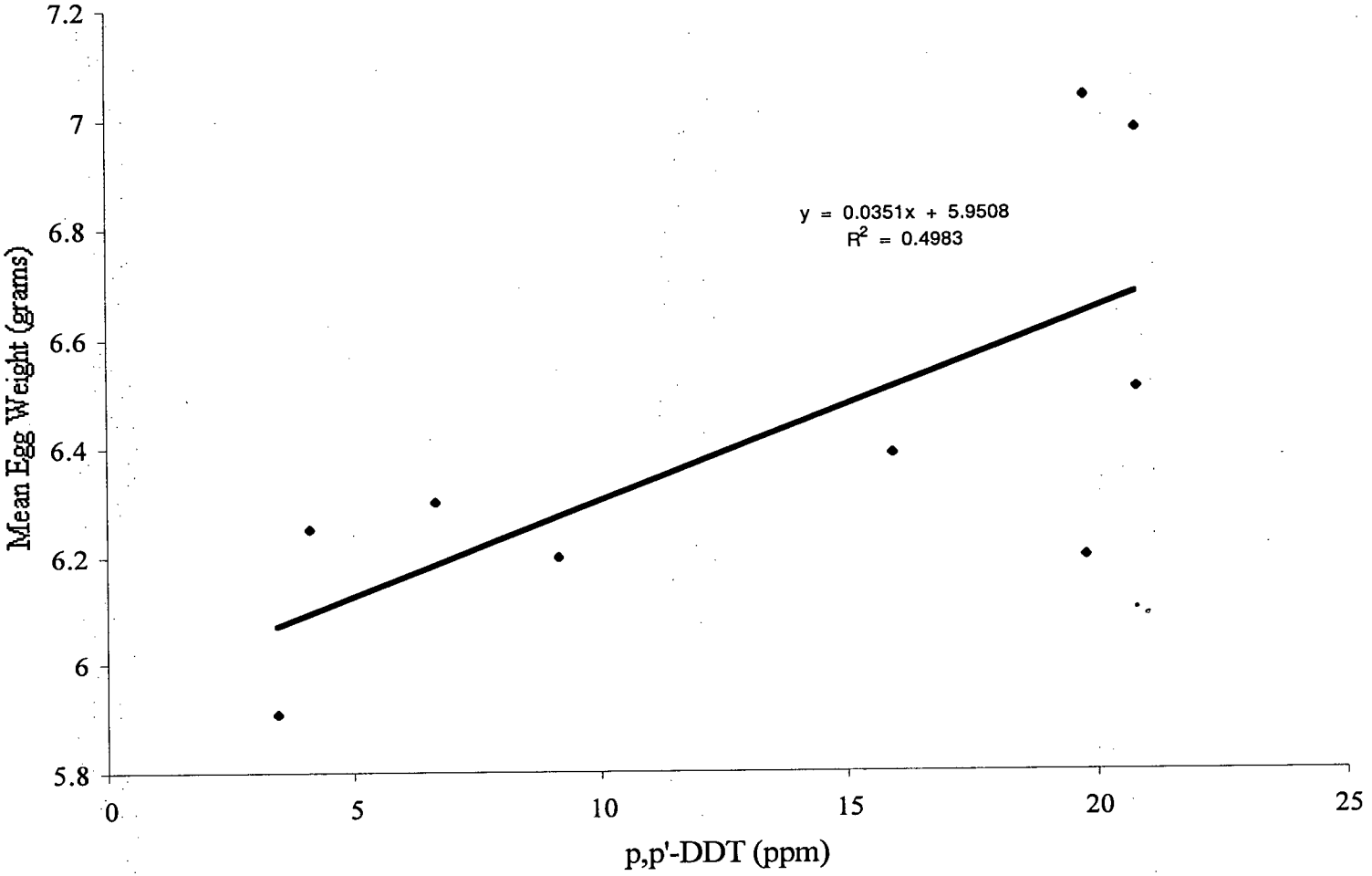

Figure 4-1: Relationship between female Okanagan American robin *in ovo* p,p'-DDT (μg/g) exposure and the mean weights of their eggs.

Table 4-2: Means (± se) and ranges of body weights and tarsus, wing, and toe lengths of the offspring of Lower Mainland and Okanagan American robin females when five and ten days old.

	Lower Mainland	Okanagan
5 day weight (g)	n = 37 23.5 (± 1.3) 10.0 - 37.3	n = 19 28.3 (± 1.9) 14.2 - 39.1
5 day tarsus (mm) *	n = 39 20.7 (± 0.7) 12.0 - 35.0	n= 19 23.3 (± 0.9) 16.0 - 30.0
5 day wing (mm) *	n = 39 21.3 (± 0.1) 11.0 - 32.0	n = 19 25.2 (± 0.2) 12.0 - 38.0
5 day toe (mm)	n = 39 15.3 (± 0.05) 9.0 - 22.0	n=19 15.5 (± 0.06) 10.0 - 19.0
10 day weight (g)	n = 23 48.2 (± 2.4) 25.6 - 69.0	n = 16 46.8 (± 3.4) 14.9 - 61.7
10 day tarsus (mm)	n = 24 33.9 (± 0.9) 20.0 - 39.0	n = 16 33.9 (± 0.9) 25.0 - 38.0
10 day wing (mm)	n = 24 54.2 (± 0.2) 27.0 - 68.0	n = 16 55.3 (± 0.3) 30.0 - 73.0
10 day toe (mm)	n = 24 19.8 (± 0.04) 15.0 - 23.0	n = 16 19.2 (± 0.04) 16.0 - 21.0

*$p < 0.05$

Okanagan females had chicks with longer tarsus ($F_{1,12} = 5.9$, $p = 0.03$) and wing ($F_{1,12} = 5.6$, $p = 0.04$) measurements at five days of age than those of Lower Mainland females. Parent type did not significantly influence the chicks' ten-day measurements.

Sex was determined for 69 of the 81 chicks hatched. In total, Lower Mainland females hatched 21 (44.7%) male chicks and 26 (55.3%) female chicks. Okanagan females had 14 (63.6%) male chicks and 8 (36.4%) female chicks. The ratio of male to female chicks was not significantly influenced by parent type.

4.3.2.4. Chick Mortality and Tissue Weights at Sacrifice

All eight of the Lower Mainland females that hatched chicks had at least one chick die or disappear within the first two weeks post-hatch, whereas only four of the seven Okanagan females (57.1%) had chicks die or disappear during this nestling stage. Thirty-seven (71.2%) chicks hatched to Lower Mainland females died during the nestling stage, 2 (3.9%) during the fledging stage, 1 (1.9%) during the juvenile stage, and 12 (23.1%) as adults (including the 11 that were sacrificed at the end of the study). Okanagan females had 15 (51.7%) chicks die as nestlings, 4 (13.8%) as fledglings, 1 (3.5%) as a juvenile, and 9 (31.0%) as adults (sacrificed at the end of the study). Chi-square tests revealed that female type influenced the number of chicks dying during the nestling stage, with more Lower Mainland females having chicks die at this early age than Okanagan females ($\chi^2 = 5.5$, $p = 0.02$). Cause of death was unknown for most of the chicks, and therefore, was not statistically analyzed. The chicks that survived to the end of the study in August, 2000 were sacrificed. Chicks that disappeared were presumed depredated, most likely by rats. A Lower Mainland male was witnessed shaking and biting two of his offspring. Six chicks were discovered cold in their nests, suggesting abandonment, but it is not known if they were abandoned prior to or after death. Of those chicks for which cause of death was not determined, 12 had wounds and/or bruising, but it is not known if these were incurred prior to or after death or how they were inflicted. Female type did not significantly influence whether or not a chick demonstrated bruises or wounds.

Of the 20 chicks that were sacrificed at the end of the study, tissue weights were available for 18 of them, six from Lower Mainland x Lower Mainland pairs, seven from Lower Mainland x Okanagan pairs, three from Okanagan x Lower Mainland pairs, and two from Okanagan x Okanagan pairs (male x female). The offspring of Okanagan males had heavier hearts (0.013 ± 0.0006 g) than those fathered by Lower Mainland (0.012 ± 0.0003 g) males ($F_{1,16} = 4.6$, $p = 0.05$), although the removal of an outlier cancels this effect. Significant male by female

interactions were found for body weight ($F_{1,16} = 6.3$, $p = 0.02$) and brain weight ($F_{1,16} = 10.3$, $p = 0.006$). Parents of the same type (Lower Mainland x Lower Mainland, 82.9 $\pm$ 2.8 g; Okanagan x Okanagan, 85.7 $\pm$ 4.2 g) had young with higher body weights at sacrifice than parents of different types (Okanagan x Lower Mainland, 75.5 $\pm$ 0.8 g; Lower Mainland x Okanagan, 79.0 $\pm$ 1.1 g). Parents of different types, however, had young with heavier brains than parents of the same type (Figure 4-2). A significant male by female interaction affected kidney ($F_{1,16} = 6.1$, $p = 0.03$) and liver ($F_{1,16} = 9.1$, $p = 0.009$) weights (Figure 4-2). The offspring of Okanagan parents had heavier kidneys and livers. The removal of an outlier led to non-significant effects on liver weight. Care must be taken when interpreting these results, as the sample size is quite small and includes siblings. The two Okanagan x Okanagan birds, for example, come from the same brood. Sex was also confirmed at this time.

4.3.3. Thyroid Hormone Levels in Breeding Birds

There were no significant differences between the Lower Mainland and Okanagan birds in plasma triiodothyronine or thyroxine levels at any of the ages tested. Triiodothyronine levels in the Okanagan birds, however, were negatively correlated with p,p'-DDT on July 20, 1999 (period 12) ($r = -0.8$, $n = 8$, $p = 0.01$). Thyroxine levels were positively correlated with both o,p'-DDT ($r = 0.7$, $n = 8$, $p = 0.05$) and o,p'-DDD ($r = 0.7$, $n = 8$, $p = 0.05$) on March 30, 1999 (period 4), but negatively correlated with p,p'-DDT ($r = -0.9$, $n = 8$, $p = 0.003$) on April 27, 1999 (period 6). There were significant time effects for triiodothyronine ($F_{11,208} = 33.0$, $p < 0.0001$; Figure 4-3) and thyroxine ($F_{11,219} = 8.1$, $p < 0.0001$; Figure 4-3). Males had higher thyroxine levels than females during testing periods 1 ($F_{1,17} = 52.2$, $p < 0.0001$), 7 ($F_{1,18} = 10.5$, $p = 0.005$), 8 ($F_{1,17} = 9.6$, $p = 0.007$), 9 ($F_{1,17} = 8.1$, $p = 0.01$), 10 ($F_{1,17} = 17.8$, $p = 0.0006$), and 11 ($F_{1,18} = 17.3$, $p = 0.0006$). Females had higher triiodothyronine levels than males during periods 1 ($F_{1,17} = 60.5$, $p < 0.0001$) and 9 ($F_{1,17} = 8.8$, $p = 0.009$).

4.3.4. Behavior

There were few behavioral differences between Lower Mainland and Okanagan birds (Table 4-3). Okanagan birds drank ($F_{1,48} = 6.9$, $p = 0.01$) more frequently than Lower Mainland birds, and Lower Mainland birds engaged in more 'other' parental care behaviors than Okanagan birds ($F_{1.10} = 5.3$, $p = 0.04$). This latter difference, however, can be attributed primarily to one bird. Same type pairs (Okanagan x Okanagan and Lower Mainland x Lower Mainland) exhibited preening behaviours significantly ($F_{1,48} = 4.1$, $p = 0.05$) more frequently (0.2 $\pm$ 0.01)

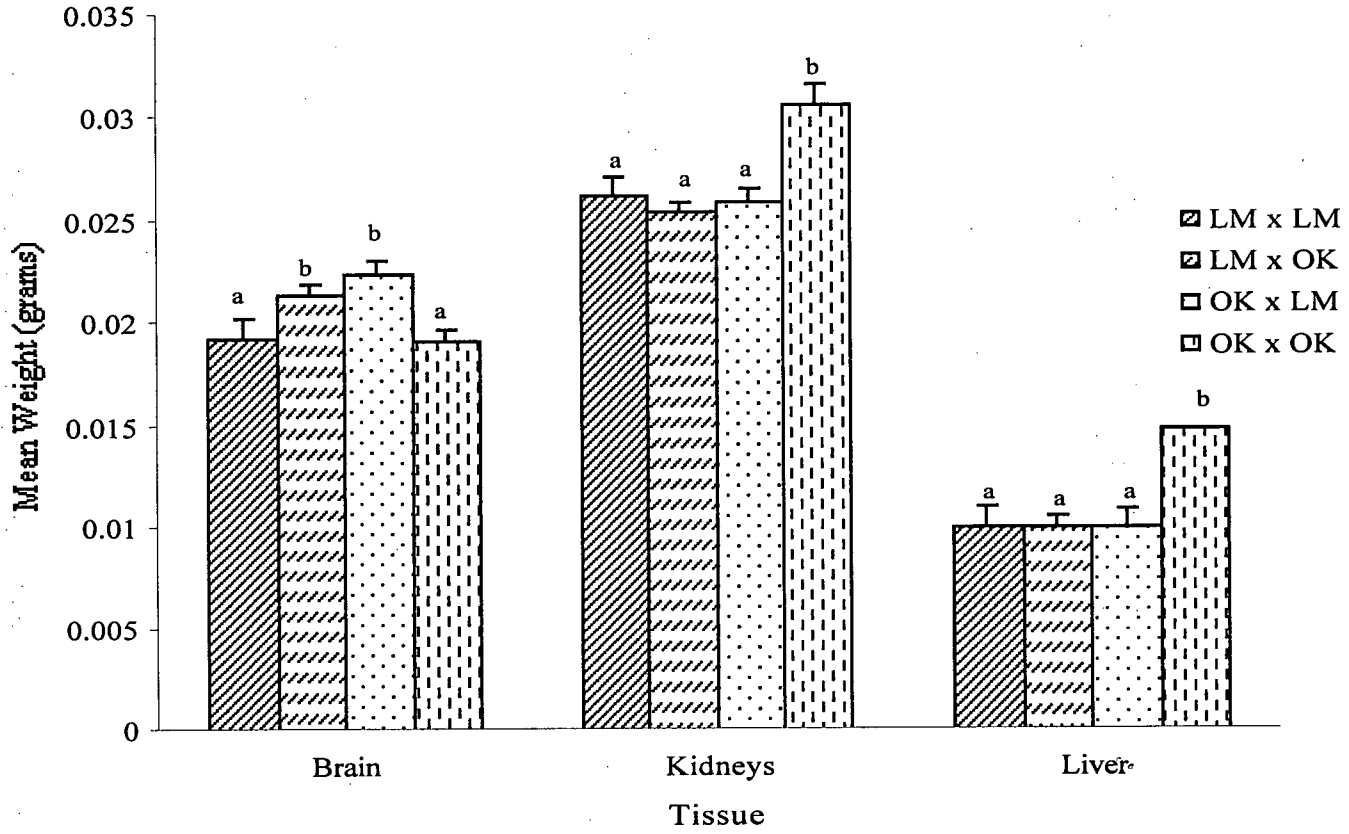

Figure 4-2: Mean (+ se) brain, kidney, and liver weights (grams) for birds hatched at San Rafael to same type and different type parents. LM = Lower Mainland, OK = Okanagan, male x female parents. For each tissue, columns with different letters are significantly different.

