UPTAKE, ELIMINATION AND TOXICITY OF AN ARSENIC-BASED PESTICIDE IN AN AVIAN SYSTEM

by

Courtney Anne Albert
B.Sc. Biology, University of Alberta, 2001

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the
Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

Summer 2006

APPROVAL

Name: Courtney Anne Albert

Degree: Master of Science

Title of Thesis:

Uptake, elimination and toxicity of an arsenic-based pesticide in an avian system

Examining Committee:

Chair: Dr. A. Kermode, Professor

Dr. T. Williams, Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. J. Elliott, Adjunct Professor
Department of Biological Sciences, S.F.U.

Dr. C. Kennedy, Associate Professor
Department of Biological Sciences, S.F.U.

Dr. R. Nicholson, Associate Professor
Department of Biological Sciences, S.F.U.
Public Examiner

---

2 June 2006
Date Approved

STATEMENT OF ETHICS APPROVAL

The author, whose name appears on the title page of this work, has obtained, for the research described in this work, either:

(a) Human research ethics approval from the Simon Fraser University Office of Research Ethics,

or

(b) Advance approval of the animal care protocol from the University Animal Care Committee of Simon Fraser University;

or has conducted the research

(c) as a co-investigator, in a research project approved in advance,

or

(d) as a member of a course approved in advance for minimal risk human research, by the Office of Research Ethics.

A copy of the approval letter has been filed at the Theses Office of the University Library at the time of submission of this thesis or project.

The original application for approval and letter of approval are filed with the relevant offices. Inquiries may be directed to those authorities.

Simon Fraser University Library
Burnaby, BC, Canada

Abstract

Monosodium methanearsonate (MSMA), an arsenic-based pesticide, has been used to control Mountain Pine Beetle (*Dendroctonus ponderosae*) populations throughout British Columbia for the past ten years. Cavity nesting forest birds have been shown to forage and breed in MSMA treated pine stands. This study examined the effects of MSMA using the Zebra Finch (*Taeniopygia guttata*) as a model passerine. The objectives were to determine the distribution as well as any sub-lethal toxic effects of this compound in both adults and nestlings. Zebra Finches were exposed to environmentally relevant concentrations of this pesticide. Adults showed dose-dependent trends in accumulation of arsenic in the blood and specific tissues, and weight loss occurred in adults in the two highest dose groups. Nestlings accumulated more arsenic in tissues than adults, and exhibited a high mortality rate at higher doses, which suggests high sensitivity to this pesticide in early life stages.

Dedication

To my family

Acknowledgements

I would first like to thank my senior supervisor, Dr. Tony Williams. Not only did Tony support me in my decision to switch to a more suitable program early on in my masters work, but he was very helpful and supportive throughout my whole degree, providing me with quick feedback and insightful comments on my experimental design, and on the results when the time came. I would also like to thank Dr. John Elliott, as his enthusiasm for the project provided me with ongoing encouragement, suggestions and support. I would like to thank the final member of my supervisory committee, Dr. Chris Kennedy, for his optimism and his willingness to sit down and help out a math-frazzled individual from time to time. I would also like to thank Dr. Christy Morrissey, postdoctoral researcher, project leader, and new mom! I consider myself fortunate to have had such a great role model in the field of biology.

I would like to thank Dr. Bill Cullen and Dr. Vivian Lai at the University of British Columbia, Department of Chemistry, for conducting laboratory analyses essential to this project, and for their continued advice and willingness to answer any questions I had. In addition, I would like to thank Sandi Lee at CWS for her support and knowledge in the field. I would also like to acknowledge the Animal Care Facility at SFU, Burnaby, for providing housing for the Zebra Finches.

Next I would like to thank the Williams Lab for all their help and support throughout the past 2.5 years. Thank you for listening to my talks and providing useful feedback; thank you for making feeding and cleaning days down at ACF most entertaining (Kat and Jim!). I would especially like to thank Tony, Oli, Kat, Jim and Emily for helping me out in the early stages of my Zebra Finch experiments.

I would like to thank my family and friends, who were there with continued support and love; my mom, and my dad and Colette for all their advice and support. A big big thank you goes out to Stephen Wells for his patience and for often lending an enthusiastic hand in the field and in the lab; Mark Wong for his work and sheer

entertainment in the field and lab; J. Dale, K. Gorman, H. Gill, L. Cesh, A. Pomeroy, J. (BBQ) Joy and W. Challenger for their advice and support. And of course, I would like to thank Finnigan for his unconditional love, and for staying awake while I practiced my conference and thesis talks for him.

Table of Contents

List of Tables

List of Figures

CHAPTER 1:
Introduction

1.1 Heavy Metals as Environmental Contaminants

Many heavy metals such as lead, cadmium, mercury and arsenic are ubiquitous in the environment and have both natural and anthropogenic sources (Hutchinson and Meema, 1987). The primary source of natural heavy metals in the environment is from the earth's crust, and a natural flux of metals occurs in the environment through processes such as weathering, erosion, biological activity and volcanic eruption (Garrett, 2000). Anthropogenic activities such as mining, manufacturing and agriculture have disrupted the natural cycle and increased the rate at which these metals are introduced into the environment (Fyfe, 1998).

All heavy metals, some more than others, have the potential to be toxic to exposed organisms. Thus, increasing quantities of metals in the environment has resulted in increased toxicological concern for exposed wildlife. Of particular concern is the ability of metals to bioaccumulate. Numerous studies have reported bioaccumulation of metals in birds (Birkhead 1982, 1983; Furness *et al.,* 1990; Heinz and Hoffman, 1998; Honda *et al.,* 1986 and 1990; Hulse *et al.,* 1980; Lee *et al.,* 1989; Maedgen *et al.,* 1982), mammals (Blus *et al.,* 1987; Ogle *et al.,* 1985; Wren, 1986 and 1988; Thies and Gregory, 1993) and fish (Campbell, 1994). Reported sub-lethal toxic effects resulting from exposure to metals or mixtures of metals in the environment include behavioural impairments (Burger and Gochfeld, 1997), decreased reproductive success (Heinz and Hoffman, 1998; Heinz

and Fitzgerald, 1993), neurotoxicity (Cory-Sletcha *et al.*,1985), decreased cholinesterase activity (Dieter and Ludke, 1975), immunotoxicity (Ilback *et al.*, 1991; Schulz *et al.*, 2002) and genotoxicity (De Flora *et al.*, 1994; Mass *et al.*, 2001).

1.2 Toxicity of environmental contaminants

Vital to understanding the toxicity of a chemical is the knowledge of how the chemical is absorbed, distributed, biotransformed, accumulated and eliminated, and the rates of such processes. It is important to assess the potential of a xenobiotic to be accumulated by specific tissues, the affinity of the xenobiotic to specific tissues, the metabolism of the xenobiotics, the rate of absorption as compared to the rate of elimination, the bioaccumulation factor and finally the half life of a xenobiotic in the environment. These characteristics are often dependent on the structural and chemical properties of a xenobiotic. Such properties include, but are not limited to, solubility in water, octanol-water partition coefficient (Kow), bioaccumulation factor and half-life (Klaassen, 2001). Biotransformation can greatly affect the toxicity of a chemical. Some chemicals are toxic in parent form and may be detoxified to a less toxic and more easily excreted metabolite through biotransformation. Alternately, some chemicals may be inherently non-toxic and may become activated by biotransformation, producing toxic metabolites (Klaassen, 2001).

Physiological effects of contaminants can occur at the cellular level, the organ level, and at the whole-organism level (Walker *et al.*, 1996). A contaminant may enter a cell in the body and disrupt cellular biochemical reactions by interacting with enzymes or receptors. For example, organochlorines tend to bind to lipoproteins and cell membranes, while heavy metals may interact with intracellular proteins such as metallothionein.

Contaminants may also have an affinity for a specific organs and tissues where they will accumulate after being circulated in the blood (Walker *et al.,* 1996). Contaminants can also express their final toxicity in an organism by affecting behaviour, such as reproductive, foraging and anti-predator behaviour. This not only affects the success of the individual but can also the success of a population (Clotfelter *et al.*, 2004). Several studies have documented the effects of contaminants on the reproductive behaviour of fish (Jones and Reynolds, 1997) and birds (Heinz, 1974; Fry, 1995; Stanley, 1994). In addition, effects on foraging behaviour in fish (Kislalioglu *et al.*, 1996) and birds and effects on antipredator behaviour in fish (Little *et al.,* 1990) and birds (Heinz *et al.,* 1979) have been shown.