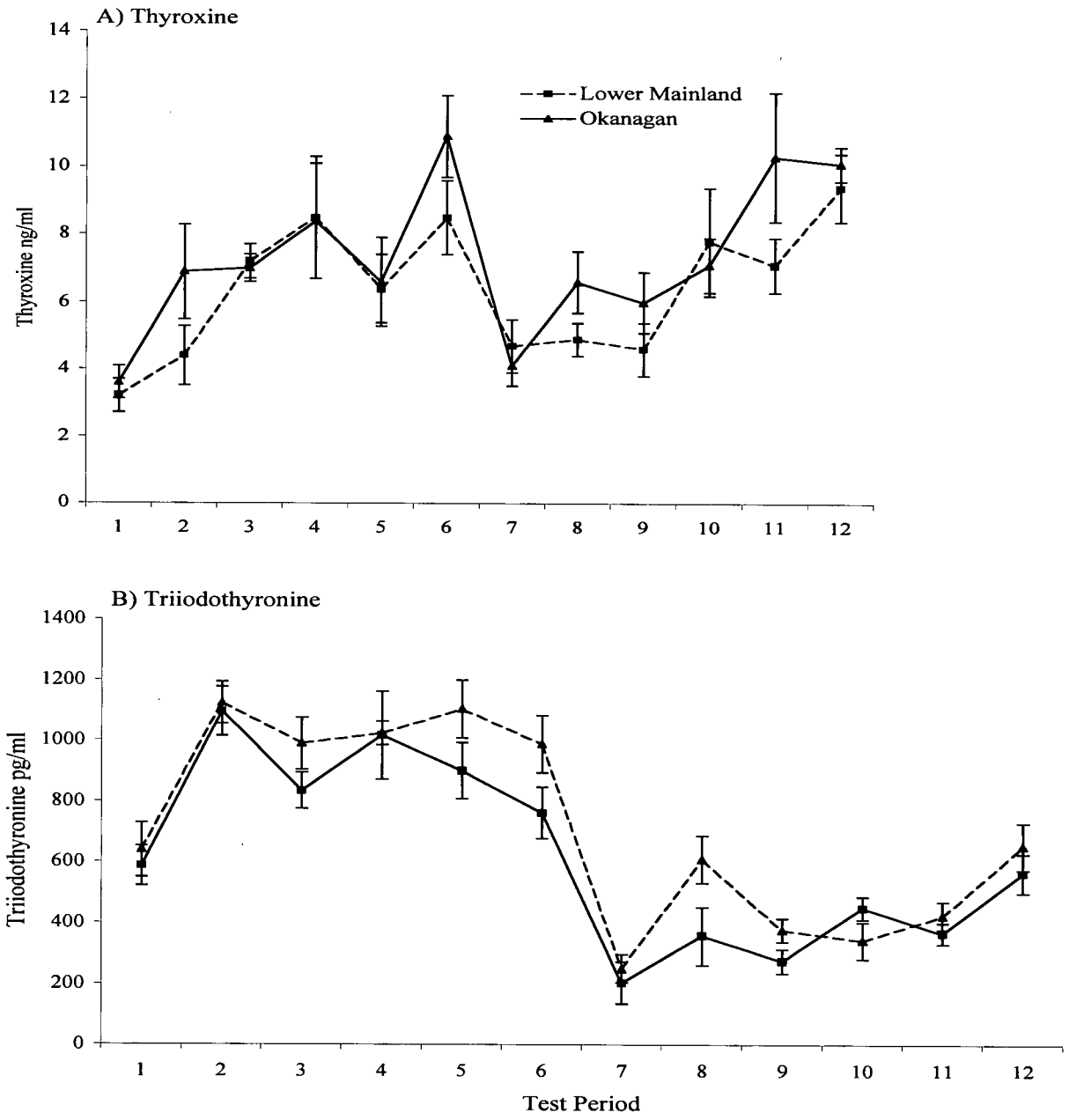

Figure 4-3: Mean (± se) differences in a) thyroxine and b) triiodothyronine levels in Lower Mainland and Okanagan robins at the various time periods tested.

than different type (Okanagan x Lower Mainland, Lower Mainland x Okanagan) pairs (0.2 ± 0.02). Different type pairs, on the other hand, exhibited chukking vocalizations more ($F_{1,48}$ = 3.9, p = 0.05) frequently (3.0 ± 0.7) than same type pairs (1.8 ± 0.3). A number of behaviors were significantly correlated with the birds' *in ovo* DDT exposures; these are listed in Table 4-4. Correlations that became non-significant when outliers were removed are indicated. Significant differences between years, pen types, and the sexes are outlined in Tables 4-5, 4-6, and 4-7.

4.4. Discussion

Although American robins were once raised as pets (Howell, 1942), this study is likely the first report of large-scale successful breeding of these birds in captivity, demonstrating that the American robin can be utilized as a model for toxicology studies. Ortega et al., (1997) reported that even in the wild, American robins are very tolerant of research activities and rarely abandoned their nests after a human disturbance. The onset of breeding, and the timing for incubation and nestling periods for the robins in this study were similar to those reported by other authors (Campbell et al., 1997; Cannings et al., 1987; Howell, 1942; Kemper, 1971; Wauer, 1999; Young, 1955) for wild robins, and there were no major differences between the Lower Mainland and Okanagan birds. The overall success rates of the captive robins in this study (Lower Mainland 63.6%, Okanagan 40.0%) were lower than those reported for free-living wild birds (Cannings et al., 1987; Elliott et al., 1994; Gill et al., 2003). However, the birds in this study were first time breeders in 1998 and their performance was expected to be substandard (Emlen, 1984; Lawton & Guidon, 1981). In 1999, when the birds went through their second breeding season, hatching and fledging rates (Lower Mainland 100%, Okanagan 75.0%) were greatly improved and were comparable to the performance of wild birds. Chick feeding, nest cleaning, and overall parental care were performed significantly more in 1999 than in 1998.

Table 4-3: Means (± se) and ranges of behaviors performed by Lower Mainland and Okanagan robins.

	Lower Mainland n = 26	Okanagan n = 24
Total parental care	0.07 (± 0.04) 0 – 0.8	0.01 (± 0.02) 0 – 0.4
Collect (nest material) (proportion)	0.03 (± 0.006) 0 – 0.1	0.04 (± 0.01) 0 – 0.3
Building (nest) (proportion)	0.03 (± 0.007) 0 – 0.1	0.04 (± 0.01) 0 – 0.3
Mounting (frequency)	0.03 (± 0.01) 0 – 0.3	0.1 (± 0.06) 0 – 1.4
Sitting (on nest) (proportion)	0.08 (± 0.03) 0 – 0.5	0.02 (± 0.01) 0 – 0.3
Feeding (chicks) (proportion)	0.06 (± 0.04) 0 – 0.8	0.02 (± 0.02) 0 - 0.4
Cleaning (nest) (proportion)	0.002 (± 0.0008) 0 – 0.02	0.001 (± 0.0007) 0 – 0.02
Other parental care * (proportion)	0.02 (± 0.004) 0 – 0.07	0.001 (± 0.001) 0 – 0.03
Aggression (frequency)	3.0 (± 0.5) 0.6 – 10.5	2.9 (± 0.5) 0.3 – 7.1
Charging (frequency)	1.9 (± 0.36) 0.2 – 9.0	2.2 (± 0.4) 0.06 – 6.3
Chasing (frequency)	0.3 (± 0.1) 0 – 3.2	0.3 (± 0.08) 0 – 1.2
Snapping (frequency)	0.3 (± 0.1) 0 – 2.2	0.2 (± 0.04) 0 – 0.8
Gaping (frequency)	0.5 (± 0.1) 0 – 3.4	0.3 (± 0.04) 0 – 0.7
Overall vocalizations (frequency)	10.8 (± 1.5) 2.7 – 35.1	8.9 (± 1.3) 1.3 – 23.4
Chirping (frequency)	4.5 (± 0.8) 0.6 – 17.2	3.1 (± 0.5) 0.4 – 9.9
Chukking (frequency)	2.7 (± 0.5) 0.2 – 10.6	2.0 (± 0.6) 0.3 – 13.5
Laughing (frequency)	2.4 (± 0.5) 0.02 – 9.5	2.6 (± 0.5) 0.05 – 10.9

Singing (frequency)	11.4 (± 3.2) 0 – 59.0	20.7 (± 6.1) 0 – 108.8
Singing (proportion)	0.1 (± 0.02) 0 – 0.3	0.1 (± 0.03) 0 – 0.5
Other vocalizations	1.2 (± 0.2) 0.3 – 4.9	1.3 (± 0.3) 0.1 – 8.6
Eating (frequency)	3.0 (± 0.2) 1.3 – 6.3	3.6 (± 0.3) 1.5 – 6.7
Drinking (frequency) *	3.2 (± 0.2) 2.1 – 5.8	4.0 (± 0.2) 2.5 – 6.1
Bathing (proportion)	0.004 (± 0.0007) 0 – 0.01	0.005 (± 0.001) 0 – 0.02
Preening (proportion)	0.2 (± 0.01) 0.09 – 0.4	0.2 (± 0.02) 0.08 – 0.4
Pecking (proportion)	0.2 (± 0.01) 0.1 – 0.4	0.2 (± 0.01) 0.1 – 0.4
Flying (frequency)	50.4 (± 5.9) 17.5 – 166.1	44.4 (± 4.8) 2.4 – 85.6
Flying (proportion)	0.5 (± 0.02) 0.3 – 0.7	0.4 (± 0.03) 0.2 – 0.7

*$p < 0.05$

Table 4-4: Significant correlations between behavior and egg DDT levels in Okanagan robins.

Behavior[a]	Contaminant	r^2	p
Building (nest)	p,p'-DDE	0.25	0.03[b]
Collecting (nest material)	p,p'-DDT	0.25	0.04[b]
	p,p'-DDE	0.25	0.02[b]
Snapping	p,p'-DDE	0.49	0.002
Overall vocalizations	p,p'-DDD	0.25	0.03
Laughing	p,p'-DDT	- 0.26	0.02
Other vocalizations	p,p'-DDE	0.64	0.0002[b]
	o,p'-DDT	0.25	0.04
	o,p'-DDD	0.36	0.004
	o,p'-DDE	0.49	0.002
Eating	p,p'-DDE	0.25	0.03
Flying (frequency)	p,p'-DDT	0.36	0.01
	p,p'-DDE	0.25	0.05
fly (proportion)	p,p'-DDD	0.25	0.04[b]
	p,p'-DDE	0.49	0.002
	p,p'-DDT	0.25	0.02

[a] Sample size = 18 birds; [b] removal of outliers renders correlation non-significant

Table 4-5: Significant effects of sex on behaviour in Lower Mainland and Okanagan robins.

Behavior	Female Mean ± se	*Male* Mean ± se	F	p
charging	1.51 (0.40)	2.57 (0.35)	9.3	0.004
chasing	0.084 (0.022)	0.51 (0.13)	25.5	< 0.0001
aggression	2.08 (0.44)	3.85 (0.46)	13.5	0.0006
laugh	1.34 (0.29)	3.65 (0.55)	12.4	0.0009
overall vocalizations	7.97 (1.18)	11.85 (1.53)	5.6	0.02
preen	0.18 (0.013)	0.21 (0.016)	4.2	0.05
other parental care	0.011 (0.0038)	0.00039 (0.00022)	24.5	0.0006
overall parental care	0.088 (0.044)	0.0045 (0.0020)	12.1	0.004

Table 4-6: Significant effects of year on behaviour in Lower Mainland and Okanagan robins.

Behavior	1998 Mean ± se	1999 Mean ± se	F	p
snap	0.44 (0.11)	0.055 (0.012)	22.9	< 0.0001
laugh	1.57 (0.11)	3.36 (0.45)	14.6	0.0004
sing	18.39 (5.60)	13.55 (4.01)	5.6	0.02
sing (proportion)	0.13 (0.033)	0.10 (0.024)	6.3	0.02
eat	3.99 (0.27)	2.63 (0.19)	16.7	0.0002
preen	0.23 (0.017)	0.16 (0.0079)	17.4	0.0001
peck	0.29 (0.013)	0.20 (0.0084)	32.3	< 0.0001
fly (proportion)	0.50 (0.029)	0.41 (0.018)	4.6	0.04
feed (chicks)	0.0029 (0.0012)	0.072 (0.040)	15.2	0.003
clean (nest)	0.00078 (0.00037)	0.0021 (0.00097)	7.5	0.03
overall parental care	0.012 (0.0046)	0.0778 (0.043)	7.9	0.01

Table 4-7: Significant effects of pen-type on behaviour in Lower Mainland and Okanagan robins.

Behavior	Indoor Mean ± se	Outdoor Mean ± se	F	p
snap	0.63 (0.19)	0.11 (0.024)	16.6	0.0002
gape	0.87 (0.28)	0.24 (0.031)	11.2	0.002
aggression	4.88 (0.88)	2.36 (0.29)	9.5	0.003
laugh	0.94 (0.25)	2.99 (0.42)	8.7	0.005
other vocalizations	2.04 (0.69)	0.99 (0.11)	5.4	0.02
eat	4.02 (0.36)	3.04 (0.21)	5.6	0.02
preen	0.28 (0.025)	0.17 (0.0065)	30.3	< 0.0001
peck	0.32 (0.019)	0.22 (0.0083)	23.5	< 0.0001
fly (proportion)	0.55 (0.043)	0.42 (0.017)	8.0	0.007

While there is abundant literature on the effects of immediate DDT exposure on reproduction in birds (Blus et al., 1997; Bryan et al., 1989; Cecil et al., 1971; Chang & Stokstad, 1975; Fox & Donald, 1980; Fry, 1995; Gish & Chura, 1970; Jefferies, 1971, 1975; Lillie et al., 1972), there is less known about the effects of *in ovo* exposure or the long-term effects carried to the next generation. Gildersleeve et al. (1985) found that *in ovo* exposure to diethylstilbestrol caused Japanese quail (*Coturnix japonica*) females to have significantly reduced oviduct weights and decreased egg production. The reproductive behaviors and social-dominance behaviors of males were also markedly attenuated. Williams (1999) investigated parental and first-generation effects of exogenous estrogens (17β-estradiol) on female reproduction in zebra finches (*Taeniopygia guttata)* and found that the mean egg mass of daughters of estradiol-treated females was larger than that of control offspring. There were no treatment effects on offspring clutch size or laying interval. Berg et al. (2001) also found *in ovo* exposure of Japanese quail to ethynylestradiol (a synthetic estrogen) resulted in various oviduct malformations. They concluded that these oviduct abnormalities could be used as biomarkers of xenoestrogen exposure in wild bird populations. The o,p' isomers of DDT and its metabolites are known to bind directly to estrogen receptors (Robinson et al., 1984) and demonstrate estrogen-like activities (Kupfer & Bulger, 1980; Gaido et al., 1997). Male Californinia gulls exposed *in ovo* to 2 mg/kg o,p'-DDT had feminized gonads, while 5 mg/kg or higher doses resulted in the development of both left and right oviducts in female gulls (Fry & Toone, 1981). This is similar to Berg et al.'s (2001) finding with *in ovo* exposure of quail to ethynylestradiol. In the robins, egg weight and size were correlated with the *in ovo* DDT levels of the mothers. These results support Williams' (1999) findings that egg mass for daughters of estradiol-treated zebra finches was larger than controls. However, the Okanagan robins here were not only exposed *in ovo* to DDT, but they were likely the offspring of birds that were also exposed to DDT *in ovo*. Despite the positive correlations between egg size and *in ovo* DDT levels, the uncontaminated Lower Mainland females laid significantly larger eggs and more eggs than the Okanagan females. Genetic differences between the two types of females likely played a significant role in egg size (Styrsky et al., 2002), and there can be a great deal of individual variation (Styrsky et al., 2002; Williams, 1999). These factors may have confounded the effects of DDT exposure.

It was found that the post-breeding gonad weights of Okanagan robins were negatively correlated with *in ovo* p,p'-DDE levels while oviduct weight was positively correlated with *in ovo* o,p'-DDT levels (see Chapter III). There was also more variation in gonad weights in birds

from low level exposures compared to high level exposures (see Figure 3-6). The majority of studies reported in the literature measured gonad weights when the birds were in full breeding condition, however, Kemper (1971) found that male robin testes weighed from 8.6 to 84.5 mg in August and September and that female ovaries ranged from 10.8 to 36.0 mg. Mean testes weight in this study was 24.7 mg (range 21.4 - 30.8 mg) for Lower Mainland males and 31.1 mg (16.1 - 49.5 mg) for Okanagan males, so they are similar to the birds Kemper studied. Ovary weights in the Lower Mainland females ranged from 9.0 to 20.3 mg (mean 13.2 mg) and 7.7 to 22.8 mg (mean 12.2 mg) in the Okanagan females. Again, these values are within the ranges found in Kemper's birds during the same time period. Unlike previous studies with laboratory birds (e.g., chickens, quail, zebra finches) (Fry & Toone, 1981; Gildersleeve et al., 1985; Berg et al., 2001; Williams, 1999), this study may be confounded by the effects of the females' own *in ovo* exposure and the *in ovo* exposure of the previous generation. Further complication comes with the fact that in many laboratory studies, birds are exposed to a single chemical or isomer while the robins were exposed to a combination of isomers. While this represents a more realistic situation, the interaction of these isomers cannot be teased apart with the given sample size.

Even though the Okanagan females laid smaller eggs than Lower Mainland females, their chicks at 5 days of age were significantly larger (as indicated by tarsus and wing length). This is interesting as hatch weight and egg weight should be highly correlated (Styrsky et al., 2002). This finding is consistent with the finding in Chapter III that Okanagan chicks at this early age were heavier than Lower Mainland chicks of the same age. However, since the Okanagan females in this study were paired with both types of males, the difference in chick size or weight cannot be attributed to genetics but to the parenting ability of the females. This hypothesis is further supported by the finding that chicks hatched by Lower Mainland females (regardless of what type of males they were paired with) suffered significantly higher mortality at this age than chicks hatched by Okanagan females. Aggrey & Cheng (1993) found that body weight in pigeon (*Columba livia*) squabs were mostly affected by the parents' parenting ability during the first two weeks after hatching. The squabs' own genetic potential to grow only expressed itself after the first two weeks of brooding. In American robins, the importance of the females' and the males' parenting roles seem to be at different stages of the rearing period. In the early stage, males were never observed brooding the chicks, although they would occasionally perch on the edge of the nest and stand guard while the female was away and both parents fed the chicks and removed fecal sacs. After fledging, the male may take over care of the young while the female begins to prepare for and lay the next clutch of eggs (Howell, 1942, Sallabanks & James, 1999; Wauer,

1999). Lower Mainland males, regardless of the type of female they were paired with, fledged significantly more (proportionally) chicks than Okanagan males. It is interesting that the role of the male is still important even when food was provided *ad libitum* and territories were set and they did not have to physically fight off intruders. Nevertheless, the co-ordination of the male and female parents was an important factor in affecting the chicks' growth. In this study, same type parents had heavier fledglings than different type parents. One would expect that chicks from different type parents should have an advantage because of hybrid vigour. Same type parents preened more than different type parents whereas different type parents vocalized (chuk) more. Although the significance of these two behaviors is not clear, the differences between parent types suggest that there may also be differences in compatability. This, in turn, suggests that there are differences between the Lower Mainland and Okanagan birds and that the robins can differentiate their own type from the other even when raised together from an early age. Like their parents (see Chapter III), the full-grown robin chicks from Okanagan parents had significantly heavier organs (standardized with body weight) than those from Lower Mainland parents. The Okanagan birds seem to be more compact compared to Lower Mainland birds. Our findings support the notion that the Okanagan and Lower Mainland robins belong to two different sub-species (Aldrich & James, 1991; Cannings, 1998).

Immediate exposure to DDT has been shown to affect behaviours in birds. In Japanese quail, herring gulls, ring doves, and merlins, DDT exposure has been shown to attenuate reproductive and aggressive behaviors and lead to reproductive failure (Fox & Donald, 1980; MaArthur et al., 1983; Fry & Toone, 1981; Fox, 1997; Bryan et al., 1989). In Bengalese finches and gray herons (*Ardea cinerea)*, on the other hand, DDT exposure led to heightened and misdirected aggression (Jefferies, 1971; Ohlendorf et al., 1978) which also led to reproductive failure. In the robins, *in ovo* DDT levels were correlated with several reproductive and related behaviours, but the results were compromised by the small sample size. As noted, many of the correlations became non-significant when outliers were removed from the analyses. These "outliers", however, were important observations. In most cases, they were individuals exposed to the highest levels of *in ovo* DDT contamination, and they expressed exaggerated behaviour. Female OK97-21C2, for example, engaged in abnormally high levels of nest material collecting and nest building but failed to complete a nest or lay any eggs. Her mate, male OK97-30C3, another highly contaminated outlier, was observed mounting his mate in abnormally high frequency. While it would be difficult to draw conclusions based on one or two individuals, it would be reasonable to propose the hypothesis that *in ovo* DDT exposure adversely affects only

highly contaminated birds, and there may be a threshold level below which American robin behaviour is not affected.

Plasma thyroid hormone levels in the Okanagan and Lower Mainland breeding birds were not significantly different during development, nor were there significant correlations with *in ovo* DDT levels (Chapter III). During the breeding season, there were also no significant differences except in period 7, when Lower Mainland birds had higher plasma triiodothyronine levels than Okanagan birds. While plasma triiodothyronine levels were negatively correlated with *in ovo* p,p'-DDT during the post-breeding period (period 12), and thyroxine levels were positively correlated with p,p'-DDT and o,p'-DDD during period 4, few conclusions can be drawn from these data. In this study, thyroxine levels peaked in late April (period 6). As a number of pairs laid eggs in early May, this peak in thyroxine may be related to increases in nest building and mating and the increased energy demands of these behaviors. It is important to note that robins often raise more than one brood in a season (Howell, 1942; Kemper, 1971; Sallabanks & James, 1999), and their gonadal hormones, and presumably thyroid hormones, would be expected to rise and fall accordingly with each brood. Thyroid hormone levels are also linked to molting activity, with maximum thyroid activity preceding or coincident with the onset of molt (Wentworth & Ringer, 1986). By early July, many birds have fledged their last brood of chicks and are preparing to molt and then migrate (Campbell et al., 1997; Kemper, 1971; Sallabanks & James, 1999; Wauer, 1999). The birds in this study did indeed exhibit a peak in thyroxine levels in July (period 11) which is when they would begin molting (Campbell et al., 1997; Howell, 1942; Kemper, 1971; Sallabanks & James, 1999).

4.5. Conclusions

Except for a couple of highly contaminated individuals, birds exposed *in ovo* and early post-hatch to DDT exposure did not exhibit detriments in their reproductive success. They demonstrated no differences in the timing of their reproductive behaviors nor in their hatching and fledging success, as compared to birds from relatively uncontaminated areas. Egg weight, length and width were positively correlated with the females' *in ovo* DDT contamination, but it is not certain whether this is an *in ovo* exposure effect or effects carried over from the previous generation. The results also suggest that the Okanagan and Lower Mainland birds were genetically different and may belong to different sub-species. Some highly contaminated birds demonstrated hyper-activity and led to the hypothesis that *in ovo* exposure to DDT adversely affects only highly contaminated birds, and there may be a threshold level below which

American robin behavior is not affected. It is also possible that after exposure to DDT for several decades, robins have developed a tolerance to it (Gill et al., 2003) or at least an ability to compensate for any detrimental effects.

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Chapter V

Infectious Disease and Immune Response in American Robins Exposed *In Ovo* and Early Post-Hatch to DDT

5.1 Introduction

The Okanagan Valley of British Columbia is an important fruit-growing region. Orchards in this area were heavily treated with the insecticide dichlorodiphenyl-trichloroethane (DDT) prior to its banning in the early 1970's (Elliott et al., 1994; Gill et al., 2003; Harris et al., 2000). Two to four treatments of 13.5 kg of technical grade DDT[2] per hectare per year were applied (Elliott et al., 1994). This organochlorine has been linked to reductions in survival, reproduction, and immune response, as well as disruptions in normal endocrine functioning, in a variety of avian species (e.g., Banerjee, 1999; Blus, 1996; Carson, 1962; Colborn et al., 1996; Fry & Toone, 1981; Jefferies, 1971; Kelce et al., 1995; Murphy, 1980; Sonnenschein & Soto, 1998; Stickel et al., 1984; WHO, 1989).

Although not used in the Okanagan Valley for approximately 30 years, DDT still persists in the soil where it is consumed and accumulated by earthworms (Harris et al., 2000). These earthworms are a favored prey of the American robin (*Turdus migratorius*), especially during the breeding season (Ehrlich et al., 1988; Sallabanks & James, 1999; Wauer, 1999). The offspring of these birds acquire significant DDT residues not only from eating contaminated earthworms offered by the parents, but also via maternal transfer of DDT into the egg yolk (Brandt et al, 1978; Cecil et al., 1972; Fox et al., 1978; Ohlendorf et al., 1978; Ottinger et al., 2001; Stickel, 1973). Robin eggs in Okanagan orchards have been reported to contain total DDT[3] levels up to 300 mg/kg (Gill et al., 2003).

DDTs have been shown to influence several aspects of the immune system in a variety of species, including birds (See Chapter I). Birds contaminated with DDTs may be more prone to succumb to infectious diseases and parasites (Banerjee et al., 1996). In 1997, ten-day-old robin nestlings from Okanagan orchards, along with controls from the British Columbia Lower Mainland, were collected and raised in captivity as part of a study on long-term effects of early DDT exposure (see Chapters III and IV). When approximately 50 to 57 days of age 13 of the

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[2] Technical grade DDT is composed of 65 to 90% p,p'-DDT, with 10 to 30% o,p'-DDT and a variety of metabolites and other products (Jefferies, 1975; Mellanby, 1992; WHO, 1989).

[3] Total DDT includes both ortho,para' and para, para' isomers of DDT and its two primary metabolites, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD).

chicks collected from the Okanagan died of apparent starvation (wasting syndrome). Coccidiosis (parasites *Eimeria* and *Isospora*) was confirmed in five of these chicks. None of the chicks collected from the Lower Mainland succumbed to this disease, suggesting that there may be differences in disease resistance and immune response between these two groups of birds. These differences may be related to the Okanagan birds' *in ovo* and early post-hatch exposure to DDT and other contaminants (Banerjee, 1999; Colmano & Gross, 1971; Grasman et al., 1996; Repetto & Baliga, 1996). However, while robins from the Lower Mainland had significantly higher heterophil to lymphocyte ratios at ten days of age compared to Okanagan robins of the same age, phytohemagglutinin tests revealed no differences in immunocompetance between the two groups. Thus, the objective of this study was to examine in more detail, and with a larger sample size, whether early DDT exposure suppressed the immune response of robin chicks from the Okanagan Valley. The goals were:

1) To replicate the 1997 experiment to see if Lower Mainland and Okangan robins were different in their response to the phytohemagglutinin skin test and differential white blood cell counts.

2) To determine if there were differences in egg size, chick size, packed red blood cell volumes, thyroid hormone levels, and corticosterone levels in response to stress, between the Lower Mainland and Okanagan chicks. These factors have the potential to influence the immune system and survival and/or serve as indicators of disruptions in normal immune responses (Harvey et al., 1986; Jefferies, 1975; Lechner et al., 2001; Maier & Watkins, 1999; Siegel, 1980; Styrsky et al., 2002).

3) To determine if the levels of DDT and DDT metabolites the chicks were exposed to *in ovo* correlated with differences in immune response and hormone levels. Blood lead levels were also assessed, as Okanagan orchards were historically treated with lead arsenate (L. Wilson, Canadian Wildlife Service, personal communication). Lead has been shown to increase susceptibility to infectious agents and impair both cell-mediated and humoral immunity at doses lower than those required for other toxic effects (Grasman & Scanlon, 1995; Lee et al., 2002).

5.2 Methods

5.2.1 Egg Contaminants

Between June 14 and July 20, 1998, 53 American robin eggs were collected from nests in orchard areas near Naramata, British Columbia in the Okanagan Valley. All eggs were collected during times of no current-use pesticide application (L. Wilson, Canadian Wildlife Service, personal communication). An additional 40 eggs (controls) were collected from park areas in the Lower Mainland; Stanley Park, Vancouver, Tinehead Park, Surrey, University of British Columbia Botanical Gardens, Vancouver, and the grounds surrounding the Canadian Wildlife Service office, Delta between April 15 and June 29, 1998. Twenty-two of the Okanagan eggs were analyzed individually and the Lower Mainland eggs were pooled (one pool of five eggs, two pools of two eggs, and one individual egg). All of the eggs were analyzed at the National Wildlife Research Center (Hull, Quebec) for contaminant levels using the same protocol as those for the eggs collected in 1997 (See Chapter III). Results were expressed in μg/g and on a wet weight basis.

5.2.2 Egg Measurements

A total of 83 eggs (50 Okanagan, 33 Lower Mainland) were weighed and measured, including those used for contaminant analyses. Where measurements were available for more than one egg per nest, nest means were used for the analyses. Egg length refers to the distance from end-to-end, while egg width refers to the measurement at the widest part of the egg (See Chapter III).

5.2.3 Chicks

Seventy-seven ten-day-old American robin nestlings were collected between June 14 and July 20, 1998, from the same Okanagan nests from which eggs were collected. Forty-eight nestlings were collected from the Lower Mainland nests, between May 1 and July 28, 1998. All nestlings were collected during periods of no current-use pesticide treatment (L. Wilson, Canadian Wildlife Service, personal communication). The birds were reared at Monika's Wildlife Shelter (Surrey, British Columbia) under the same conditions as those collected in 1997 (See Chapter III). All birds were euthanized at the end of the study in October 1998. Sex was determined post-mortem based on morphology. All treatment and housing protocols were approved by the University of British Columbia Animal Care Committee (Certificate # A97-0043).

5.2.4 Chick Measurements

Forty-eight Lower Mainland chicks from 25 nests were weighed and measured, along with 77 Okanagan chicks from 31 different nests. Chicks were weighed and body measurements taken approximately ten days post-hatch, following the same protocols as those used for 1997 birds (See Chapter III). When more than one chick was collected from a nest, means per brood were used for the analyses. Body weights and morphometric measurements were not taken at later stages.

5.2.5 Thyroid Hormones

Thyroid hormones (plasma triiodothyronine and thyroxin concentrations) were assessed by Tracy Marchant at the University of Saskatchewan, Saskatoon, Saskatchewan, in plasma samples collected from 14 Lower Mainland (8 broods) and 58 Okanagan (22 broods) birds when they were ten-days-old. The same protocol as that used for the 1997 birds were used (See Chapter III).

5.2.6 Immune Response

5.2.6.1 White Blood Cell Counts

Blood smears were collected from the birds when they were ten-day-old nestlings (47 Lower Mainland chicks from 25 broods, 73 Okanagan chicks from 30 broods) and 50 - 57 day old juveniles (43 Lower Mainland birds from 22 broods, 70 Okanagan birds from 29 broods), using the same protocol as that used for the 1997 birds (See Chapter III).

5.2.6.2 Phytohemagglutinin Skin Test

Forty-one Lower Mainland (21 broods) and 69 Okanagan (31 broods) juveniles of both sexes were used for the phytohemagglutinin test, using the same protocol as described in Chapter III.

5.2.6.3 Hematocrits

When the birds were 10 (38 Lower Mainland nestlings from 21 broods, 76 Okanagan nestlings from 31 broods) and 50 – 57 days of age (43 Lower Mainland birds from 22 nests, 70 Okanagan birds from 29 broods), blood samples were collected in order to determine packed blood cell volumes. Three 40 μl heparinized capillary tubes of blood were collected from each bird following puncture of the brachial vein with a 27-gauge needle. The ends of the tubes were plugged with putty and then centrifuged at 11,000 rpm for five minutes. Averages of the three hematocrits and brood means were used for the analyses.

5.2.6.4 Positive Controls

Six juvenile robins admitted to Monika's Wildlife Shelter from areas around the Lower Mainland and not treated with any type of drug were included as positive controls for the immune tests. These birds were orally dosed with dexamethasone once a day for two days prior to and on the day of the phytohemagglutinin injections. Two birds were dosed with 1.5 mg/kg body weight and four with 3.7 mg/kg body weight.

5.2.7 Stress Response

Stress response in 43 Lower Mainland (22 broods) and 70 Okanagan (29 broods) birds was determined using changes in corticosterone levels during a restraint stress test (Gaunt & Oring, 1999) when the birds were between 50 and 57 days of age (juveniles). Blood samples were collected immediately upon capture of the birds (time 0), after five minutes (time 5), ten minutes (time 10), thirty minutes (time 30), and sixty minutes (time 60). Between sample collections, the birds were held in a box with a cloth cover. Blood samples were taken and processed in the same way as for the hematocrits, but only two capillary tubes of blood were collected from five minutes on. Plasma samples were extracted and analyzed using a radioimmuno-assay at the University of Saskatchewan, following the protocols outlined in Kloepper-Sams et al. (1994) and Bortolotti et al. (1996).