1.3 Arsenic in the Environment

Arsenic is an element found in soil, water, air, plants and all living tissue (Woolson, 1975). Large quantities of arsenic are released into the environment anthropogenically, primarily through agricultural use, including the application of pesticides (Woolson, 1975). The increase in evironmental arsenic has become a concern to non-target organisms in both aquatic and terrestrial environments. Previous studies have reported accumulation of arsenic in animals in areas of high anthropogenic activity, and acute and chronic toxicity due to arsenic exposure in laboratory animals (NAS 1977; NRCC 1978; Hughes 2002).

The chemistry of arsenic is complex, having trivalent and pentavalent, inorganic and organic forms. Arsenic may undergo oxidation, reduction and

biomethylation as a result of interaction with microorganisms in soil, freshwater or seawater (Cullen and Reimer, 1989). Biotransformation of arsenic can occur *in vivo*, resulting in monomethyl arsenic (MMA (V)) and dimethylarsenic (DMA (V)), with the liver as the primary site for methylation in most tested species (Marafante, 1985). DMA (V) is the primary metabolite for most mammals (Yamauchi and Yamamura, 1985).

The species of arsenic distributed within tissues and excreted depends on the chemical species administered. Several studies have examined the tissue distribution and chemical speciation of animals dosed with inorganic arsenic. Rats dosed with inorganic arsenic appear to have predominantly dimethylated arsenic in the blood, liver and kidney, and excrete primarily inorganic arsenic (Odanaka *et al.*, 1980), whereas rats dosed with MMA (V) have shown MMA (V) as the predominant form of arsenic in the liver and kidney (Suzuki *et al.*, 2003), and excrete primarily MMA (V) and DMA (V) (Yoshida *et al.*, 1998; Suzuki *et al.*, 2003). Similarly, rats dosed with DMA (V) show DMA (V) as the primary form in the liver (Suzuki *et al.*, 2003) and initially excrete predominantly DMA (V) (Yoshida *et al.*, 1997; Suzuki *et al.*, 2003). In addition, tissue retention of arsenic may operate depending on whether the administered arsenic is in the methylated or inorganic form. Methylated arsenicals tend to be eliminated faster, and have less affinity for, and therefore less accumulation in, the tissues and blood (Hughes and Kenyon, 1998). An inhibition of arsenic methylation in mice and rats has been shown to increase tissue retention (Marafante *et al.,* 1985). Rats dosed with inorganic arsenic retained 70-80% of the given dose (Vahter, 1981) while rats

dosed with DMA (V) retained only 10-20% (Stevens, 1977). These results suggests that the amount of arsenic being taken up and distributed to the tissues or excreted highly depends on the form administered, and that methylated arsenicals are more readily excreted than inorganic arsenic.

The metabolism of arsenic plays an important role in the toxicity of this metalloid (Hughes 2002). In the past it was thought that the biomethylation of inorganic arsenic was solely a detoxification process because DMA (V) is less toxic and more readily excreted than inorganic arsenic (Marafante *et al.,* 1987; Hughes and Kenyon, 1998). However, it has since been discovered that this metabolic pathway involves a reduction to trivalent methylarsenicals such as monomethyl arsenous acid (MMA (III)) and dimethyl arsenous acid (DMA (III)), both of which have direct genotoxic actions (Mass *et al.* 2001). In addition, MMA (III) is more acutely toxic than arsenite (As (III)) (Cullen *et al.,* 1989; Petrick *et al.* 2000; Styblo *et al.* 1995) and DMA (V) has carcinogenic properties (Wei *et al.,* 1999). Thus, methylation of arsenic is not solely a detoxification method, and may in some cases be bioactivating.

1.4 Mountain Pine Beetle (*Dendroctonus ponderosae*)

The Mountain Pine Beetle (MPB) (*Dendroctonus ponderosae*) is a species of beetle native to North America (Furniss and Carolin, 1980). The MPB attacks primarily lodgepole pine (*Pinus contorta*) (Dost, 1995). Female adult MPBs initially attack pines by seeking out mature, large diameter trees in the mid to late summer (Raffa ,1987). Beetles bore into the tree and infect the tree with their symbiotic fungi. If the attack is

successful, the fungus will impede the tree's circulatory system by blocking xylem translocation of defensive resin, weakening the tree's resistance to the spreading fungus (Raffa, 1987). In British Columbia the mountain pine beetle is the most damaging forest pest, killing extensive tracts of mature pine trees (Furniss and Carolin, 1980; Dost, 1995). The lodgepole pine is the most abundant conifer in British Columbia and is an important timber source, with large-scale mortality of the lodgepole pine posing a long-term threat to British Columbia's forest economy as well as challenges to forest managers and planners (Cole *et al.*, 1985; Patriquin *et al.*,2005).

Management strategies have been implemented to control Mountain Pine Beetle infestations, including preventative methods such as small patch harvesting of susceptible pine trees, and direct control methods such as semiochemical baiting, falling and burning and the use of pesticides (Dost, 1995). In British Columbia, monosodium methanearsonate (MSMA) has been used as a pesticide to control the mountain pine beetle (Dost, 1995). The most common method is to use MSMA in combination with semiochemical baiting. Specific trees within a susceptible pine stand are baited with an aggregation pheromone in early to mid summer, just before beetle flight. Baiting the trees predisposes them to attack. In late summer, forest surveyors will determine whether the baiting has been successful and the baited trees have been attacked. MSMA is then applied to the baited and attacked trees, with the intention of killing all of the beetles, and preventing spread of beetles to neighboring trees within the stand (Dost, 1995). Various factors can alter the effectiveness of the treatment, such as moisture level (Woolson, 1975) and timing of treatment (Machlauchlan *et al.,* 1988).

Despite efforts to control the mountain pine beetle, British Columbia is experiencing the largest outbreak in recorded history (Patriquin *et al.*, 2005). The province has implemented an action plan with the goal to mitigate the impacts of the epidemic on forests and communities. The role MSMA will play in this plan has yet to be explicitly stated.

1.5 Zebra Finches as a Model Laboratory Species

Birds have been used successfully as bioindicators or monitors to determine environmental contamination (Furness, 1993). However, assessing toxic effects of specific metals can be challenging using wild populations of birds due to several confounding factors. While blood is relatively easy and safe to obtain, blood samples provide information only on short-term exposure of metals (Furness, 1993). Tissue sampling is often necessary to determine longer term exposure and physiological and histological effects of contaminants, however, it is neither practical nor ethical to kill large numbers of wild birds for this purpose (Furness, 1993). Background levels of metals in the tissues due to natural environmental exposure and not pollution, as well as seasonal changes in body mass can both affect perceived body burdens (Furness, 1993). Furthermore, to fully understand the kinetics and possible toxic effects of a chemical, it is important to collect data on the absorption and elimination rates, which would likely be challenging in a field situation.

Therefore, in order to gain a complete understanding of the toxic effects of arsenic-based pesticides to wild bird populations, laboratory studies using a suitable model species are necessary. Our goal in this study was to determine the effects of arsenic-based pesticides on cavity nesting forest birds such as woodpeckers and

chickadees. Past studies looking at effects of contaminants on birds in a laboratory setting have generally used quail or ducks as an avian species (Heinz, 1979; Hoffman, 1991; MMA Task Force Three, 1993). However, results obtained from these species may not be properly extrapolated to the cavity nesting forest birds described in this study due to various differences in both behaviour and physiology. Laboratory colonies of Zebra Finches have successfully been used in several areas of research, including toxicology (Scheuhammer, 1987; Dauwe *et al.,* 2002; Gill *et al.,* 2004). Zebra Finches are small granivorous passerines. Wild populations of Zebra Finches are distributed throughout Australia and many of the islands that make up the Lesser Sundas archipelago (Zann, 1996). They are grass seed specialists, and rarely eat insects, even during the breeding season. However, they have been found to switch to green, half-ripe seeds on grasses rather than dry seeds on the ground during breeding to increase nutrient quality (Zann, 1996).

In this study, Zebra Finches proved to be tolerant of handling and experimental manipulation, such as oral dosing, blood sampling, and immune function testing. Furthermore, they breed rapidly and successfully in the laboratory, despite continuous handling. Thus, Zebra Finches were a suitable laboratory model avian species for determining toxic effects of an arsenic-based pesticide to cavity-nesting forest birds.

1.6 Thesis Structure

The research described in this thesis addresses two main questions, which will be discussed in the following two chapters. All research was conducted using a laboratory colony of Zebra Finches (*Taeniopygia guttata*) as a model species to represent passerine songbirds. Chapter two describes the uptake, elimination and sub-lethal toxicity of

environmentally relevant concentrations of monomethylarsonic acid (MMA (V)) in the adult Zebra Finch. MMA (V) is equivalent to monosodium methanearsonate (MSMA) at physiological pH, and is also the form of arsenic detected in mountain pine beetles exposed to MSMA. Chapter three describes the effects of MMA (V) on the growth and general health of nestling Zebra Finches. Parameters measured include weight gain per day, body mass and tarsi length as a measure of overall skeletal size at day 21 (fledging), and immune function using the phytohaemagglutinin (PHA) cell-mediated immune function test. Chapter three also attempts to describe the sensitivity of nestlings to environmentally relevant concentrations of MMA (V) as compared to the adults. These data are important for understanding general toxicity of MMA (V) in birds, as well as possible effects on reproduction and survival of avian populations exposed to MMA (V) via ingestion of contaminated bark beetles.

CHAPTER 2:
Uptake, elimination and toxicity of monosodium methanearsonate (MSMA) in adult Zebra Finches (*Taeniopygia guttata*): implications for insectivorous passerines in MSMA-treated forests of British Columbia, Canada

2.1 Introduction

Monosodium methanearsonate, or MSMA, is the salt of methylarsonic acid (MMA), and is a highly water-soluble crystalline solid containing arsenic in the pentavalent form. MSMA has been used in the past as an herbicide and defoliant, and has been recently used to control Mountain Pine Beetle (MPB) (*Dendroctonus ponderosae*) outbreaks in pine forests across British Columbia. The commercial formula of MSMA used in British Columbia is called Glowon®, and approximately 960 kg (6.6 lbs MSMA/gallon Glowon®) of this product has been used annually in British Columbia (Dost, 1995). In the Cascades Forest District alone, over 60,000 trees were treated between 2000 and 2004 (Morrissey *et al.*, 2006). Despite the heavy usage of this compound, there is little knowledge of the fate or effects to exposed non-target avian wildlife. Laboratory studies using terrestrial mammals show high variation in acute toxicity of MSMA, with LD_{50} values ranging from 102 mg/kg for domestic rabbits (*Oryctalagus cuniculus*) over a 14 day period (Jaghabir *et al.*, 1988) to 250 mg/kg for cattle (Dickenson, 1972) and 1800 mg/kg for white mice (duration of treatment not specified) (Dickenson, 1972). Sublethal effects of MSMA generally include gastroenteritis, diarrhea and depressed activity level in rabbits (*Oryctalagus cuniculus*) (Jaghabir *et al.*, 1988) as well as decreased hematocrit

and blood glucose levels in white-footed mice (*Peromyscus leucopus*) (Judd, 1979) and reduced reproductive capabilities in laboratory strains of mice (Prukop and Savage, 1986). There are limited data on the toxicity of MSMA to birds. For 17-week-old Bobwhite Quail (*Colinus virginianus*) the acute 96 hour LD_{50} was 834 mg/kg MSMA. Oral acute 96 hour LD_{50} values in 10-day-old Bobwhite Quail were approximately 650 mg/kg/day (MMA Task Force Three, 1993).

Very few studies have examined tissue distribution and speciation of MSMA in any terrestrial animal. Studies using laboratory or domesticated mammals suggest that orally administered MSMA can increase arsenic levels transiently, sometimes to high concentrations, in specific tissues, but most of the arsenic (> 90%) is rapidly excreted, and that tissue levels decrease to normal upon cessation of dosing (Yamauchi *et al.*, 1988; Shariatpanahi and Anderson, 1984). In addition, intravenous injection with methylated arsenicals such as metabolites of MSMA including dimethylarsinic (DMA (V)) and monomethylarsonic acid (MMA (V)) can result in uptake by the liver in intact forms, and excretion in urine and feces shortly after in the same intact forms (Suzuki *et al.,* 2004).

It is clear that the toxicity of MSMA varies with animal species. Additionally, the toxicity of this compound depends on the formation of toxic metabolites during *in vivo* metabolism. It is well known that the toxicity of arsenic depends on the species of arsenic, the oxidation state and animal species exposed. In addition, there is plenty of evidence that trivalent arsenicals are more cytotoxic than pentavalent arsenicals (Yamauchi and Fowler, 1994; Styblo *et al.*, 2000) and among the methylated arsenicals, methylarsonous acid (MAs^{III} or MMA (III)) has been shown to be the most toxic species (Cullen *et al.*, 1989; Petrick *et al.*, 2000; Styblo *et al.*, 2000; Mass *et al.*, 2001).

Biotransformation of MSMA and other methylated arsenicals occurs following ingestion by an animal, and DMA is the most common metabolite and the primary excretory form in mammals (Yamauchi and Yamamura, 1985). Therefore, while MSMA may be less acutely toxic than other arsenic species, oral administration of MSMA may produce more toxic metabolites, such as MMA (III), during biotransformation to DMA *in vivo* (Figure 2.6).

Exact mechanisms of toxicity of MSMA to exposed animals are not well known. In general, arsenic is a toxic metalloid with multiple effects. It has been suggested that arsenic is directly toxic through binding to sulfhydryl groups in proteins and altering their functions (Aposhian, 1989). The speculated mechanism of toxicity for inorganic pentavalent arsenic (arsenate) involves the replacement of phosphate in many biochemical reactions, which may be due to the similar structure and properties of arsenate and phosphate (Dixon, 1997). The result is arsenolysis, a term used to describe the uncoupling of *in vitro* formation of adenosine-5′-triphosphate (ATP) at both the substrate and mitochondrial level, ultimately reducing ATP levels (Hughes, 2002). The mechanism of toxicity for inorganic trivalent arsenic (arsenite) involves the interaction with critical sulfhydryl groups or thiols on molecules such as glutathione (GSH) (Scott *et al.*, 1993) inhibiting biochemical reactions and ultimately leading to toxicity (Hughes, 2002). Interestingly, it has also been suggested that GSH may actually decrease the toxicity of arsenic through the direct binding, reducing its toxic potential in other reactions (Huang *et al.*, 1993). Methylated trivalent arsenicals such as MMA (III) can inhibit enzymes such as GSH reductase (Styblo *et al.*, 1997), altering cellular redox reactions and leading to cytotoxicity (Hughes, 2002). Carcinogenicity of arsenic is well

known in humans and speculated in many animals (Hughes, 2002). Arsenite has been shown to be genotoxic, inducing deletion mutations in hamster-human hybrid cells (Hei *et al.*, 1998). Methylated pentavalent arsenicals have also been shown to be genotoxic, with effects such as excess tetraploidy (Kuroda *et al.*, 2004) and mitotic arrest (Eguchi *et al.*, 1997). In addition, DMA (V), MMA (III) and DMA (III) have been shown to increase cell proliferation in the development of cancer (Wanibuchi *et al.*, 1996; Vega *et al.*, 2001), and DMA (V) has been shown to be a tumor promoter in several organ tissues of the mouse and rat (Yamamoto *et al.*, 1995; Wanibuchi *et al.*, 1996).

In British Columbia, the primary non-target species of concern with MSMA exposure are woodpeckers and other cavity nesting forest birds that feed on the Mountain Pine Beetle. Woodpeckers are known to have winter diets of up to 99% beetle adults and larvae (Crockett and Hansley, 1978), with Three-toed (*Picoides tridactylus*) and Hairy (*Picoides villosus*) woodpeckers ingesting many thousands of beetles and larvae per day (Koplin, 1972). In areas where Mountain Pine Beetle outbreaks occur, woodpeckers locally increase in number (Rust, 1929 and 1930; Baldwin 1960; Koplin, 1972), which suggests that they are attracted to areas of high beetle density. Recent studies in the Merritt forest district of British Columbia have shown that arsenic can accumulate in beetles in MSMA treated stands of pine trees, and that Three-toed and Hairy woodpeckers forage in those pine stands (Morrissey *et al.*, 2006).

The primary goal of the research described in this study was to determine the uptake, elimination and toxicity of MSMA in passerines using the Zebra Finch (*Taeniopygia guttata*) as a model species. Our main objectives were a) to measure uptake, excretion and metabolism of arsenic in adult birds orally dosed with

different concentrations of MMA (V) (0, 8, 24 and 72 μg/g, respectively), b) to determine speciation of arsenic in tissues and excreta of Zebra Finches orally dosed with MMA (V), and c) to speculate on the implications for passerines exposed to MSMA. We hypothesize that if birds were metabolizing MMA (V) we would expect to see metabolites such as DMA in the excrement. If little to no metabolism of the administered MMA (V) occurred we would expect to see MMA (V) excreted in its original form. MMA (V) was used in this study as it is equivalent to MSMA at gastrointestinal pH (Dost, 1995), and is the primary form of arsenic detected in the bark beetle samples collected in MSMA treated pine stands in British Columbia (Morrissey *et al.*, 2006).