5.2.8 Blood Lead

Lead levels were analyzed in blood samples (14 Lower Mainland and 30 Okanagan) collected when the birds were ten days old. Analyses were conducted at the National Wildlife Research Center using graphite furnace spectrometry. Values were expressed as μg/g, dry weight.

5.2.9 Tissue Weights

Following completion of the study (October 1998) the birds were euthanized and dissected. Collected tissues (gall bladder, heart, spleen, liver, kidneys, adrenal glands, gonads, thyroid glands, thymus, and bursa) were weighed when fresh and then frozen or stored in formalin for future analyses. Sex was also determined at the time of sacrifice.

5.2.10 Statistics

Differences between Lower Mainland and Okanagan eggs, nestlings, and juveniles were analyzed using one-way analyses of variance (ANOVAs) and nest means. Tissue weights at time of sacrifice were corrected for body weight by dividing the tissue weight by the body

weight minus the tissue weight. Data were common log transformed, where necessary, in order to increase normality. Pair-wise correlations were conducted to determine the effects of egg p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, p,p'-DDE, and o,p'-DDE[4] in the Okanagan birds only. Contaminant non-detects and zeros were replaced by 0.00005 (half the detection limit) for the correlations. Outliers were determined using the Mahalanobis outlier distance test. P values less than or equal to 0.05 were considered significant. Where possible, data from the 1998 eggs and chicks were compared to those from 1997 (see Chapter III) using the model:

$$Y_{ijk} = \mu + L_i + T_j + (LT)_{ij} + E_{ijk}$$

where Y is the parameter being measured, L refers to whether the bird was from the Okanagan or Lower Mainland, T refers to the year the egg or chick was collected (1997 or 1998), and LT is the two-way interaction between type and year. All statistics were conducted using JMP version 3.2.1 software (SAS Institute, Cary, North Carolina). Values expressed are ranges and means $\pm$ standard error.

5.3 Results

5.3.1 Egg Contaminants

Table 5-1 illustrates the means, standard errors, and ranges for moisture content, lipid content, p,p'-DDT, p,p'-DDD, p,p'-DDE, o,p'-DDT, o,p'-DDD, o,p'-DDE, and the ratios of DDE to DDT for the eggs collected in 1998 and 1997. The complete listing of organochlorine and polychlorinated biphenyls analyzed is available in Appendices I and II. In both years, eggs from the Okanagan contained significantly higher levels of p,p'-DDE ($F_{1,58} = 134.7$, $p < 0.0001$), p,p'-DDT ($F_{1,58} = 83.4$, $p < 0.0001$), and p,p'-DDD ($F_{1,58} = 57.6$, $p < 0.0001$) than eggs from the Lower Mainland. Only p,p'-DDE ($F_{1,58} = 8.3$, $p = 0.006$) levels were higher in 1997 than in 1998 eggs. The p,p'-DDT levels were also higher in 1997 than in 1998, but only if outliers were removed. The Lower Mainland and Okangan eggs showed no significant differences in DDE:DDT ratios. However, when outliers were removed, Lower Mainland eggs had a significantly higher ratio than the Okanagan eggs. In both years, the para,para' isomers were found in all Lower Mainland (although only minute amounts in pooled egg samples) and Okanagan eggs, but the ortho,para' isomers were only found in the Okanagan eggs. O,p'-DDE

[4] Ortho, para' and para,para' isomers were evaluated separately as they have been shown to have very different effects (e.g., Gaido et al., 1997; Kelce et al., 1995; 1998; Sohoni & Sumpter, 1998).

Table 5-1: Means (± standard error) and ranges of DDTs, moisture, and lipids (μg/g) in American robin eggs collected from the Lower Mainland and Okanagan Valley of British Columbia.

	1997 Lower Mainland n = 3	1998 Lower Mainland n = 4	1997 Okanagan n = 31	1998 Okanagan n = 22	Total Lower Mainland n = 7	Total Okanagan n = 53
% water	82.0 (0.2) 81.6-82.3	82.0 (0.3) 81.1-83.3	82.8 (0.3) 80.2-85.3	81.7 (0.4) 77.9–85.1	82.0 (0.2) 81.1-83.3	82.4 (0.2) 77.9-85.3
% lipid	5.7 (0.2) 5.4-6.1	5.3 (0.3) 3.8-6.1	4.3 (0.2) 1.5-5.8	5.0 (0.2) 3.7–7.3	5.4 (0.2) 3.8-6.1	4.6 (0.1) 1.5-7.3
p,p'-DDT	0.01 (0.01) 0.1-0.2	0.07 (0.008) 0.05-0.09	12.1 (1.6) 0.9-30.5	6.9 (1.8) 0.2-37.3	0.1 (0.02) 0.05-0.2	9.9 (1.2) 0.2-37.3
p,p'-DDE	1.9 (0.7) 0.9-3.2	0.8 (0.4) 0.3-1.9	51.7 (8.7) 10.0-245.0	26.2 (2.9) 5.4-59.1	1.3 (0.4) 0.3-3.2	41.1 (5.5) 5.4-245.0
p,p'-DDD	0.009 (0.004) 0.003-0.02	0.006 (0.0008) 0.005-0.008	1.0 (0.3) 0.06-8.7	0.7 (0.3) 0.02-6.5	0.007 (0.002) 0.003-0.02	0.9 (0.2) 0.02-8.7
o,p'-DDT	ND[2]	ND	0.05 (0.01) ND-0.2	0.05 (0.02) ND-0.3	ND	0.05 (0.009) ND-0.3
o,p'-DDE	ND	ND	0.002 (0.0006) ND-0.01	0.004 (0.001) ND-0.02	ND	0.006 ± 0.004 ND – 0.07
o,p'-DDD	ND	ND	0.003 (0.0007) ND-0.02	0.004 (0.003) ND-0.07	ND	0.003 (0.001) ND-0.07
DDE:DDT[1]	12.8 (3.7) 8.0-20.1	11.3 (4.2) 6.2-23.7	6.8 (2.0) 0.7-63.1	11.4 (2.3) 0.7-38.5	11.9 (2.6) 6.2-23.7	8.7 (1.5) 0.7-63.1

[1] ortho,para' and para,para' isomers

[2] ND = not detected * $p < 0.05$

was found in 62.3% of the Okanagan eggs, o,p'-DDD in 56.6%, and o,p'-DDT in 81.1%. There were no significant differences between the Lower Mainland and Okanagan eggs in moisture and lipid content, and there were no significant correlations of these parameters with egg contaminant levels. Eggs collected in 1998 had significantly ($F_{1,64} = 8.1$, $p = 0.006$) higher lipid contents than the 1997 eggs.

5.3.2 Thyroid Hormones

In 1998, 10-day old Okanagan broods had significantly higher triiodothyronine levels than Lower Mainland birds ($F_{1,28} = 5.7$, $p = 0.02$; Figure 5-1), but there were no significant differences in thyroxine levels (Figure 5-1), nor were there significant correlations with DDT exposure in the Okanagan chicks. Thyroid hormone levels were measured in much older birds in 1997. As thyroid hormone levels can change dramatically over time (Wentworth & Ringer, 1986), statistical comparisons between years would not be meaningful.

5.3.3 Immune Response

5.3.3.1 Hematocrits

No significant differences were found in hematocrit values between the Lower Mainland and Okanagan broods at ten days or 50 - 57 days. When the Okanagan birds were juveniles, however, their hematocrit values were positively correlated with o,p'-DDT ($r = 0.5$, $n = 22$, $p = 0.02$) and o,p'-DDE ($r = 0.5$, $n = 22$, $p = 0.02$), but negatively correlated with p,p'-DDE ($r = - 0.4$, $n = 22$, $p = 0.05$). A comparison of the two ages revealed that hematocrit values were considerably higher when the birds were juveniles than when they were nestlings ($F_{1,101} = 323.7$, $p < 0.0001$; Figure 5-2). Hematocrits averaged 45.0 and 49.8 in the 1.5 mg/kg dexamethasone controls, which was not significantly different from the Okanagan and Lower Mainland juveniles. Hematocrits were not collected from the 1997 birds.

5.3.3.2 White Blood Cell Ratios

At 10 days of age, the Lower Mainland nestlings had higher eosinophil/heterophil to lymphocyte/monocyte ratios than their Okanagan counterparts ($F_{1,126} = 15.5$, $p = 0.0001$). The difference was consistent in both years. The white blood cell ratios were significantly correlated with p,p'-DDD egg levels ($r = - 0.3$, $n = 55$, $p = 0.03$) for the Okanagan birds. As juveniles (1998) and post-breeding adults (1997), the birds showed no differences in their white blood cell

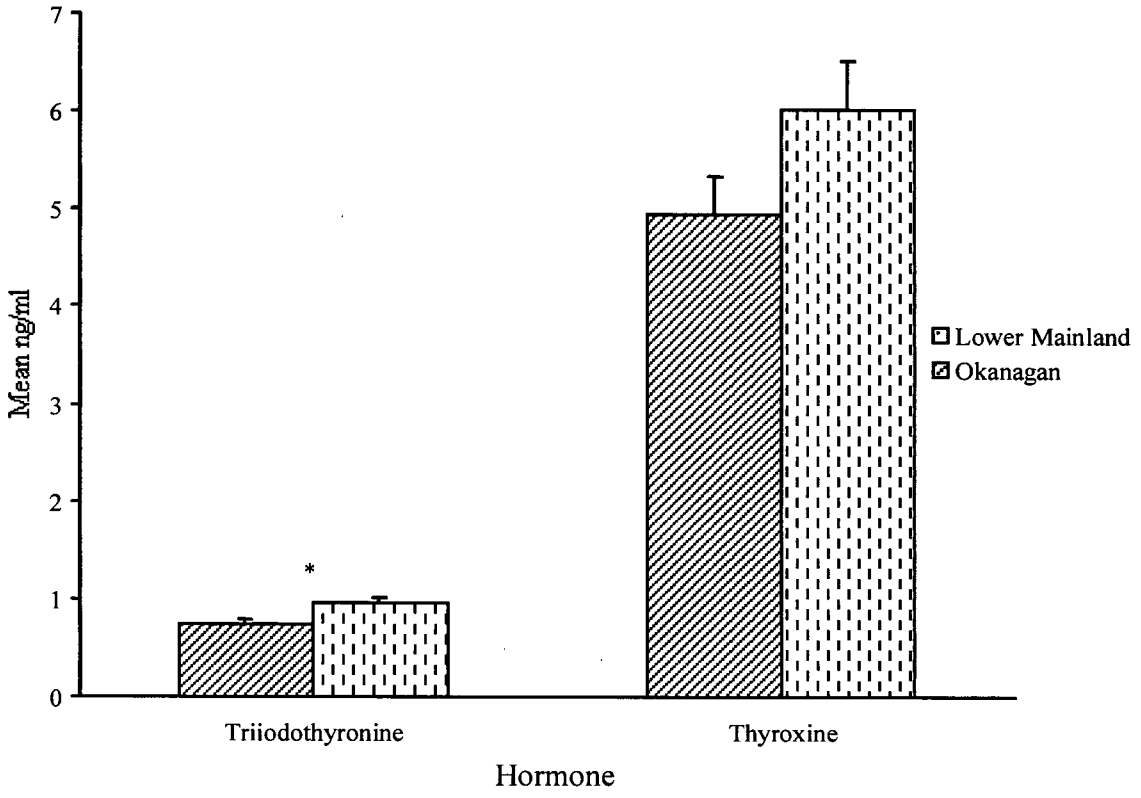

Figure 5-1: Mean (+ se) triiodothyronine and thyroxine levels (ng/ml) in the blood of 1998 Lower Mainland and Okanagan birds at ten days of age. * $p < 0.05$

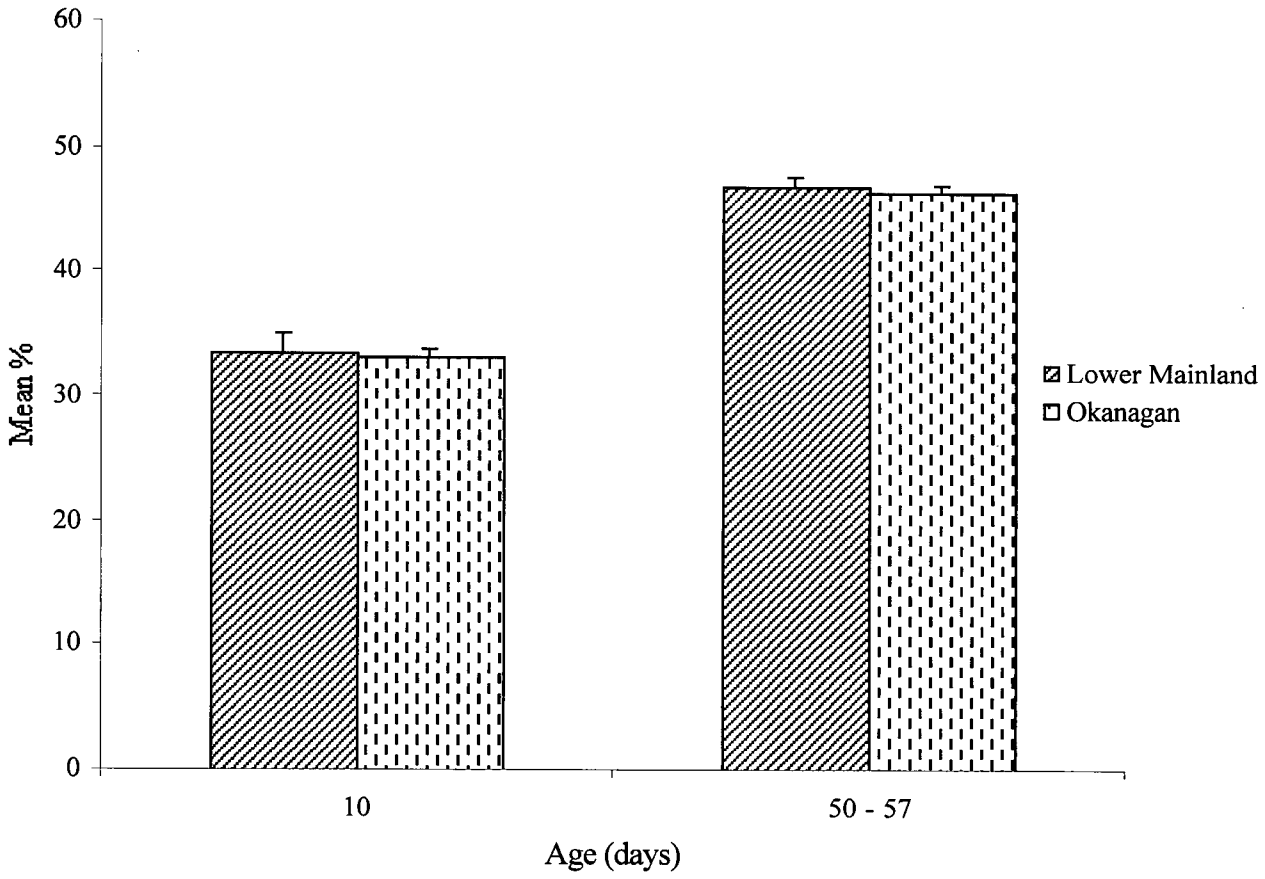

Figure 5-2: Mean (+ se) hematocrit values for 1998 Lower Mainland and Okanagan robins when 10 and 50 –57 days of age.

ratios. While juveniles demonstrated no significant correlations with DDTs, post-breeding adults showed a positive correlation with p,p'-DDT ($r = 0.4$, $n = 25$, $p = 0.03$). A comparison of the two ages (1998 birds only) revealed that the birds had significantly higher ratios as nestlings than as juveniles ($F_{1,104} = 94.0$, $p < 0.0001$; Figure 5-3). White blood cell ratios were available for the two 1.5 mg/kg positive controls (0.8 and 0.5), and they did not differ significantly from the Okanagan and Lower Mainland birds.