2.2 Methods

2.2.1 Animals and husbandry

The study was conducted using a laboratory colony of Zebra Finches (*Taeniopygia guttata*) maintained at the Simon Fraser University Animal Care Facility, Burnaby, British Columbia. Finches were maintained in Canadian Committee on Animal Care (CCAC) accredited facilities, with a constant 14 hr light: 10 hr dark cycle at 19-23 °C and 35-55% humidity. Experiments and animal husbandry were conducted under a Simon Fraser University Animal Care Committee permit (666B-03). All work was done following CCAC guidelines. For these experiments, birds were held in cages (61 x 46 x 41 cm) with 3-6 birds per cage. All birds were allowed free access to a mixed seed diet (Panicum and white millet, 50:50; approximately 12.0% protein, 4.7% lipid; Just For Birds, Vancouver) and water, grit and cuttlefish bone *ad libitum*.

2.2.2 Experimental groups and dosing procedure

For this study, 21 female Zebra Finches were randomly assigned to one of four experimental groups: a) low dose, 8.0 μg MMA/g BW/day (1 cage x 6 birds); b) medium dose, 24.0 μg MMA/g BW/day (1 cage x 6 birds); c) high dose, 72.0 μg MMA/g BW/day (1 cage x 6 birds); and d) a control group dosed with de-ionized distilled water only (1 cage x 3 birds). See section 2.2.3. for dosage rationale.

Technical grade methylarsonic acid (MMA (V)) was used in this study. Solution concentrations were calculated assuming a Zebra Finch would receive 0.1 ml of the MMA (V) solution each day, and this was based on a 15 g Zebra Finch. A stock solution of the high (72 μg/g) concentration was made and serial dilutions were carried out to make the medium (24 μg/g) and low (8 μg/g) solutions. For the stock solution, the compound was dissolved in de-ionized distilled water (0.648g of MMA (V) per 60 ml water for stock solution). In order to validate the dosing solution concentrations, the solutions were analysed (University of British Columbia, Dr. William Cullen) prior to administration. Birds were fasted overnight and dosed between 08:00-10:00 PST for 14 days. Approximately 0.1 ml of the MMA-water solution was administered via intubation. Exact administered volumes of the solution were separately calculated within each dose group for each bird depending on individual body mass. Birds were orally dosed daily using a micropipette for the early stages and a syringe with a gavage attachment once birds reached an appropriate size (approximately 10 days old). Spillage was negligible and we can assume there were no discrepancies in the amount of MMA solution administered to the birds. Birds were given food and water between 10 and 20 minutes

after being dosed. Body mass and general health of each bird was observed and recorded daily. Wax paper was laid down immediately after dosing to collect excreta.

Excreta samples were collected daily, approximately 24 hours after being dosed, and pooled per cage (each cage represented a separate dose group). Excreta were collected using acid-washed (10 % nitric acid for 24 hours) plastic spoons, and stored in sterile plastic bags. Excreta samples were frozen immediately after collection and freeze dried prior to analysis. It is important to note that birds excrete urine and feces together. Therefore we use the term "excreta" to represent both urine and feces.

2.2.3 Dosage Rationale and Environmental Relevance

Dose calculations were based on arsenic concentrations in bark beetles collected in the Merritt forest district, where MSMA has been used extensively, and on daily food requirements for three species of woodpeckers. Mountain Pine Beetle samples contained up to 350 μg/g dry weight total arsenic, with the monomethylarsonic acid (MMA(V)) species contributing to over 90% of the total (Morrissey *et al.*, 2006). Therefore, the dosing scheme used in this experiment was based on 0, 50, 150 and 450 μg/g in beetle prey to encompass the range of MMA (V) concentrations found in the bark beetle samples. Elemental arsenic represents 54% of the total molecular weight of MMA (V). Therefore, our actual elemental arsenic doses are approximately half of the administered MMA (V) doses. However, woodpeckers are actually ingesting primarily (> 90%) MMA (V) in the bark beetles. Thus, in order to remain consistent with our environmental relevance in this experiment, we based the dosing scheme on total ingested MMA (V).

Based on information on daily food requirements of wild insectivorous birds provided by Nagy (2001), woodpeckers require an average of approximately 10.5 g of

dry matter intake (DMI) per day. This average daily DMI was based on the average body mass of Three-Toed (*Picoides tridactylus*) (65.7g), Black-Backed (*Picoides arcticus*) (69.3g) and Hairy (*Picoides villosus*) (66.3g) woodpeckers (Dunning, 1993), which was calculated to be 67.1g. So, for example, in order to calculate a daily dose for the lowest dose group (50 μg/g) we multiplied the MMA (V) concentrations in the bark beetles by an average daily DMI (10.5 g/day), which gives a total daily MMA (V) intake of 525 μg/day for woodpeckers. We then divided the estimated total daily MMA(V) intake by the average body mass woodpecker (67.1 g) (Nagy, 2001). This yields a daily dose of approximately 8 μg arsenic/g body mass/day. Therefore, dosages were 0, 8, 24 and 72 μg/g MMA (V) per day. These doses were extrapolated to the laboratory colony of Zebra Finches based on their individual mass. Volumes administered were calculated daily based on their daily mass.

2.2.4 Blood and tissue sample collection

After 14 days of MMA (V) dosing all 21 birds were anesthetized via an intramuscular injection of 50 μl ketamine and xylazine solution (50:50 by volume; Associated Veterinary Products, Abbotsford, British Columbia) followed by exsanguination. Whole blood samples were collected to determine total arsenic content, and were taken via both the jugular and brachial veins using heparinized pipettes and transferred to heparinized centrifuge tubes. Within four hours of being collected, a subset of the blood samples (1 blood sample from each dose group, total 3 blood samples) were separated into plasma and red blood cells by centrifuging for 15 minutes at 5,000 r.p.m. (Baxter CanLab Biofuge 13) and immediately frozen at –20 C. Red blood cell samples were then freeze dried prior to analysis.

Additional blood samples were taken using a heparinized capillary tube to determine hematocrit and leucocrit. Hematocrit is a measure of the percentage of red blood cells to the total volume of a blood sample (Ots *et a.l*, 1998), and leucocrit is a measure of the percentage of white blood cells to the total volume of a blood sample (Wardlaw and Levine, 1983). Blood was kept cold and within 4 hours of collection, the blood was centrifuged for 3 minutes at 5,000 r.p.m. and the height of the leucocrit, hematocrit and total sample was then measured using a digital caliper (0.01mm). To remain consistent, all measurements of hematocrit and leucocrit were made by the same researcher.

Liver, kidney, brain and carcass tissues were obtained through dissection and were frozen at – 20 °C prior to being freeze dried and analysed for arsenic.

2.2.5 MMA (V) analysis

Arsenic species analyzed included MMA (V), which is the original dosing compound; DMA (V), which is the metabolite of MMA and is the predominant excretory form in mammals; and inorganic arsenic (As (V)).

Solutions, blood, excreta, organ and carcass tissues were sent to Dr. William Cullen's Chemistry Laboratory at the University of British Columbia, BC, where they were measured for arsenic content and speciation by Vivian W. M. Lai. Solutions were analysed before dosing to confirm MMA (V) concentrations. For total arsenic analysis, whole blood samples were analysed for each individual bird in all four dose groups, whereas excreta samples comprised pooled samples from 6 birds per cage/treatment. Excreta samples from each treatment group on MMA (V) treatment days 1, 7 and 14

were analysed for total arsenic. Organ samples were also pooled (2 birds per single analysis) to ensure adequate levels of arsenic for detection. Organ samples were analysed for all dose groups. Carcass samples were analysed individually. For arsenic speciation analysis, one plasma sample from each treatment group was analysed (n=3 plasma samples). Control samples were not analysed for speciation. Only excreta samples from the highest dose group (72 μg/g) from treatment days 1, 7 and 14 and only organs from the highest dose group were analysed for arsenic speciation.

Once organs were extracted from the bodies, the feathers, feet and beaks were removed and the remaining carcasses were freeze-dried. Individual dried carcasses were then homogenized using a food processor, taking all precautions necessary to avoid cross contamination of different dose groups.

Freeze-dried red blood cells, excreta, organ and carcass tissue samples were weighed into centrifuge tubes (either 15 mL or 50 mL) and 5 mL of a methanol/water mixture (1:1, v/v) was added to each tube. Tubes were then sonicated for 10 min and centrifuged (3,000 rpm) for 10 min, and the supernatant was removed by means of a Pasteur pipette and placed in a round bottom flask. This extraction procedure was repeated four times for each sample. The combined supernatants were evaporated to dryness and dissolved in 10 mL of deionized water prior to further analysis. Extracts were stored at -20°C and transferred to the cold room (~ 4°C) on the day of analysis (Lai *et al.*, 2004).