5.3.3.3 Phytohemagglutinin Skin Test

Wing index measurements in the 1998 Lower Mainland birds ranged from 0.1 to 1.14 (0.7 ± 0.05), whereas in the Okanagan birds it ranged from 0.3 to 1.0 (0.6 ± 0.03). There were no significant differences in response to the phytohemagglutinin test between Lower Mainland and Okanagan birds. No significant correlations with *in ovo* DDT exposure were found either. Wing index measurements for the positive controls ranged from 0.2 to 0.9 (0.5 ± 0.1) and were not significantly different from the Okanagan and Lower Mainland birds. The 1998 birds demonstrated a greater response to this test than did the 1997 birds ($F_{1,71} = 8.2$, $p = 0.006$). As age, sex and year effects were confounded, it could not be determined which of these factors were causing the difference. However, there were no significant differences between males and females in 1998. Thus, the difference between 1997 and 1998 may be attributable to age differences.

5.3.4. Stress Response

There were no significant differences between Lower Mainland and Okanagan broods in plasma corticosterone level at any time during the restraint test. There were also no significant correlations with DDT exposure. The removal of one Okanagan outlier, however, renders the differences between Lower Mainland and Okanagan birds in plasma corticosterone at times 0 ($F_{1.26} = 7.0$, $p = 0.01$) and 5 ($F_{1.27} = 4.7$, $p = 0.04$) statistically significant. Although corticosterone levels were lower in the Okanagan birds than in the Lower Mainland birds during the first five minutes of the test, both groups reached similar peak levels at time 30 (Figure 5-4). Removal of the outlier also resulted in significant positive correlations between egg p,p'-DDT and corticosterone levels at time 5 ($r = 0.6$, $n = 21$, $p = 0.009$) and time 10 ($r = 0.5$, $n = 21$, $p = 0.03$), o,p'-DDT and time 5 ($r = 0.5$, $n = 21$, $p = 0.01$) and time 10 ($r = 0.6$, $n = 21$, $p = 0.01$), and o,p'-DDE and time 5 ($r = 0.6$, $n = 21$, $p = 0.008$) and time 10 ($r = 0.5$, $n = 21$, $p = 0.02$). There

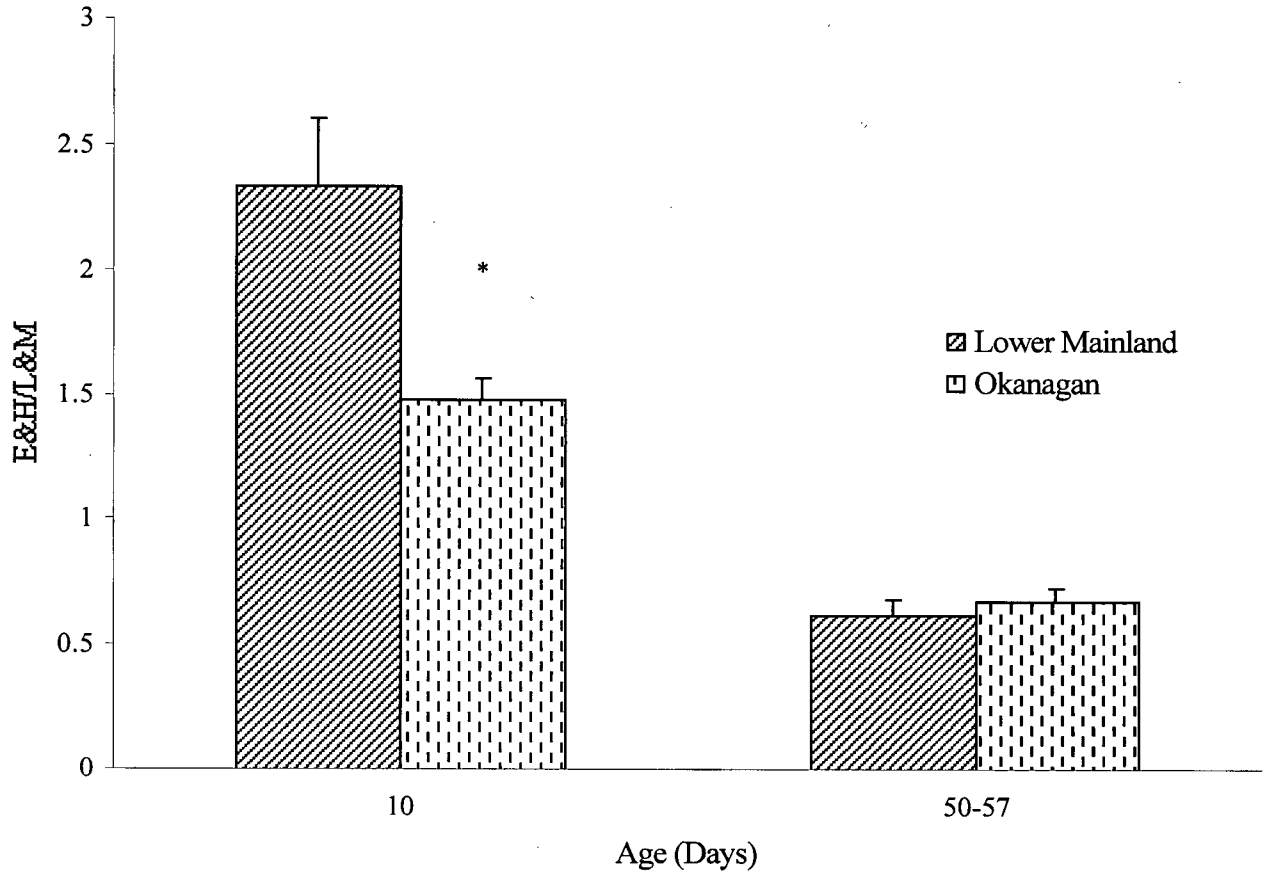

Figure 5-3: Mean (+ se) eosinophil + heterophil / monocyte + lymphocyte ratios for 1998 Okanagan and Lower Mainland birds when 10 and 50 – 57 days old. * $p < 0.05$

were significant time differences in corticosterone levels ($F_{4,144} = 36.7$, $p < 0.0001$; Figure 5-4). Stress response was not tested in the birds collected in 1997.

5.3.5 Blood Lead

Blood lead levels ranged from 0.09 to 1.5 μg/g (0.4 ± 0.1 μg/g) in the 1998 Lower Mainland chicks and 0.2 to 1.3 μg/g (0.7 ± 0.06 μg/g) in the Okanagan chicks. These levels were significantly higher in the Okanagan than in the Lower Mainland birds ($F_{1,41} = 9.0$, $p = 0.005$). Pair-wise correlations were conducted between blood lead levels in the ten-day old chicks and white blood cell ratios, hematocrits, thyroid hormone levels, body weights, and tarsus lengths at the same age. No significant correlations were found. A nominal logistics comparison between blood lead levels and whether or not the bird was treated for mycoplasmosis was also non-significant. Blood lead levels were not determined for the birds collected in 1997.

5.3.6 Infectious Diseases and Parasites

Mycoplasma gallisepticum and/or *Mycoplasma synoviae* infections were diagnosed in eight Lower Mainland and 46 Okanagan robins in 1998, using the hemagglutination inhibition test at the Animal Health Monitoring Lab, British Columbia Ministry of Agriculture, Food, and Fisheries, Abbotsford, British Columbia. These birds were treated with Baytril (enrofloxazin) until they no longer showed symptoms of infection (i.e., swollen eyes, nasal discharge, cough), with the first treatments beginning on July 29, 1998. Plasma samples from the birds at ten-days of age were negative for mycoplasma, therefore, the birds acquired the infection while in captivity. In addition, one Lower Mainland bird and four Okanagan birds were suspected of suffering from aspergillosis (*Aspergillus fumigatus*) infections. Tapeworms were found in 16 Lower Mainland and 43 Okanagan birds post-mortem, and unidentified nematodes were found in five Lower Mainland and ten Okanagan birds.

5.3.7 Mortality

Of the 38 1998 Lower Mainland birds studied, 36 (94.7%) survived to the end of the study. One Lower Mainland chick died of unknown causes when 53 days of age, and one was euthanized when 64 days of age. Fifty-eight of 64 (90.6%) Okanagan chicks survived until the end of the study. Two Okanagan chicks died of unknown causes when 67 and 69 days of age. Three Okanagan birds were euthanized; one at 41 days, one at 58 days, and one at about the same time as the others were sacrificed. One 55 day old Okanagan chick likely died as a result

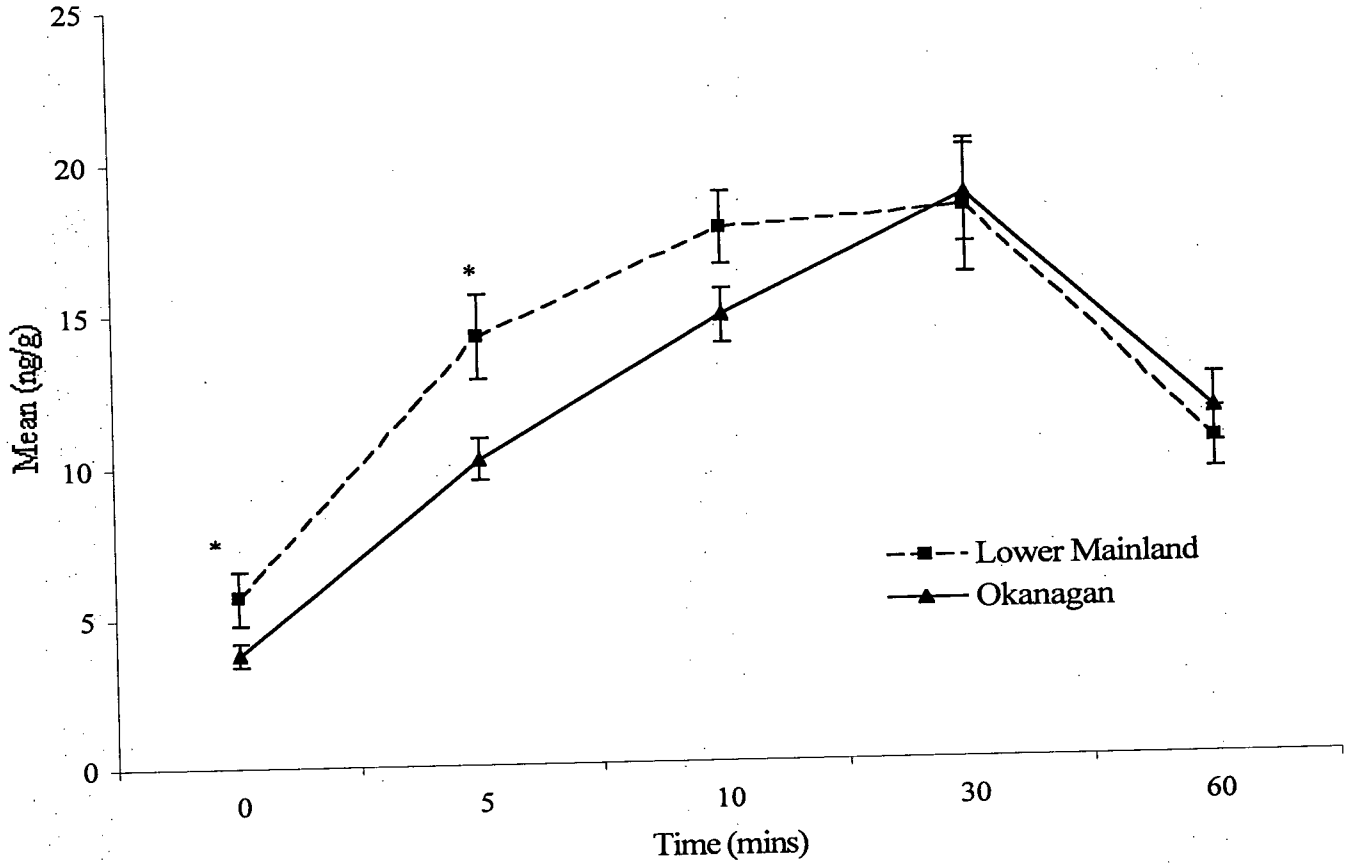

Figure 5-4: Mean (± se) plasma corticosterone levels (ng/ml) in 1998 Lower Mainland and Okanagan juvenile robins during a restraint stress test. Outlier removed. * $p < 0.05$.

of a mycoplasma infection.

5.3.8 Egg Measurements

Lower Mainland eggs (n = 68) weighed 6.5 ± 0.2 grams, averaged 29.3 ± 0.3 mm long, and 21.0 ± 0.1 mm wide. Okanagan eggs (n = 110) weighed 6.6 ± 0.1 grams, averaged 29.0 ± 0.1 mm long and 21.0 ± 0.1 mm wide. There were no significant differences between Lower Mainland and Okanagan eggs in weight, length, or width, nor were there any significant correlations with contaminant levels within the Okanagan eggs. Eggs collected in 1997 and 1998 were of similar weights and widths, but eggs collected in 1997 were longer than those collected in 1998 ($F_{1,173} = 3.9$, $p = 0.05$). The removal of outliers, however, rendered this result non-significant.

5.3.9 Chick Measurements

There were no significant differences between 10-day old Lower Mainland and Okanagan robins in body weights, tarsus lengths, or wing lengths at this age and there were no differences in the measurements taken in 1997 and 1998. Lower Mainland nestlings (n = 58) weighed 58.6 ± 1.0 grams (34.0 – 76.0 grams) at ten days, had 37.4 ± 0.2 mm (32.8 to 41.5 mm) long tarsus, 21.4 ± 0.2 mm (19.0 to 26.0 mm) middle toes, and 67.5 ± 1.8 mm (46.0 – 127.0 mm) wing length. Corresponding measurements in Okanagan nestlings (n = 72) were 60.3 ± 0.6 grams (50.1 – 75.1 grams), 37.1 ± 0.2 mm (33.9 to 40.6 mm), 14.9 ± 0.6 mm (6.7 to 23.0 mm) and 66.2 ± 0.7 mm (50.7 – 78.0 mm), respectively. Okanagan chicks had shorter middle toes than Lower Mainland chicks ($F_{1,122} = 384.1$, $p < 0.0001$), and chicks collected in 1998 had longer wings ($F_{1,121} = 13.7$, $p = 0.0003$) and middle toes ($F_{1,122} = 257.9$, $p < 0.0001$) than those collected in 1997. There was a significant year by type interaction for toe length ($F_{3,122} = 297.3$, $p < 0.0001$), with Okanagan birds collected in 1997 having significantly shorter middle toes than Lower Mainland birds. There was no such difference in the 1998 birds. Body weight was positively correlated with egg p,p'-DDE levels ($r = 0.4$, $n = 50$, $p = 0.007$) and wing length was negatively correlated with p,p'-DDD levels ($r = -0.3$, $n = 50$, $p = 0.02$).

5.3.10 Tissue Weights

Table 5-2 illustrates the body and corrected tissue weights for the 1998 Lower Mainland and Okanagan birds at sacrifice. Okanagan birds had significantly heavier livers ($F_{1,48} = 13.9$, $p = 0.0005$), kidneys ($F_{1,48} = 7.0$, $p = 0.01$), and spleens ($F_{1,48} = 5.2$, $p = 0.03$) than Lower Mainland birds. The removal of outliers rendered the difference between Lower Mainland and

Table 5-2: Means (± se) and ranges of body and tissue weights (grams) of 1998 Okanagan and Lower Mainland broods at time of sacrifice.