Plasma samples were cleaned using microcon® centrifugal filter devices (model YM-3, 3,000 NMWL, Millipore) by centrifugation for 100 minutes at 14,000 x g.

Cleaned samples were stored at -20°C and transferred to the cold room (~ 4°C) on the day of analysis (Lai *et al.*,2004).

Plasma and tissue sample extracts were analysed for arsenic speciation using High Performance Liquid Chromatography Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS). Arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of known standards. Quantification was done by comparing peaks with those of matching standards (Lai *et al.*, 2004)

Solid RBC, tissue and fecal samples were weighed into glass test tubes (outer diameter 16 mm). 2 ml of nitric acid and three Teflon boiling chips were added to each tube. The samples were heated in a test tube block heater at temperatures increasing stepwise from 70 to 150 °C and then cooled overnight. Hydrogen peroxide (2 ml) was added into the samples on the next day and the samples were heated to 150 °C until they were evaporated to dryness. The residue was redissolved in 4 ml of an aqueous solution containing 1% (v/v) nitric acid and 5 ppb Rhodium. The samples were mixed thoroughly by using a vortex mixer and filtered (0.45 um). The samples were stored at 4 °C until analysis. Digested RBC, excreta and tissue samples were then analysed for total arsenic using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Lai *et al.*, 2004)

All reagents used were of high purity, suitable for HPLC-ICP-MS analysis. Quality assurance included the analysis of the standard reference materials: dogfish muscle (DORM-2) from National Research Council, Canada and *fucus* sample (IAEA-

140) from International Atomic Energy Agency. Kelp powder was also used as a laboratory standard (Lai *et al.*, 2004).

Throughout the literature, monomethylarsonic acid is abbreviated as MMA, MAA, MMAA and MMAs. To avoid any confusion, we will use MMA throughout this paper. In addition, our analysis cannot distinguish between different valences. Therefore, we report MMA (V) and DMA (V) to represent both valences (III) and (V). The concentrations are expressed in μg/g based on dry weights (dw).

2.2.6 Statistical Analyses

A Shapiro-Wilks W test (Version 5.0, JMP, SAS Institute Inc 2003) showed that the blood arsenic data deviated significantly from normality, and therefore data were $\log_{10}$ transformed prior to analysis to meet assumptions of normality for subsequent parameteric statistical analyses. Analysis of variance (ANOVA) was applied to determine if a difference existed in total blood arsenic and percent hematocrit and leucocrit in birds exposed to control, low, medium and high levels of MMA (V). Where differences were found, Tukey's Honestly Significant Difference (HSD) tests were used to identify which treatment levels differed significantly in their response to MMA (V) exposure. To determine whether body mass changed with time among dose groups, a repeated measures ANOVA was conducted.

2.3 Results

2.3.1 General health and body mass

Zebra Finches dosed with MMA (V) did not show any signs of lethargy or diarrhea, and there was no change in the appearance or quantity of feces. One bird in the high dose group lost a large amount of weight and appeared to have a very swollen tongue by the 9th day of dosing. Mass loss over the 14-day treatment period was significantly related to MMA (V) dose (repeated measures ANOVA, $F_{3,20} = 3.85$, $P < 0.05$). Birds in the control and low MMA (V) dose groups showed no change in body mass over the 14-day treatment period (paired t-test, $P > 0.05$ in both cases). In contrast, birds dosed with 24 μg/g MMA (V) ($t_6 = 2.73$, $P < 0.05$) and 72 μg/g MMA (V) ($t_6 = 5.09$, $P < 0.01$) showed significant mass loss (Table 2.1).

Hematocrit did not differ among treatment groups (ANOVA, $P > 0.05$; Table 2.3, Fig. 2.4). The mean hematocrit was 56.7% (± 2.3%). Similarily, leucocrit did not differ among treatment groups (ANOVA, $P > 0.05$; Table 2.1, Fig. 2.1), and the mean leucocrit value was 6.3% (± 1.5%).

2.3.2 Total arsenic residues

Total blood arsenic varied significantly among the four dose groups, in a dose-dependent manner (ANOVA, $F_{3,53} = 31.7$, $p < 0.0001$; Fig.2.2). Each of the three MMA (V) treatment groups was significantly different from the control, and all treatment groups were significantly different from each other ($P < 0.05$ in all cases; Fig.2.2). The mean ratio of arsenic concentrations in the blood for low, medium and high dose groups was 1:3:9, close to that of the dosing solutions, i.e. 1:3:8. Control birds had trace amounts of

arsenic in the blood, which can likely be attributed to background levels resulting from "natural" exposure of arsenic in drinking water and food.

All organ tissues contained total arsenic residues that increased with increasing dose (Table 2.2). To ensure detection, organs from two individuals were pooled, so each value represents one pooled sample, and therefore no statistical analyses were possible (Figure 2.3). Brain tissue contained the highest levels of arsenic, followed by the kidney and finally the liver (3.7, 1.6 and 1.1 μg arsenic/g dw, respectively for the highest dose group).

Total arsenic in carcass tissues showed a difference in mean total arsenic between dose groups, with carcasses from the high dose group (72 μg/g) having significantly higher total arsenic than all other treatment groups (ANOVA, $F_{3,19} = 14.7$, $P < 0.001$; Figure 2.4). Average total arsenic and standard error for control (0 μg/g, n = 3), low (8 μg/g, n = 6), medium (24 μg/g, n = 5) and high (72 μg/g, n = 6) dosed birds was 0.24 ± 1.91 μg/g, 1.33 ± 1.36 μg/g, 3.13 ● 1.49 μg/g and 12.31 ● 1.36 μg/g arsenic, respectively.

On average, the Zebra Finches in our study retained in the tissues and organs less than 1% of the total arsenic detected over the 14 day period. The majority of the remaining arsenic is likely excreted, as the fecal samples contained the highest total arsenic concentrations. Overall, total arsenic in excreta samples increased with dose group (Figure 2.5). Excreta was pooled per cage, so again each value comprised n=1 and therefore no statistical analyses were conducted. Total arsenic detected in excreta samples from control Zebra Finches was a consistent 0.2 μg/g for days 1, 7 and 14. Low dose birds showed an initial increase in arsenic from day 1 to day 7, starting at 14.5 μg/g and increasing to 44.8 μg/g. This was followed by a slight decrease to 39.6 μg/g on day 14.

Medium dose group birds show the same trend, starting at 36 μg/g , increasing to 69.4 μg/g and decreasing to 40.2. Total arsenic in excreta samples of high dose birds, however, peaked initially at 191.6 μg/g, followed by a drastic drop to 60.8 μg/g by day 7 and then increased once again to 151.7 μg/g by day 14.

2.3.3 Arsenic Speciation

For birds in all three dose groups, MMA (V) was the predominant form of arsenic in the blood plasma, while only trace amounts of the metabolite, DMA, was detected in the medium and high dose groups (1-2%). No inorganic arsenic was found in the plasma of birds in any of the three dose groups (Table 2.3)

DMA (V) was the major form of arsenic found in the liver (83%) and kidney (61%) tissues of high dose group birds, with MMA (V) as the other predominant form detected in these tissues (Table 2.4). The brain tissue contained the original unmetabolized MMA (V) form (57%), along with a relatively large amount of DMA (V) (43%). Inorganic arsenic was only detected in the liver tissue and in trace amounts (5%).

Excreta samples from high dose birds on day 1, 7 and 14 contained arsenic primarily in the original MMA (V) form. DMA (V) was detected on day 1 in small amounts (8%), increased by day 7 (17%) and decreased by day 14 (4%). The birds excreted no inorganic arsenic during this time period.

2.4 Discussion

Female Zebra Finches dosed with MMA (V) showed body mass loss at medium (24 μg MMA/g) to high doses (72 μg MMA/g). Methylated arsenicals were detected in the blood, excreta and organ tissues in all treatment groups, illustrating that these compounds

can bioaccumulate in finches exposed to MMA (V) over a period of 14 days, at levels found in bark beetle samples in MSMA treated stands of trees. Moreover, there was a significant dose response in all samples analyzed for total arsenic. The predominant species of arsenic excreted as well as accumulated in the blood and brain tissue was MMA (V), while DMA (V) was the predominant form found in the liver and kidney tissues.

2.4.1 General Health Effects

Zebra Finches showed significant body mass loss when dosed with 24 and 72 μg/g MMA (V) during the experimental time period of 14 days (Table 2.3). Aside from this general weight loss response, one bird in the high dose group lost a much larger amount of weight and appeared to have a very swollen tongue by the 9th day of dosing. However, since this was a single occurrence, we cannot be sure this was the result of MMA (V) treatment.