	Lower Mainland n = 21	Okanagan n = 30
body	81.7 ± 1.1 66.5 – 88.9	81.8 ± 1.3 56.5 – 90.0
gall bladder	0.001 ± 0.0001 0.0004 – 0.003	0.001 ± 0.00007 0.0004 – 0.002
spleen	0.002 ± 0.0002 0.001 – 0.004	0.003 ± 0.0003* 0.001 – 0.008
bursa	0.0009 ± 0.0001 0 – 0.002	0.0007 ± 0.00007 0.0002 – 0.002
adrenal glands	0.0002 ± 0.00002 0.00006 – 0.0004	0.0002 ± 0.00001 0.0001 – 0.0003
gonads	0.0002 ± 0.00002 0.0001 – 0.0004	0.0002 ± 0.00002 0.00006 – 0.0006
thymus	.001 ± 0.00007 0.005 – 0.002	0.001 ± 0.00006 0.0003 – 0.002
liver	0.03 ± 0.0008 0.03 – 0.04	0.04 ± 0.002* 0.03 – 0.08
heart	0.01 ± 0.0003 0.009 – 0.02	0.01 ± 0.0002 0.01 – 0.02
thyroid glands	0.0003 ± 0.00002 0 – 0.0005	0.0003 ± 0.00002 0.00006 – 0.0004
kidneys	0.02 ± 0.0004 0.01 – 0.02	0.02 ± 0.0005* 0.02 – 0.03

* $p < 0.05$

Okanagan birds' spleens non-significant, but did not affect the liver and kidney differences. Among the Okanagan birds, positive correlations were found between bursa weights and p,p'-DDE ($r = 0.5$, $n = 21$, $p = 0.02$), liver and p,p'-DDD ($r = 0.6$, $n = 22$, $p = 0.002$), liver and o,p'-DDD ($r = 0.7$, $n = 22$, $p = 0.007$), kidneys and p,p'-DDD ($r = 0.7$, $n = 22$, $p = 0.0007$), and kidneys and o,p'-DDD ($r = 0.7$, $n = 22$, $p = 0.001$). Significant negative correlations were found between the gall bladder and p,p'-DDE ($r = -0.5$, $n = 22$, $p = 0.02$) and adrenal glands and o,p'-DDT ($r = -0.4$, $n = 21$, $p = 0.05$). Only the gall bladder correlation was significant after the removal of outliers. Direct comparisons with the 1997 birds were not possible, as the 1998 birds were sacrificed as juveniles and the 1997 birds were sacrificed as two to three year old adults. A total of 19 Lower Mainland males, 27 Lower Mainland females, 41 Okanagan males, and 37 Okanagan females were sacrificed.

5.4 Discussion

In both years (1997 and 1998), eggs collected from orchard areas of the Okanagan Valley contained significantly higher levels of various DDT isomers than eggs from the Lower Mainland. Robins from the Okanagan were more susceptible to diseases and parasites, and suffered higher mortality than their Lower Mainland conspecifics when reared together in captivity. There was no evidence, however, that the Okanagan birds that became infected came from nests with higher levels of *in ovo* DDT exposure. At 10 days of age, right after collection from their respective nests, Okanagan robins in both years had significantly lower heterophil to lymphocyte ratios than Lower Mainland robins. In 1998, Okanagan birds had significantly higher plasma triiodothyronine levels than Lower Mainland birds, but hematocrit and thyroxine measurements were not different between the two types of birds. Except for the heterophil/lymphocyte ratios, none of the parameters measured at this age were correlated with *in ovo* DDT levels. The correlation between 10-day heterophil/lymphocyte ratios and p,p'-DDD although significant, was marginal ($r = - 0.03$).

Injecting 2 or 4 mg/kg p,p'-DDE into Japanese quail (*Coturnix japonica*) eggs resulted in chicks hatched with higher leukocyte numbers (Quinn et al., 2002). Caspian terns (*Sterna caspia*) from the Great Lakes showed increasing heterophil/lymphocyte ratios with increasing DDE levels (Grasman et al., 1996). Campbell (1994) concluded that in general, an excess of either endogenous or exogenous glucocorticoid could lead to slight to moderate leukocytosis, heterophilia, and lymphopenia, which would result in higher heterophil/lymphocyte ratios. Smits

and Williams (1999) found higher ratios in 10-day old zebra finches (*Taeniopygia guttata*) fed dexamethasone (a synthetic glucocorticoid and immunosuppressant) compared to controls. They found that stress due to handling increased total leukocrits, but dexamethasone increased the heterophil component while decreasing the lymphocyte component. In the present study, we did not have 10-day old dexamethasone birds to serve as controls, but 10-day old Okanagan robins had significantly lower heterophil/lymphocyte ratios as compared to Lower Mainland robins of the same age. Unfortunately, only differential white blood cell counts were conducted, not total leukocrit analyses. By heterophil/lymphocyte ratio measurements, the immunocompetence of young Okanagan nestlings did not seem to have been compromised by *in ovo* DDT exposure.

Jefferies and French (1969) reported that the thyroid glands of feral pigeons (*Columbia livia*) fed 3 to 36 mg DDT/kg/day for six weeks were twice as heavy as those of controls. The thyroids of dosed birds had smaller follicles, less colloid, and hyperplastic epithelia, regardless of the dose level. Similar results were found in birds dosed with DDE (Jefferies, 1975). However, contaminant-induced alterations in thyroid hormones in wildlife, experimental and field studies have produced divergent findings (see review by Rolland, 2000). Furthermore, the relationship between thyroid hormones and immune response is mediated by many endogenous and exogenous factors and may not be easily predictable (Fowles et al., 1997; Erf & Marsh, 1989; Williamson et al., 1990; Smits et al., 2002). In general, animals with low thyroid hormone levels demonstrated decreased cell-mediated and humoral immune response (Klecha et al., 2000; Smits et al., 2002). In the present study, 10-day old Okanagan robins had significantly higher plasma triiodothyronine levels than same age Lower Mainland robins in 1998 while showing no difference in plasma thyroxine levels. Thus, there is no evidence that the cell mediated or humoral immune responses of young Okanagan nestlings have been compromised by low thyroid hormone levels. In fact, the Okanagan robins may be showing mild hyperthyroidism as was found previously in pigeons and Bengalese finches (*Lonchura striata*) exposed to a variety of DDT dosages (Jefferies, 1975). Unfortunately, because of logistic reasons, the phytohemagglutinin skin test was not performed on the birds when they were nestlings. Phytohemagglutinin tests administered to juveniles (1998) and adults (1997) revealed that there was no difference between the Okanagan and Lower Mainland robins in their T-lymphocyte mediated immune response.

No significant differences were found in hematocrit values between the Lower Mainland and Okanagan broods at ten days or 50 - 57 days, nor were these values significantly different in the dexamethasone controls. In Smits and Williams' (1999) study, 10-day old zebra finch

exposed to dexamethasone also did not have different hematocrit values than controls. These authors commented that hematopoiesis of red cells in birds is a very active process during the late embryonic and early post-hatch periods and a full complement of red blood cells does not occur until some time between mid-nestling and post-fledging stage. This is consistent with our finding that hematocrit values were considerably higher when the birds were juveniles than when they were nestlings. Hematocrit values taken from juvenile Okanagan birds were negatively correlated with ortho-DDT isomers (o,p'-DDT and o,p'-DDE) but positively correlated with a para-DDT isomer (p,p'-DDE). The significance of these correlations remains to be determined.

Initial analysis of the stress response test of juvenile robins revealed that there was no significant difference between the Okanagan and Lower Mainland birds in their corticosterone response. One Okanagan female, which had a very high plasma corticosterone level, was found dead the day after the test and was also identified as an outlier. After the exclusion of this individual from the data set, it was found that Lower Mainland juvenile robins had a significantly lower corticosterone response than Okanagan juveniles during the first 5 minutes of exposure to the stress stimulus. This may be an indication that Lower Mainland robins had higher baseline corticosterone levels than Okanagan birds. There was however, no difference in their peak response level as well as the time to achieve this level.

The initial corticosterone response was positively correlated with many of the *in ovo* DDT isomers. Eastern bluebird (*Sialia sialis*) nestlings that were restrained and then challenged with adrenocorticotropic hormone exhibited negative correlations between corticosterone and DDE levels (Mayne et al., 2002). This study, however, was dealing with direct rather than *in ovo* DDT exposure. They were also concerned with the level of corticosterone response rather than the rate of response. The findings in this study seem to be novel and deserve further investigation.

While DDTs have been shown to influence several aspects of the immune system in a variety of species, including birds, and *in ovo* exposures may result in mortality, reduced hatchability, wasting syndrome, skeletal abnormalities, and impaired differentiation of the reproductive and nervous systems in offspring (Fry, 1995; Jefferies, 1971; Banerjee et al., 1996). This study, however, revealed no evidence that in captive Okanagan robins, the wasting syndrome, parasite infections, and mortality were related to compromised immunocompetence due to *in ovo* DDT exposure.

In 1998, blood lead levels were assayed in 10-day old robin nestlings and Okanagan robins had significantly higher blood lead than Lower Mainland birds. Lead has been shown to

increase susceptibility to infectious agents and impair both cell-mediated and humoral immunity (Grasman & Scanlon, 1995; Lee et al., 2002). However, Grasman and Scanlon (1995) also concluded that in Japanese quail, lead suppressed antibody-mediated immunity only at dosages that also caused clinical lead poisoning. Fair and Ricklefs (2002), combining dosing lead with an immunological challenge, also found that lead did not affect antibody production or cell-mediated immune response in Japanese quail. In the robin study, blood lead levels were not correlated with white blood cell ratios, hematocrits, or thyroid hormone levels. There was also no relationship between blood lead level and parasite/disease infection. Therefore, there is no evidence in this study that blood lead levels found in the Okanagan birds was affecting their immunocompetence.

By eliminating alternative hypotheses, this study strengthened the notion put forth in Chapter III, that Lower Mainland and Okanagan robins are genetically different, and that the difference between the two in disease/parasite susceptibility was mainly because of the adaptability of Lower Mainland robins to local parasite and disease vectors.

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Chapter VI

General Discussion

The goal of this study was to examine whether *in ovo* and early exposure to dichlorodiphenyltrichloroethane (DDT) have any delayed or long-term effects on the growth and survival, immune response, behavior, reproductive success, and stress response of American robins (*Turdus migratorius*). This was done by comparing contaminated Okanagan birds with uncontaminated Lower Mainland controls, and correlating contamination levels of Okanagan eggs with a number of parameters in their clutch mate(s). The design of the study was based on three assumptions: 1) that the Okanagan and Lower Mainland birds were of similar genetic background, 2) the level of contamination in all the eggs in a clutch was similar, and 3) among Okanagan birds, parental care and post-hatch DDT exposure (from hatching to about ten-days of age) were not significant variables.

Although American robins were once raised as pets (Howell, 1942) and are used for short term captive studies (e.g. Wellehan et al, 2001; Richter et al, 2000; Levey & Karasov, 1992; Wong & Desser, 1978), this study is the first to conduct long term observations and likely the first to report large-scale successful breeding of these birds in captivity, demonstrating that the American robin can be utilized as a non-domesticated passerine model for long term, multi-generation toxicology studies.

6.1 Egg Contaminants

American robin eggs from orchard areas in the Okanagan Valley of British Columbia contain high levels of DDT and its metabolites (Elliott et al., 1994; Gill et al., 2003). Eggs collected from the Okanagan for this study in 1997 and 1998 demonstrated total DDT levels ranging from 5.71 to 277.62 μg/g, as compared to levels of 0.36 to 3.39 μg/g in eggs collected from control areas of the British Columbia Lower Mainland. The majority of the contaminant burden found in Okanagan robin eggs comes from the metabolite p,p'-dichlorodiphenyldichloroethylene (DDE), but dichlorodiphenyldichloroethane (DDD), the other main metabolite of DDT, was also found. Para,para'-DDE is considered the final breakdown product of DDT in living birds (Poland et al., 1972), and it tends to be lost quite slowly (Stickel, 1973). Ortho,para' isomers were not found in any of the eggs collected from the Lower

Mainland, and only trace amounts were found in the eggs from the Okanagan. Ortho,para' -DDT composes only 10 to 30% of technical grade DDT (Jefferies, 1975; Mellanby, 1992; WHO, 1989) and it tends to be stored less readily and eliminated more quickly than p,p'-DDT (Stickel, 1973). As robins likely get the bulk of their contaminant loads from the foods they eat, the unequal concentrations of the various isomers and metabolites may also be influenced by DDT metabolism within their invertebrate prey species. Earthworms, in particular, have been shown to play a role in the initial degradation of DDT by converting it to DDE and DDD (Edwards & Jeffs, 1974; Gish & Hughes, 1982). These differences in the levels of the various forms of DDT are important as different isomers and metabolites have been shown to have different effects (Sohoni & Sumpter, 1998; Sonnenshein & Soto, 1998).

As expected, robin eggs from orchards in the Okanagan had significantly higher levels of DDTs than those collected from reference sites within the Lower Mainland. The residue levels found in these eggs were surprisingly high considering DDT has not been used in the Okanagan for approximately thirty years, but were similar to levels found in other studies of Okanagan birds (Elliott et al., 1994; Gill et al., 2002; Harris et al., 2000). Also surprising, were the low DDE to DDT ratios found in the eggs from both the Okanagan and the Lower Mainland. These ratios confirm the view that DDT degrades very slowly (Colborn et al., 1993; Murphy, 1980; Repetto & Baliga, 1996). The Okanagan likely serves as a DDT sink or hot-spot due to heavy historical use (Elliott et al., 1994; Gill et al., 2003; Harris, 2000). It has been predicted that ratios should exceed 20:1 following 15 to 20 years without DDT application (Elliott et al., 1994), but this was not found in the present study.

Although the highest levels of contamination in the robin eggs came from DDTs, these birds were also exposed to a variety of other current-use and historical chemicals. For example, lead arsenate was used extensively as a pesticide prior to the introduction of DDT. One of the ways lead exerts its toxic effects is by acting as a porphyrinogenic agent causing defective hemoglobinization and anemia (Bunyan & Stanley, 1982). The organochlorines, chlordane, heptachlor, mirex, aldrin, and dieldrin were banned many years ago but still show up in soils and animal tissues. Other organochlorines such as endosulfan, dicofol, lindane, and methoxychlor are currently in use (Crisp et al., 1998). Appendices I and II list the organochlorine chemicals evaluated for this study. Aside from the DDTs, the only organochlorines found at appreciable levels in the robin eggs were *cis*- and *trans*-nonachlor and tris(4-chlorophenyl)methanol (TCPM). *Cis*- and *trans*-nonachlor are components of the cyclodiene pesticide chlordane. TCPM is a relatively newly discovered organochlorine that has been found in a number of

species. Its origins are not clear, however, it is believed to be an impurity in DDT (de Boer, 2000). A number of organochlorine pesticides have been linked to increased mortality, reduced reproductive success, altered immune functioning, and other detrimental effects in both laboratory and wildlife studies (Banerjee et al., 1996; Barnett & Rodgers, 1994; Blus & Henny, 1997; Murphy, 1980; Stickel, 1973; Street, 1981; Thomas, 1975; Voccia et al., 1999).

Although not intentionally released into the environment, polychlorinated biphenyls are ubiquitous contaminants that have been shown to have a number of detrimental effects on birds and other animals. Polychlorinated biphenyls can influence thyroid glands and hormones, the immune system, the thymus, the liver, thyroid functioning, and a variety of other systems (Bunyan & Stanley, 1982; Cheek et al., 1999; Fernie et al., 2001; Hoffman et al., 1996; Jefferies, 1975). The most toxic polychlorinated biphenyl congeners are polychlorinated biphenyl 126, 77, and 169 (Hoffman et al., 1996). A number of polychlorinated biphenyl congeners, as well as the polychlorinated biphenyl mixture Aroclor 1260 were measured in the robin eggs collected for this study (Appendices I and II). Although non-DDT organochlorines and polychlorinated biphenyls were found in low levels, it cannot be ruled out that some of these chemicals may exert an effect at very low concentrations and/or they may interact with the DDTs (Fossi, 1998; Stickel, 1973).

The majority of insecticides used today are organophosphates and carbamates. Both of these chemical classes act by inhibiting cholinesterase activity (Bunyan & Stanley, 1982). Although these chemicals are much less persistent than the organochlorines, many of them are considered highly toxic, with LD_{50} levels less than 25 mg/kg in several species of birds (Fluetsch & Sparling, 1994; Murphy, 1980). Cholinesterase inhibition greater than 20% is indicative of exposure, while inhibition greater than 50% can be lethal (Gill et al., 2000). As these chemicals are often applied during the breeding season they can reduce reproduction and recruitment by increasing mortality, altering adult behavior, or decreasing food supplies (Fluetsch & Sparling, 1994; Gill et al., 2000; Graham & DesGranges, 1993; Patnode & White, 1991; Rondeau & DesGranges, 1995; Thomas, 1975). They may also influence the immune system (Banerjee et al., 1996; Barnett & Rodgers, 1994; Street, 1981; Voccia et al., 1999), the thyroid gland and its functioning (Bishop, Van Der Kraak et al., 1998; Mayne et al., 2002), and other systems. Although not measured in this study, the organophosphates azinphos-methyl (Guthion) and diazinon, and the carbamate carbaryl (Sevin), among others, were sprayed in the Okanagan orchards from which the robin eggs and nestlings were collected. Eggs and chicks were not

collected on spraying days, but may have been exposed to these pesticides just prior to collection (L. Wilson, personal communication).