These results add to the literature on health effects of MSMA exposure in laboratory animals. Documented effects include decreased hematocrits (although we did not find this in our study) and blood glucose levels (Judd, 1979) and impaired female nest-building behaviour (Lopez and Judd, 1979) in white-footed mice, as well as lowered reproductive capabilities of male mice (Prukop and Savage, 1986). Rabbits dosed with 30 and 60 mg/kg MSMA showed clinical symptoms of weakness, reduction of activity, gastroenteritis, diarrhea, depressed blood pressure and altered electrocardiogram (ECG) results (Jaghabir *et al.*, 1988). A study using dogs reported that administration of capsules containing 0, 2, 8 and 35 mg/kg/day over a 52-week period resulted in gastrointestinal irritation, frequent vomiting and diarrhea in the two highest dose groups (MMA Task

Force Three, 1993). Based on the health parameters measured in the present study, Zebra Finches dosed with MMA (V) displayed less of a response to MMA (V) exposure than other laboratory studies, with no effects on percent hematocrit or leaucocrit and a decrease in body mass in only the two highest dose groups. There are several possible physiological explanations for the relatively low acute toxicity seen in the Zebra Finches.

As previously mentioned, Zebra Finches are completely granivorous birds (Zann, 1996). Plants are a food source that produces secondary metabolites that can cause negative effects such as impaired digestion and direct toxicity (Dearing *et al.,* 2005). Herbivores have had to evolve mechanisms to mitigate the effects of these plant secondary metabolites (PSMs). One of these mechanisms is the ability to regulate the absorption of PSMs by gut cells via a glycoprotein transporter (P-gp) and a biotransformation enzyme (cytochrome p450 3A), which can work together or separately to reduce the amount of PSMs absorbed (Sorenson and Dearing, 2003). In addition to enzyme activity in the gut wall, hepatic monooxygenase activity has also been shown to be greater in herbivorous species compared to carnivorous species of birds as a result of contaminant exposure. Riviere *et al.* (1985) observed a marked increase in monooxygenase activity from the primarily herbivorous quail (*Coturnix coturnix*) liver compared to that of a bird of prey, the buzzard (*Buteo buteo*) when exposed to a PCB mixture. Cytochrome p450 has also been shown to increase in response to oral administration of MMA (V) in rats (Brown *et al.*, 1998). These molecular mechanisms appear to be unique to herbivores, and may perhaps decrease the sensitivity of herbivores and granivores such as Zebra Finches, to xenobiotics such as monomethylarsonic acid (MMA (V)) compared with species with an insectivorous diet such as the wild cavity

nesting birds discussed in this paper. Additional studies have concluded that Zebra Finches appeared to be less sensitive to xenobiotics such as organophosphate pesticides compared with other songbird species (Gill, 2003). Thus, while Zebra Finches were a suitable model species to use in laboratory studies due to their robustness and breeding success, they may be less sensitive to contaminant exposure when compared with wild insectivorous birds. This must be taken into account when extrapolating the results to wild species.

An additional explanation for the comparatively low sensitivity to MMA (V) compared with other laboratory animals may be the duration of treatment. It is possible that a 14-day dosing period is not adequate time for some health effects such as changes in immune function to become apparent. Thus, future studies using larger sample size are warranted with emphasis on determining effects of long-term exposure to MMA (V) on immune function and general health.

In general, signs of acute toxicity were not observed in the Zebra Finches. Several factors could have contributed to the results seen in this study. As mentioned earlier, arsenic toxicity involves the interaction with critical sulfhydryl groups or thiols on molecules such as glutathione (GSH) (Scott *et al.*, 1993) inhibiting biochemical reactions and ultimately leading to toxicity (Hughes, 2002). However, it has also been shown that GSH may decrease the toxicity of arsenic through the direct binding, reducing its toxic potential in other reactions (Huang *et al.*, 1993). GSH is an important component in the metabolic conversion of arsenic to methylated forms (Styblo *et al.*, 1995; Zakharyan 1995; Yamauchi and Fowler, 1994). It has been suggested that during methylation of inorganic arsenic to methylated arsenicals, GSH and other thiol containing molecules

may become initially depleted, leading to increased toxicity of arsenic (Ramos *et al.*, 1995). In our study, since we were dosing with MMA (V), further methylation to form DMA (V) may not require as much GSH, and these pools of thiol containing proteins may not be depleted. This is further supported by our speciation results, in which we see relatively little methylation to DMA (V). These results may explain our lack of observed acute toxicity.

Another possible explanation for the lack of observable signs of acute toxicity in the Zebra Finches may be due to a possible induction of metallothioniens as a response to MMA (V) dosing. Metallothioneins (MT) are metal-binding proteins with several sulphur groups. MTs are of low molecular weight, and can act to prevent the cellular toxicity of many metals (Cherian, 1995). Arsenicals effectively induce hepatic MT, and MMA (V) has been shown to be the most effective inducer of MT (Albores *et al.*, 1992; Kreppel *et al.*, 1993). Other factors can cause MT induction such as acute stress or exposure to various organic chemicals (Dunn *et al.*, 1987). It has been shown that a decrease in dietary calcium can increase the accumulation of non-essential metals such as lead and cadmium, as well as increase marker proteins such as metallothioneins, in Zebra Finches (Scheuhammer, 1996). It is unknown whether a decrease in dietary calcium, or additional stress factors in Zebra Finches causes an increase in arsenic uptake or MT induction. However, this information would be useful in our understanding of the toxicity of this compound in Zebra Finches, and requires further study.

One marked sub-lethal effects of MMA (V) treatment we observed in adult Zebra Finches was significant body mass loss in the two highest dose groups (up to 15% of initial body mass over 14 days). There are a number of possible reasons for this. Firstly,

diarrhea can result from ingested MSMA (Jaghabir *et al.*, 1988), which could lead to dehydration as a component of mass loss. However, there were no observable differences in quantity or texture of feces or between dose groups of Zebra Finches and, furthermore, dehydration would increase hematocrit and we found no change in hematocrit with MSMA treatment. Secondly, it has been shown that ingestion of MSMA causes gastrointestinal inflammation (Jaghabir *et al.*, 1988). This may result in a decrease in appetite and subsequently decreased food intake, or malabsorption of food and nutrients, perhaps resulting in a decrease in overall body mass over a two week period. Further studies measuring food intake during MMA (V) treatment would be useful in determining the cause of this marked mass loss.

2.4.2 Absorption, accumulation and distribution of arsenic in blood and tissues

The goal of this study was to determine the distribution of MMA (V) in the Zebra Finch using a general mass balance approach by obtaining fecal samples as well as tissue samples. The exact amount of MMA (V) absorbed by the Zebra Finches is unknown. Similar studies show species differences in absorption of MMA. In sheep and goats, 90% of an oral dose of MMA is rapidly absorbed (Shariatpanahi and Anderson, 1984), while approximately only 34% of an oral dose of MMA is shown to be absorbed by the hamster (Yamauchi *et al.*, 1988).

On average, Zebra Finches dosed with environmentally relevant concentrations of 0, 8, 24 and 72 μg/g MMA (V) for 14 days excreted more than 90% of the overall total arsenic detected, with low levels of arsenic in the blood and tissues. Fecal samples from the low and medium dose groups show small amounts of arsenic on day 1, with a large increase by day 7. Results from the low and medium dose groups suggest that Zebra

Finches are initially accumulating the ingested MMA (V), and excreting less. They may then reach a threshold where tissues become saturated, at which point they start to excrete much more of the ingested MMA (V) with little absorption. Results from the high dose group do not exhibit this trend, and birds appear to excrete large amounts of the ingested MMA (V) on both days 1 and 14, with a decrease on day 7.

Zebra Finches accumulated arsenic in a dose dependent manner in all tissues, with up to 6.02 μg/g arsenic in the blood, 1.1 μg/g arsenic in the liver, 1.6 μg/g arsenic in the kidney, 3.7 μg/g arsenic in the brain and 21.04 μg/g in carcass tissues. Our results are in accord with similar studies. Broiler chickens dosed with 100 and 500 mg/kg arsinilic acid accumulated up to 2.3 and 8 mg/kg arsenic in the liver tissue, and 0.15 and 0.67 mg/kg arsenic in muscle tissue, respectively (VanderKop and MacNeil, 1989). Moreover, mallards fed 25, 100 or 400 mg/kg sodium arsenate accumulated arsenic in a dose-dependent manner, with arsenic in adult mallard livers ranging from 0.49 to 6.6 mg/kg (Stanley *et al.*, 1994).