Birds and other animals are rarely exposed to a single chemical, but rather mixtures of them (Bishop, Boermans et al., 1998; McArthur et al., 1983; Tyler et al., 1998; Vos et al., 2000). A variety of other substances were also sprayed in the Okanagan orchards, such as dormant oil, fertilizers, growth hormones, and herbicides. All of these could have potential impacts on the robins, either directly (toxicity) or indirectly (reduced food supply or cover).

6.2 Similarities and Differences Between the Lower Mainland and Okanagan Robins

During the course of the study, many differences between the Okanagan and Lower Mainland robins were observed (see Table 6-1 for summary). Because not all of the parameters were measured in the same individuals and at the same age, and because of small sample sizes (e.g., for many parameters, clutch or brood were used to avoid psuedo-replication), it was often not possible to integrate the *in ovo* DDT levels into a regression analysis. Thus, correlational analyses using the individual DDT isomers were conducted. These analyses were based on the assumption that the level of contamination in all the eggs in a clutch was similar. While most previous studies also followed this assumption, there have been suggestions that contaminant loads may vary between eggs within a clutch (Ohlendorf et al., 1985; Ottinger et al., 2001). Although occasionally more than egg was collected from a nest for this study, contaminant analyses were only conducted on one egg per nest. Therefore, comparisons within a clutch were not possible. Significant correlations are summarized in Table 6-2.

6.2.1 Egg and Chick Measurements

Although all of the eggs collected for this study were within the size ranges described by Howell (1942), females from the Lower Mainland laid heavier and wider eggs than their Okanagan counterparts. Among the Okanagan females, egg size was positively correlated with their *in ovo* DDT exposure. The occurrence of larger eggs being laid by more contaminated females may be related to the estrogenic effects (Williams, 1999) of various DDTs (Fry, 1995; Sohoni & Sumpter, 1998; Tyler, 1998), however, this does not account for the fact that Lower Mainland females, as a whole, laid even larger eggs. There are a number of factors that can play

Table 6-1: Significant differences found between Lower Mainland and Okanagan birds and eggs in 1997 and 1998.

Chapter	Parameter	Effect
III	o,p' isomers	found only in Okanagan eggs
	egg lipid levels	Lower Mainland > Okanagan
	10 day middle toe length	Lower Mainland > Okanagan
	body weight at 2 months	Okanagan > Lower Mainland
	body weight at 7-9 months	Lower Mainland > Okanagan
	tarsus length at 2 months	Lower Mainland > Okanagan
	tarsus length at 5 months	Lower Mainland > Okanagan
	white blood cell ratios at 10 days	Lower Mainland > Okanagan
	coccidiosis infections	Okanagan > Lower Mainland
	juvenile deaths	Okanagan > Lower Mainland
	heart weight at sacrifice	Okanagan > Lower Mainland
IV	males fledging chicks	Lower Mainland > Okanagan
	egg weight	Lower Mainland females > Okanagan females
	egg width	Lower Mainland females > Okanagan females
	offspring tarsus length at 5 days	Okanagan females > Lower Mainland females
	offspring wing length at 5 days	Okanagan females > Lower Mainland females
	offspring deaths at nestling stage	Lower Mainland females > Okanagan females
	offspring body weight at sacrifice	same type parents > different type parents
	offspring brain weight at sacrifice	different type parents > same type parents
	offspring kidney weight	Okanagan x Okanagan parents heaviest
	offspring liver weight	Okanagan x Okanagan parents heaviest
	drinking behavior	Okanagan > Lower Mainland
	preening behavior	same type pairs > different type pairs
	chukking vocalizations	different type pairs > same type pairs
V	egg DDT levels	Okanagan > Lower Mainland
	10 day triiodothyronine levels	Okanagan > Lower Mainland
	10 day white blood cell ratios	Lower Mainland > Okanagan
	corticosterone levels time 0	Lower Mainland > Okanagan

	corticosterone levels time 5	Lower Mainland > Okanagan
	blood lead levels	Okanagan > Lower Mainland
	mycoplasmosis infections	Okanagan > Lower Mainland
	tapeworms and nematodes	Okanagan > Lower Mainland
	middle toe length 10 days	Lower Mainland > Okanagan
	liver weight at sacrifice	Okanagan > Lower Mainland
	kidney weight at sacrifice	Okanagan > Lower Mainland

Table 6-2: Significant correlations between Okanagan egg contaminant levels and other parameters.

DDT Isomer/Metabolite	Parameter	Direction of Correlation
Chapter III		
o,p'-DDD	egg lipid levels	negative
p,p'-DDE	10 day body weight	positive
p,p'-DDT	post-breeding white blood cell ratios	positive
p,p'-DDE	gonad weight at sacrifice	negative
o,p'-DDT	oviduct weight at sacrifice	positive
Chapter IV		
p,p'-DDT	egg weight	positive
p,p'-DDD	egg length	positive
p,p'-DDE	egg length	positive
p,p'-DDT	egg width	positive
o,p'-DDT	egg width	positive
p,p'-DDT	triiodothyronine levels, period 12	negative
o,p'-DDT	thyroxine levels, period 4	positive
o,p'-DDD	thyroxine levels, period 4	positive
o,p'-DDT	thyroxine levels, period 6	negative
p,p'-DDE	snapping	positive
p,p'-DDD	overall vocalizations	positive
p,p'-DDT	laughing	negative
o,p'-DDT	"other" vocalizations	positive
o,p'-DDD	"other" vocalizations	positive
o,p'-DDE	"other" vocalizations	positive
p,p'-DDE	eating	positive
p,p'-DDT	flying (frequency)	positive
p,p'-DDE	flying (frequency)	positive
p,p'-DDT	flying (proportion)	positive
p,p'-DDE	flying (proportion)	positive

Chapter V		
o,p'-DDT	juvenile hematocrits	positive
o,p'-DDE	juvenile hematocrits	positive
p,p'-DDE	juvenile hematocrits	negative
p,p'-DDD	10 day white blood cell ratios	negative
p,p'-DDT	post-breeding white blood cell ratios	positive
p,p'-DDT	corticosterone levels, times 5 and 10	positive
o,p'-DDT	corticosterone levels, times 5 and 10	positive
o,p'-DDE	corticosterone levels, times 5 and 10	positive
p,p'-DDE	10 day body weight	positive
p,p'-DDE	10 day wing length	negative
p,p'-DDE	gall bladder weight at sacrifice	negative

a role in egg size including genetics, female condition (Styrsky et al., 2002), and individual differences (Williams, 1999).

Few differences were found in the morphometric measurements of the Okanagan and Lower Mainland robins and their offspring. Okanagan chicks collected in 1997 had significantly shorter middle toes than the 1997 Lower Mainland birds and both groups collected in 1998, and the young of Okanagan females had longer tarsi and wings at five-days-of-age. None of these differences persisted into adulthood. While some of this discrepancy may be due to changes in measuring practices or in the people conducting the measurements, it is also possible that environmental conditions or parental care played a role. Body weights and wing measurements were similar to those reported by Howell (1942), although tarsus lengths in this study were slightly larger. The majority of studies do not report toe lengths, so the significance of this size difference is not clear.

It is possible that *in ovo* DDT exposure influenced the growth of the Okanagan chicks. For example, body weight at ten days of age was positively correlated with p,p'-DDE egg levels and wing length was negatively correlated with p,p'-DDD in the 1997 Okanagan birds. These birds also showed a delay in their tarsus growth relative to the Lower Mainland controls. It is not possible, unfortunately, to separate the effects of contaminant exposure from that of parental care, genetics, and other factors, so one can not be certain what role DDT played in the growth of these birds.

Overall, the male to female ratio of the chicks collected in 1997 and 1998 was 0.76 for the Lower Mainland birds and 1.15 for the Okanagan birds, which is not a significant difference. There were also no significant differences in the sex ratios of the offspring of the 1997 birds. Lower Mainland females had a male to female ratio of 0.81 whereas Okanagan females showed a 1.56 ratio. Sex ratios in the wild are not widely reported (Sallabanks & James, 1999), however, Young (1955) suggested that the average is 1:1. Although DDT may feminize and DDE demasculinize birds, this was likely not an issue in this study as most of the chicks were sexed genetically and the feminizing/demasculinizing effects of DDT and DDE most likely act on sexual differentiation of the brain, gonads, and secondary sexual characteristics, not on the genes themselves (McLachlan & Arnold, 1996). Complete sex reversal has also not been witnessed in any bird species studied (Fry & Toone, 1981).

6.2.2 Behavior and Reproduction

In Chapter III it was reported that the 1997 Okanagan and Lower Mainland birds displayed no significant differences in their abilities to build nests, lay eggs, hatch eggs, or fledge chicks. The timing of reproductive events was also similar between the two groups and previously studied birds (Gill et al., 2003; Kemper, 1971). The reproductive success of these robins improved dramatically during their second breeding season. This may be due to experience, changes in the pens, different mates, and/or environmental conditions.

Few differences were found in the behaviors of the 1997 Lower Mainland and Okanagan birds. Okanagan birds drank more frequently than Lower Mainland birds, same type pairs (Lower Mainland x Lower Mainland, Okanagan x Okanagan) preened more than different type pairs (Lower Mainland x Okanagan, Okanagan x Lower Mainland), and different type pairs exhibited more chukking vocalizations than same type pairs. The importance of these differences, however, is unclear. While drinking and preening have obvious survival benefits, the purpose of chukking has not been well established. It is also not known if excessive displays of these behaviors indicate boredom or frustration, or actually represent dehydration, feather parasites, or other conditions.

Despite the fact that there were few differences between the two groups, within the Okanagan birds several behaviors were significantly correlated with *in ovo* DDT exposure. The fact that some of the most highly contaminated birds demonstrated high frequencies of certain behaviors (e.g., nest building, mounting) suggests that there may be threshold levels below which robin behavior is not affected. These thresholds likely vary depending on sex, age, the behavior tested, and even individual differences. In order to test this, one would need sufficient sample sizes of birds with similar contaminant levels or treat birds or eggs with DDTs in order to examine dose-response curves.

6.2.3 Hormones

Although Okanagan birds had higher triiodothyronine levels than their Lower Mainland counterparts at ten days of age, no significant differences in plasma triiodothyronine or thyroxine were found at any other age tested. There were also no significant correlations with *in ovo* DDT exposure for the Okanagan chicks. The Okanagan chicks may have been experiencing a mild hyperthyroidism, as seen in other species exposed to a variety of DDT dosages (Jefferies, 1975). Although thyroid hormones play a role in growth (Bolander, 1994; Nelson, 2000; Singh et al., 1968) and immunity (Jefferies, 1975), among other processes, these were not systematically tested. There were, however, significant differences across the testing periods, which is not

surprising given that thyroid hormone levels can vary dramatically over time (Wentworth & Ringer, 1986).

Corticosterone levels were evaluated in the 1998 birds during a restraint stress challenge. Plasma levels of corticosterone were significantly lower in the Okanagan birds than in the Lower Mainland birds during the first five minutes of the test. This suggests that baseline corticosterone concentrations were lower in the Okanagan birds. Both groups, however, displayed similar peaks in corticosterone at 30 minutes. It is not clear why the Okanagan birds had lower corticosterone levels at the beginning of the test. It may be related to genetic differences or perhaps DDT exposure. Corticosterone levels at five and ten minutes were positively correlated with both p,p'-DDT and o,p'-DDT exposure. How much of the measured corticosterone is bound, rather than free, is also not known and could be influencing these group differences.

6.2.4 Immunity

Immune response was tested using differential white blood cell counts and the phytohemaggluttinin skin test. At ten days of age, Lower Mainland chicks had higher eosinophil/heterophil to lymphocyte/monocyte ratios than Okanagan birds. As these ratios were negatively correlated with p,p'-DDD levels, it is possible that *in ovo* exposure played a role in this effect. Whether or not the Okanagan birds were at a disadvantage in terms of immune response can not be determined from this test, as lymphocyte and heterophil numbers increase in response to different infectious agents (Siegel, 1980). The higher levels of lymphocytes in the Okanagan birds may have made them more resistant to viral infections but more susceptible to bacterial invasion. The heterophil to lymphocyte ratio quantifies the balance between the nonspecific, fast-acting defenses of heterophils and the antigen-specific, slower acting defenses of lymphocytes (Grasman et al., 1996). No significant differences in white blood cell ratios were found between the birds when they were tested as juveniles or adults. There were, nonetheless, differences between the different testing periods. Both juveniles and adults demonstrated dramatically lower white blood cell ratios than they did as nestlings. These age differences are likely the result of normal changes in the immune system (Smits & Williams, 1999; Sturkie & Griminger, 1986).

The phytohemagglutinin skin test reflects a complex series of physiological events (Grasman et al., 1996; Lochmiller et al., 1993). The delayed (24 hour) response tested here is due to a local influx of T cells recruiting inflammatory cells to the challenge site (Parmentier et

al., 1998; Smits et al., 1999). Although Grasman et al. (1996) found a decrease in wing index measurements with increasing DDE levels in gulls and terns, the robins in this study showed no differences in response, despite their high levels of DDE exposure. This was true both of the 1998 juveniles and the 1997 adult males. It is possible that *in ovo* contamination had no effect on T lymphocyte mediated immunity in these birds. However, as dexamethasone treatment did not influence the phytohemagglutinin response of the 1998 positive controls, it is possible that this particular immune test is not appropriate for robins.

Hematocrit values were not significantly different between the Lower Mainland and Okanagan birds when they were nestlings or juveniles. Values were, however, significantly higher in the birds when they were juveniles than when they were ten-day-old nestlings. Significant correlations with egg contaminants were found in the birds as juveniles, which suggest that DDT exposure has the potential to impact packed red blood cell volumes.

Okanagan robins appeared to be far more susceptible to infectious disease than the Lower Mainland birds. In 1997, 13 Okanagan birds died as a result of starvation, likely due to the intestinal coccidiosis parasites *Eimeria* and *Isospora*. None of the Lower Mainland birds, housed under the same conditions, were affected. Two of the infected birds were shown to carry protozoan blood parasites (*Leucocytozoon*). In 1998, eight Lower Mainland and 46 Okanagan birds were treated for infections caused by *Mycoplasma gallisepticum* and/or *Mycoplasma synoviae*. One Lower Mainland and four Okanagan birds were suspected of having aspergillosis (*Aspergillus fumigatus*), and tapeworms and unidentified nematodes were discovered in several birds, especially those from the Okanagan.

Captive birds are prone to a number of diseases and parasites, and one would expect that all the birds would be equally susceptible. It is possible that Okanagan birds succumbed to these infections more readily because their early DDT exposure compromised their immune systems in some way that was not readily apparent given the tests used in this study. Genetic differences may also have played a role. As the birds were all raised in the Lower Mainland, it may be that the Okanagan birds did not have the ability to cope with the particular infectious agents found in this area. The Lower Mainland birds, in contrast, may have developed immunities to them over successive generations. Perhaps if the birds had been raised in the Okanagan, the Lower Mainland birds would have been more susceptible. As all of the birds were juveniles at the time of infection, they may have been less able to mount adequate immune responses than if they had become infected as adults. It is not known if these infections or the Baytril treatments influenced other aspects of the study.

6.2.5 Mortality and Tissue Weights

Cause of death for many of the birds in this study was not determined. Nearly 37% of the birds that were collected in 1997 survived until the time of sacrifice in 2000, whereas the rest died as a result of accidents, depredation, disease (coccidiosis), or unknown causes. Rats (*Rattus norvegicus*) were the main predators in the robin pens, and accidental deaths included drownings in water dishes, complications during blood sampling, and a variety of other misfortunes. There were significant differences in cause and age of death between the Lower Mainland and Okanagan birds, likely due to the starvation deaths of juvenile Okanagan birds. *In ovo* DDT levels did not seem to influence either cause or age of death.