The high excretion and low tissue retention results of the present experiment are similar to those reported by previous studies. Oral administration of a single 50 mg/kg BW dose of MMA to hamsters resulted in excretion of 94.5% (urine + feces) of the administered dose (Yamauchi *et al.*, 1988). Rats injected with MMA also directly excreted the compound in its original form, with low uptake into the tissues (Suzuki *et al.*, 2004). Similarly, sheep and goats orally dosed with 10 mg MSMA/kg BW excreted over 90% of the arsenic within 120 hours (Shariatpanahi and Anderson, 1984) and mice injected with 8.04 nmol/kg and 804 mumol/kg MMA showed rapid elimination as well, with low tissue retention (< 2% of dose) (Hughes and Kenyon, 1998). In all species

studied, concentrations in the tissues were low. When considering possible toxic effects of a chemical administered orally, it is important to assess the oral bioavailability of the chemical, as it is not necessarily the dose that drives ultimate toxicity but the amount of chemical at the site of action, such as the liver, kidney or brain (Klaassen, 2001). Zebra Finches and other species studied show low concentrations of MMA (V) in tissues with most of the arsenic being excreted in the original MMA (V) form. These results could suggest that the MMA (V) is not readily taken up by the gastrointestinal tract and is instead excreted unchanged with only trace amounts being absorbed by the GI tract and redistributed to the tissues and metabolized. Alternatively, since DMA (V) is detected in small amounts in the tissues, MMA (V) may be readily taken up by the gastrointestinal tract and then primarily eliminated in the bile with only trace amounts of the compound being taken up and metabolized by the tissues.

While there appears to be trends in tissue retention and elimination patterns of MMA (V) among species, there are differences in specific target tissues and patterns of distribution among species. For example, most of the remaining arsenic in the Zebra Finches was found in the carcass, followed in order of decreasing total arsenic by the blood, brain, kidney, and liver. In hamsters dosed with MMA (V), the highest tissue concentration of total arsenic was in the kidney, followed by the spleen, lung, skin, liver, muscle, hair and brain (Yamauchi *et al.*, 1988). In contrast, mice injected with MMA (V) had the highest arsenic levels in the carcass, followed by the liver, kidney and the lung (Hughes and Kenyon, 1998). Of the organs analyzed, the brain had the most arsenic in the Zebra Finches. Arsenic has the ability to cross the blood-brain barrier. It has been to shown to accumulate in the brain and cause neurotoxic effects in rats by altering ATP

metabolism and influencing neurons and glia (Nagaraju and Desiraju, 1993; Valkonen *et al.*, 1983). Future studies should be conducted to determine any neurotoxic effects of MMA (V) to pesticide-exposed passerines.

Timing of sampling may play a role in the distribution of arsenic in the blood and tissues. Ingested methylated arsenicals have biological half-lives of 30 hours (Klaassen, 2001). In the present study, Zebra Finch tissues and blood were sampled after two weeks of MMA (V) dosing, approximately 24 hours after the last dose. In a similar study, hamsters dosed with a single oral dose of 50 ug/g MMA were sampled after 1, 6, 12, 24, 72 and 120 hours after administration (Yamauchi *et al.,* 1988). Peak arsenic tissue concentrations occurred between 6 and 12 hours after administration, with maximum concentrations of 0.21 ug/g in the liver, 1.99 ug/g in the kidney and 0.04 ug/g in the brain. These concentrations dropped back to control levels by 120 hours (Yamauchi *et al.*, 1988). Tissue distribution results from the present study may have differed had we sampled at different times. Sampling 24 hours after the last dose may have yielded slightly lower tissue residue concentrations than if we had samples 6 or 12 hours post dose. Zebra Finches may have had time to metabolize and excrete much of the last dose by the time we collected tissues.

2.4.3 Arsenic Speciation

Monomethylarsonic acid (MMA (V)) is the predominant form of arsenic found in the plasma and feces of Zebra Finches dosed with MMA (V), while the tissues had much higher levels of DMA (V). The minimal excretion of DMA (V) following MMA (V) administration is in accord with related studies. The hamster (Yamauchi *et al.,* 1988) and human (Buchet *et al.,* 1981) excrete primarily MMA (V) following MMA (V)

administration. Hughes and Kenyon (1998) suggest that this lack of methylation to DMA (V) *in vivo* may be due to the rapid elimination of MMA (V) in urine, or the low uptake into tissues with high methylating capacities. The authors suggest that MMA (V) may not have time to undergo methylation to DMA (V), and that this methylation may not occur as quickly as methylation from arsenite to MMA (V) (Hughes and Kenyon, 1988). If the Zebra Finches are indeed absorbing the MMA (V), this may hold true for the current study, since large amounts of MMA (V) were excreted after only one dose (Figure 2.5)

A large portion of the arsenic taken up by the liver and kidney was methylated, as these tissues contained primarily DMA (V) (83% and 61% respectively). This is to be expected as these organs, especially the liver, are where most metabolism of toxins takes place. This is especially true after oral ingestion, as opposed to intravenous or intraperitoneal injection of MMA (V), where the compound is absorbed through the gastrointestinal tract and then passes to the liver before being distributed to the tissues and other organs (Marafante *et al.,* 1985). The species of arsenic distributed in the tissues and excreted by animals depends on the arsenic biotransformation abilities of individual species as well as non-biological factors, such as the chemical species of arsenic administered.

Arsenic is metabolized in the liver by way of enzymes called methyltransferases, and studies have shown that there are differences in these enzymes between animal species (Healy *et al.*, 1999). Zebra Finches in this study showed the ability to methylate MMA (V) to DMA (V), as DMA (V) was the predominant arsenic species in the liver and kidney. Similarly, other animals such as the rabbit, hamster and pigeon all have these methyltransferases in the liver (Zakharyan *et al.*, 1995 and 1996). The rat also appears to

have these enzymes (Buchet and Lauwerys 1985 and 1988; Styblo *et al.*, 1995 and 1996). In contrast, animals such as the guinea pig, marmoset monkey, squirrel monkey and tamarin lack these enzymes and are unable to methylate arsenite or MMA (V) (Vahter *et al.*, 1982; Zakharyan *et al.*, 1996).

One limitation of our study in understanding the possible toxicity of this pesticide to Zebra Finches is the inability to distinguish between oxidation states. Elucidation of valency is important, as this drives the toxicity of arsenic species. Generally, trivalent arsenicals are more cytotoxic than pentavalent arsenicals. More specifically, trivalent inorganic arsenic (As (III)) is more acutely toxic than pentavalent inorganic arsenic (As (V)) (Yamauchi and Fowler, 1994; Styblo *et al.*, 2000), as shown both *in vitro* and *in vivo*. Among methylated arsenicals, methylarsonous acid (MAs^{III} or MMA (III)) has been shown to be the most toxic species (Cullen *et al.*, 1989; Petrick *et al.*, 2000; Styblo *et al.*, 2000).

Based on the postulated metabolic pathway, MMA (V) would first be reduced to its trivalent analog MMA (III), followed by further methylation to DMA (V) and possible reduction to DMA (III) (Fig. 2.6; Cullen *et al.*, 1984). Glutathione (GSH) and other thiols serve as reducing agents, and S-adenosylmethionine (SAM) is the main methyl donor (Buchet and Lauwerys, 1988; Styblo *et al.,* 1995). In the present study, both MMA and DMA were detected in the blood, feces and organ tissues of Zebra Finches dosed with MMA (V). Thus, we suggest that Zebra Finches were exposed to the highly toxic intermediate, MMA (III), during metabolic conversion of MMA (V) to DMA (V). However, since MMA (III) is very unstable, it is likely that any MMA (III) produced was quickly methylated to DMA (V). As discussed earlier, MMA (III) has the ability to

inhibit enzymes such as GSH reductase (Styblo *et al.*, 1997), altering cellular redox reactions and leading to cytotoxicity (Hughes, 2002). This may explain the observed toxic effects, namely weight loss, in the Zebra Finches.

Inorganic arsenic detected in the liver tissue (5%) can be attributed to background levels resulting from natural exposure to arsenic in food and drinking water. Although there was no inorganic arsenic detected in fecal samples or other tissues, this is the most parsimonious explanation, as demethylation of MMA (V) to inorganic arsenic is highly unlikely (Yamauchi *et al.*, 1988; Marafante *et al.*, 1987).

2.4.4 Environmental Relevance

Blood sample results from woodpeckers exposed to MSMA via ingestion of contaminated bark beetles in the Merritt forest district in British Columbia ranged from 0.03 to 3.73 μg arsenic/g dw blood (Morrissey *et al.*, 2006). Mountain Chickadees (*Poecile gambeli*) in the same district had total blood arsenic levels of up to 2.20 μg/g (Morrissey *et al.*, 2006). Zebra Finches dosed with presumably the same environmentally relevant concentrations of MMA (V) had total blood arsenic values ranging from 0.05 to 6.02 μg/g. Based on these similar total blood arsenic results, we can assume that woodpeckers are accumulating similar tissue concentrations of arsenic in the field, and could be experiencing similar health effects, such as decreased body mass, when exposed to similar high concentrations of 24 and 72 μg/g MSMA. Health parameters examined in other studies that were not examined in the present study may be occurring in both the woodpeckers and their captive counterparts.