The birds collected in 1998 were sacrificed less than six months after being taken into captivity, so it is not surprising that more than 90% of them survived to the time of sacrifice. Three of the birds in this group died of unknown causes, four were euthanized due to debilitating injuries, and one Okanagan bird is believed to have died as a result of a mycoplasma infection.

Among the offspring hatched by the 1997 birds, more Lower Mainland females than Okanagan females had chicks die within the first two weeks post-hatch. Again, cause of death was often not known. Chicks that disappeared were likely depredated by rats. Several chicks demonstrated bruises and wounds. Although predators may have inflicted these injuries, predators would more likely carry the chick away and eat it. One male was witnessed shaking and biting his offspring, and some females were seen pecking at young in the nest. Thus, at least some of these injuries may have been due to parental aggression. It is not known for sure how many of the injuries were incurred prior to as opposed to after death nor why the parents behaved so aggressively.

Heart weights, and to some extent liver weights, were higher in 1997 Okanagan birds than in the Lower Mainland birds. The gonads of Okanagan birds from heavily p,p'-DDE contaminated nests were lighter than those from less contaminated nests, whereas oviducts were heavier in birds from nests with high levels of o,p'-DDT. As p,p'-DDE is known to act as an anti-androgen (Gaido et al., 1997; Kelce et al., 1995; 1998), it is not surprising that high levels would suppress gonad size, at least in males. Altered gonadal morphology has been shown in gulls and chickens exposed *in ovo* or during development to DDTs (Balasubramaniam & Sundararaj, 1993; Burlington & Lindeman, 1950; Fry & Toone, 1981; Stickel, 1973). Ortho,para'-DDT and other forms of DDT, on the other hand, are estrogen agonists and have

been shown to influence the female reproductive system in a number of species (Fry & Toone, 1981; Gaido et al., 1997; McLachlan & Arnold, 1996; Stickel, 1973).

The livers and kidneys of the 1998 Okanagan birds were heavier than those of the Lower Mainland birds. While enlarged hearts and livers have been linked to the effects of DDT on thyroid gland functioning (Jefferies, 1975), no significant differences were found in thyroid gland weights. There could, however, have been morphological differences in the thyroid glands that were not examined. Although heavier hearts, livers, and kidneys in the Okanagan birds may represent genetic differences between the two groups of birds, the birds collected in 1997 did not exhibit the same asymmetries as the 1998 birds. Age may play a role in this year effect, as the 1997 birds were sacrificed as adults but the 1998 birds were sacrificed as juveniles.

Among the offspring of the 1997 birds, the young of Okanagan parents tended to have heavier kidneys than those of Lower Mainland or mixed type parents. Same type parents had young with heavier body weights at the time of sacrifice, whereas different type parents had young with heavier brains. Although these results suggest parental influences on offspring physiology long after the birds have become independent and second generation effects of early contaminant exposure, the sample size was quite small and included siblings. Thus, no clear conclusions can be drawn.

6.3 Problems

Like all research, this study had its share of difficulties. Although not all factors can be controlled in an experiment, there are some things that could perhaps have been done differently and some things that could not be helped. For example, the birds in this study were exposed to a variety of chemicals in addition to DDT. It would be virtually impossible to find a wild species that was not previously exposed to pesticide contamination to some extent.

Collecting eggs and chicks from nests in the wild is a time-consuming and costly enterprise. These problems were exacerbated by the fact that peak egg laying periods do not occur at the same time in the Okanagan and Lower Mainland (Campbell et al., 1997). Sample sizes may not be equal and it may not be possible to always get both an egg and chicks from the same nests as a result of timing problems, difficulty finding the nests, and depredation of nests. Although it would have been nice to analyze contaminant levels in all of the eggs individually, this was not possible due to the high costs involved and the fact that very low levels of DDTs were expected to be found in the Lower Mainland eggs.

Inconsistencies between years may have played a role in some of the reproduction and behavior results found in this study. Not only were the 1997 birds inexperienced during their first breeding season, so was the experimenter. Thus, some of the behavioral scoring may have changed slightly between years. The pens underwent major renovations prior to the second breeding season including the elimination of the indoor pens, improvements to the outdoor pens to ensure more consistency between pens, and higher vigilance against rats. Environmental effects such as temperature and weather conditions may have influenced the outcome of this study, but they were not taken into account as both Lower Mainland and Okanagan birds would have been exposed to the same conditions.

The original intent of this study was to examine the effects of DDT as an endocrine disruptor. Unfortunately, there was a miscommunication with the laboratory conducting the hormone assays and the plasma samples were analyzed for thyroid hormones rather than estradiol and testosterone. While this opened an exciting new avenue for research, it required a dramatic shift in the focus of the study. Given some of the differences seen in reproduction and behavior, it would have been interesting to relate them to differences in gonadal hormone levels.

The main problem of concern in this study is the possibility that genetic differences between the Lower Mainland and Okanagan birds may overshadow any effects early DDT exposure may have had. It is likely that the Lower Mainland and Okanagan birds represent two different subspecies of American robin (Aldrich & James, 1991; Sallabanks & James, 1999). While this may be an arbitrary distinction, based on geographical location and differences in morphometric measurements and plumage (Sallabanks & James, 1999), it could potentially account for many of the differences seen between the two groups. It would be interesting to see how closely related the two groups are genetically. In order to avoid this problem, one would have to dose relatively clean Lower Mainland birds with DDT or use only birds from the Okanagan. Elliott et al. (1994) compared birds from conventionally managed Okanagan orchards that had been treated with DDT to ones from organic orchards that had not been exposed to pesticides for at least five years. The present study, however, does demonstrate that the two sub-species can inter-breed.

6.4 Implications

Despite its shortcomings, this study provided valuable insights into the effects of DDTs on a model passerine. The effects of direct exposure to DDT, in its various forms, have been

broadly studied, but studies on the more long-term, latent effects as seen in second-generations have been sparse. This study indicated that long-term, latent effects do exist but may be subtle, and may primarily be seen in highly contaminated birds. Although it is not clear how much influence genetics had on the findings presented here, based on the number of significant correlations with *in ovo* DDT exposure, it is obvious that DDTs have the potential to play a role in several aspects of these birds' lives. The robins in this study are interesting in that they were exposed not only to *in ovo* contamination via the egg yolk, but also directly through their ingestion of contaminated earthworms during their first ten days post-hatch. Thus, they may demonstrate both long-term effects, and as chicks, relatively immediate effects. The long-term effects of DDT exposure may rely on a number of different mechanisms or pathways. DDTs may exert their influences through their alterations of enzymes, genes, hormones, neurotransmitters, and other cells, resulting in changes in the growth and development of organs, tissues, and glands. These changes, in turn, may affect survival, reproduction, behavior, and other functions. Alterations in one system can have profound effects on other systems. Therefore, it is unlikely that the effects early DDT exposure had on these birds can be attributed to particular mechanisms.

On a population level, American robins from the Okanagan do not appear to be detrimentally affected by early DDT exposure. They continue to thrive and reproduce despite their high levels of contamination. After several generations of DDT exposure, robins in the Okanagan may have developed a tolerance or resistance to the effects of DDT, similar to that seen in other species (WHO, 1989). This ability to accumulate DDTs, however, makes these birds potential sources of secondary poisoning for their predators, in much the same way as the earthworms they themselves consume. This is especially problematic for susceptible raptorial species such as the peregrine falcon *(Falco peregrinus*; DeWeese et al., 1986; Mora, 1997). DDT levels as low as 1 μg/g can be detrimental to peregrines (Mora, 1997), and levels found in robins and other prey species are often considerably higher than that (Blus, 1996; Harris et al., 2000)

A number of questions have arisen from this study that warrant further investigation. For example, it would be worthwhile to see if the collected tissues from these birds still contain detectable levels of DDTs. It has been suggested that in some species it can take up to 988 days for 95% of a bird's body burden of DDT to be eliminated (Stickel et al., 1984). It would also be interesting to determine if DDT exposure during development influenced the histology of the gonads, brain, thyroid, and adrenal glands, as these are all potential targets of DDT and

alterations in their development can have profound effects on the animal throughout life (Biesmann & von Faber, 1981; Fry & Toone, 1981; Jefferies, 1975; Kelce et al., 1998; Latimer & Siegel, 1974; Lorenzen et al., 1999; Parmigiani et al., 1998; Spyker, 1975). Dosing studies could be employed in order to determine if there are, in fact, threshold levels above which robin behavior, reproduction, and other functions are detrimentally affected. Dosing the control birds with DDT/DDE or feeding them earthworms from the Okanagan would demonstrate whether all robins are somewhat immune to the effects of DDTs or if this is a trait found only in birds that have been exposed over several decades.

Raising the birds in captivity allowed for the measurement of a number of parameters that would have been very difficult to study in the field. Even more detailed studies could be undertaken in a future captivity study in order to monitor hormone levels, examine behaviors such as mate choice and aggression against intruders, and look at other immune system parameters. Although time-consuming, it would also be interesting to monitor robins in the wild after they have fledged. Adults and chicks could be banded and their reproductive success observed over several years, assuming they return to the same breeding areas.

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Appendix I

Mean organochlorine and polychlorinated biphenyl (PCB) levels (μg/g, wet weight) found in American robin eggs collected from the Okanagan and Lower Mainland in 1997.

	Okanagan† **n = 31**	**Lower Mainland†** **n = 3**
1,2,4,5-tetrachlorobenzene	< 0.00010 (0)	< 0.00010 (0)
1,2,3,4-tetrachlorobenzene	< 0.00010 (0)	< 0.00010 (0)
pentachlorobenzene	< 0.00010 (0)	< 0.00010 (0)
α-hexachlorocyclohexane	< 0.00010 (0)	< 0.00010 (0)
β- hexachlorocyclohexane	< 0.00010 (0)	< 0.00010 (0)
γ- hexachlorocyclohexane	< 0.00010 (0)	< 0.00010 (0)
hexachlorobenzene	0.00010 (1)	0.00053 (2)
octachlorostyrene	< 0.00010 (0)	< 0.00010 (0)
heptachlor epoxide	0.00033 (3)	0.0024 (3)
oxychlordane	0.00086 (2)	0.0030 (1)
trans-chlordane	< 0.00010 (0)	< 0.00010 (0)
cis-chlordane	< 0.00010 (0)	< 0.00010 (0)
trans-nonachlor	0.0026 (11)	0.0022 (3)
cis-nonachlor	0.0026 (8)	0.0090 (2)
dieldrin	< 0.00010 (0)	< 0.00010 (0)
photomirex	< 0.00010 (0)	< 0.00010 (0)
mirex	< 0.00010 (0)	< 0.00010 (0)
tris (4-chlorophenyl) methanol	0.055 (15)	0.00070 (1)
p,p'-DDT	12.08 (31)	0.14 (3)
p,p'-DDE	51.66 (31)	1.89 (3)
p,p'-DDD	1.01 (31)	0.0090 (3)
o,p'-DDT*	0.049 (23)	< 0.00010 (0)
o,p'-DDE*	0.0024 (14)	< 0.00010 (0)

o,p'-DDD*	0.0025 (15)	< 0.00010 (0)
total organochlorines	64.87	2.05
trichlorobiphenyls[1]	0.00029 (3)	< 0.00010 (0)
tetrachlorobiphenyls[2]	0.0018 (2)	0.0016 (1)
pentachlorobiphenyls[3]	0.021 (31)	0.020 (3)
hexachlorobiphenyls[4]	0.043 (31)	0.058 (3)
heptachlorobiphenyls[5]	0.032 (31)	0.048 (3)
octachlorobiphenyls[6]	0.011 (15)	0.011 (3)
nonachlorobiphenyls[7]	0.0040 (4)	< 0.00010 (0)
total PCBs	0.11	0.14
Aroclor 1260	0.10 (30)	0.092 (3)

[†]Numbers in brackets represent the number of samples containing each chemical at levels greater than 0.00010 μg/g

*non-detects replaced with zeros in order to determine means

[1]trichlorobiphenyls = PCB 16/32, 17, 18, 22, 28, 31, 33/20

[2]tetrachlorobiphenyls = PCB 42, 44, 47/48, 49, 52, 56/60, 64, 66, 70/76, 74

[3]pentachlorobiphenyls = PCB 85, 87, 92, 95, 97, 99, 101/90, 105, 110, 118

[4]hexachlorobiphenyls = PCB 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158

[5]heptachlorobiphenyls = PCB 170/190, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187

[6]octachlorobiphenyls = PCB 194, 195, 196/203, 200, 201, 202

[7]nonachlorobiphenyls = PCB 206, 207, 208

Appendix II

Mean organochlorine and polychlorinated biphenyl (PCB) levels (μg/g, wet weight) found in American robin eggs collected from the Okanagan and Lower Mainland in 1998.

	Okanagan† **n = 31**	**Lower Mainland†** **n = 3**
1,2,4,5-tetrachlorobenzene	< 0.00010 (0)	< 0.00010 (0)
1,2,3,4-tetrachlorobenzene	< 0.00010 (0)	< 0.00010 (0)
pentachlorobenzene	< 0.00010 (0)	< 0.00010 (0)
α-hexachlorocyclohexane	< 0.00010 (0)	< 0.00010 (0)
β- hexachlorocyclohexane	< 0.00010 (0)	< 0.00010 (0)
γ- hexachlorocyclohexane	< 0.00010 (0)	< 0.00010 (0)
hexachlorobenzene	0.00045 (8)	0.00088 (2)
octachlorostyrene	< 0.00010 (0)	0.00033 (1)
heptachlor epoxide	0.00060 (8)	0.073 (1)
oxychlordane	0.0010 (5)	0.0019 (1)
trans-chlordane	< 0.00010 (0)	< 0.00010 (0)
cis-chlordane	< 0.00010 (0)	< 0.00010 (0)
trans-nonachlor	0.0023 (17)	0.0015 (4)
cis-nonachlor	0.00014 (3)	0.0011 (1)
dieldrin	< 0.00010 (0)	< 0.00010 (0)
photomirex	< 0.00010 (0)	0.0043 (1)
mirex	< 0.00010 (0)	0.011 (1)
tris (4-chlorophenyl) methanol	0.042 (19)	0.00085 (2)
p,p'-DDT	6.91 (22)	0.071 (4)
p,p'-DDE	26.22 (22)	0.85 (4)
p,p'-DDD	0.66 (22)	0.0063 (4)
o,p'-DDT*	0.046 (20)	< 0.00010 (0)
o,p'-DDE*	0.0040 (19)	< 0.00010 (0)

o,p'-DDD*	0.0043 (15)	< 0.00010 (0)
total organochlorines	33.89	0.95
trichlorobiphenyls[1]	< 0.00010 (0)	< 0.00010 (0)
tetrachlorobiphenyls[2]	< 0.00010 (0)	< 0.00010 (0)
pentachlorobiphenyls[3]	0.0046 (22)	0.0070 (4)
hexachlorobiphenyls[4]	0.0097 (22)	0.028 (4)
heptachlorobiphenyls[5]	0.0064 (22)	0.016 (4)
octachlorobiphenyls[6]	0.0013 (8)	0.0047 (4)
nonachlorobiphenyls[7]	< 0.00010 (0)	< 0.00010 (0)
total PCBs	0.022	0.055
Aroclor 1260	0.020 (21)	0.039 (4)

[†]Numbers in brackets represent the number of samples containing each chemical at levels greater than 0.00010 μg/g

*non-detects replaced with zeros in order to determine means

[1]trichlorobiphenyls = PCB 16/32, 17, 18, 22, 28, 31, 33/20

[2]tetrachlorobiphenyls = PCB 42, 44, 47/48, 49, 52, 56/60, 64, 66, 70/76, 74

[3]pentachlorobiphenyls = PCB 85, 87, 92, 95, 97, 99, 101/90, 105, 110, 118

[4]hexachlorobiphenyls = PCB 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158

[5]heptachlorobiphenyls = PCB 170/190, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187

[6]octachlorobiphenyls = PCB 194, 195, 196/203, 200, 201, 202

[7]nonachlorobiphenyls = PCB 206, 207, 208