Although the aim of this experiment was to simulate field exposure conditions in a laboratory setting, woodpeckers and other cavity nesting forest birds are more likely to experience long-term exposure to MSMA rather than a 14-day period. In a review by Scheuhammer (1987), several studies demonstrate that long-term exposure of birds to low-levels of heavy metals such as aluminum, cadmium, mercury and lead that does not result in severe acute toxicity can result in subtle but marked toxic responses, such as reproductive dysfunction, behavioural abnormalities, and increased susceptibility to additional stressors such as disease. Zebra Finches exposed to levels of MMA (V) detected in bark beetle samples in MSMA treated stands of trees exhibited mass loss over the sub-acute time period of 14 days. However, longer-term exposure to this compound may cause profound effects that may otherwise go undetected in 14 days. Therefore, the effects of long-term exposure of MSMA and its metabolites on uptake, elimination and toxicity should be further studied. If the goal is to determine population level effects of chronic exposure to methylated arsenicals, studies carried out over more than one generation may be beneficial. The result of such exposure may better provide an understanding of the consequences for forest birds exposed to MSMA as well as forestry management implications.

2.4.5 Conclusions

Female Zebra Finches orally dosed with environmentally relevant concentrations of MMA (V) largely excreted this compound. Trace amounts of MMA (V) as well as the metabolite DMA (V) accumulated in the liver, kidney, brain and blood. MMA (V) was primarily excreted in its original form. Some of the MMA (V) was methylated *in vivo* to form DMA, which was found predominantly in the liver and kidney, as well as

appreciable amounts in the brain. The high elimination and low tissue retention results were in accord with other MMA dosing studies using different species, however, tissue distribution results differed slightly from these studies.

Accumulation of MMA (V) and DMA (V) resulted in significant mass loss in the two highest dose groups throughout the 14 day period. Hematocrit and leucocrit levels were unaffected by MMA (V) exposure. Further studies are required to determine longer-term health effects of this compound in an avian system.

Table 2.1 **Comparison of mass change, and haematological parameters in Zebra Finches dosed with MMA (V) for 14 days. Values are expressed as the mean ± standard error, and samples size is stated in parentheses. Significant values are in bold.**

Dosage Group (μg/g)	Mean Body Mass Day 1 (g)	Mean Body Mass Day 14 (g)	Mean Change in Mass (g)	Hematocrit (%)	Leucocrit (%)
0 (control)	15.65 ± 0.75 (3)	15.18 ± 0.95 (3)	0.47 ± 0.59 (3)	56.33 ± 2.77 (3)	4.67 ± 1.86 (3)
8 (low)	14.69 ± 0.53 (6)	14.16 ± 0.67 (6)	0.53 ± 0.42 (6)	54.97 ± 1.96 (6)	9.00 ± 1.32 (6)
24 (medium)	14.91 ± 0.53 (6)	13.74 ± 0.67 (6)	1.17 ± 0.42 (6)	56.98 ± 1.96 (6)	4.83 ± 1.32 (6)
72 (high)	14.87 ± 0.53 (6)	12.53 ± 0.67 (6)	2.24 ± 0.42 (6)	59.35 ± 2.40 (4)	5.5 ± 1.62 (4)
P - value	0.764	0.155	0.029	0.577	0.137

Table 2.2 **Concentrations of total arsenic detected in tissue samples of Zebra Finches dosed with MMA (V). Liver, kidney and brain values represent a pooled sample of two birds. Carcass values are expressed as the mean ± standard deviation with sample sizes in parentheses.**

	Tissue Residues (µg/g)			
Dose Group (µg/g)	**Liver**	**Kidney**	**Brain**	**Carcass**
0 (control)	0.05	0.1	0.05	0.24 ± 1.91 (3)
8 (low)	0.1	0.3	0.2	1.33 ± 1.36 (6)
24 (medium)	0.1	0.4	0.7	3.13 ± 1.49 (5)
72 (high)	1.1	1.6	3.7	12.31±1.36 (6)

Table 2.3 **Species of arsenic in plasma of adult female Zebra Finches dosed with MMA (V) for 14 days, in percentage of total arsenic detected in a plasma sample.**

	Total Arsenic (%)		
Dosage group (µg/g MMA (V))	**MMA**	**DMA**	**As**
8 (low)	100 %	0%	0%
24 (medium)	98%	2%	0%
72 (high)	99%	1%	0%

Table 2.4 **Species of arsenic in liver, kidney and brain tissues of Zebra Finches dosed with 72 µg/g MMA (V) for 14 days. Values are expressed as percentage of total arsenic detected in the tissue sample.**

	Total Arsenic (%)		
Tissue	**MMA**	**DMA**	**As**
Liver	12 %	83 %	5 %
Kidney	39 %	61 %	0 %
Brain	57 %	43 %	0 %

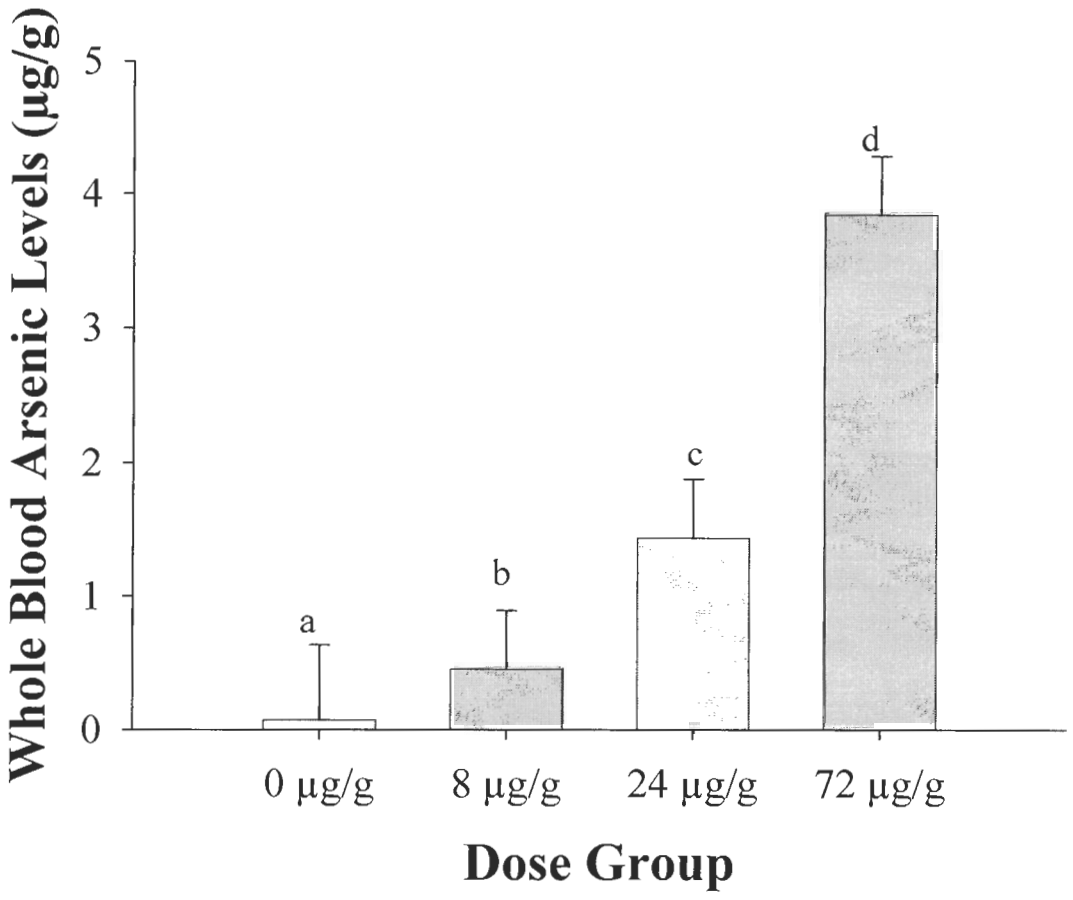

Figure 2.1 **Total arsenic detected in whole blood samples (µg/g dry weight blood) from Zebra Finches orally dosed with 8, 24 and 72 µg/g MMA (V) and a control. Mean blood values were analysed using analysis of variance (ANOVA). Values given are the mean ± standard error. Different letters indicate significant difference at $p < 0.05$.**

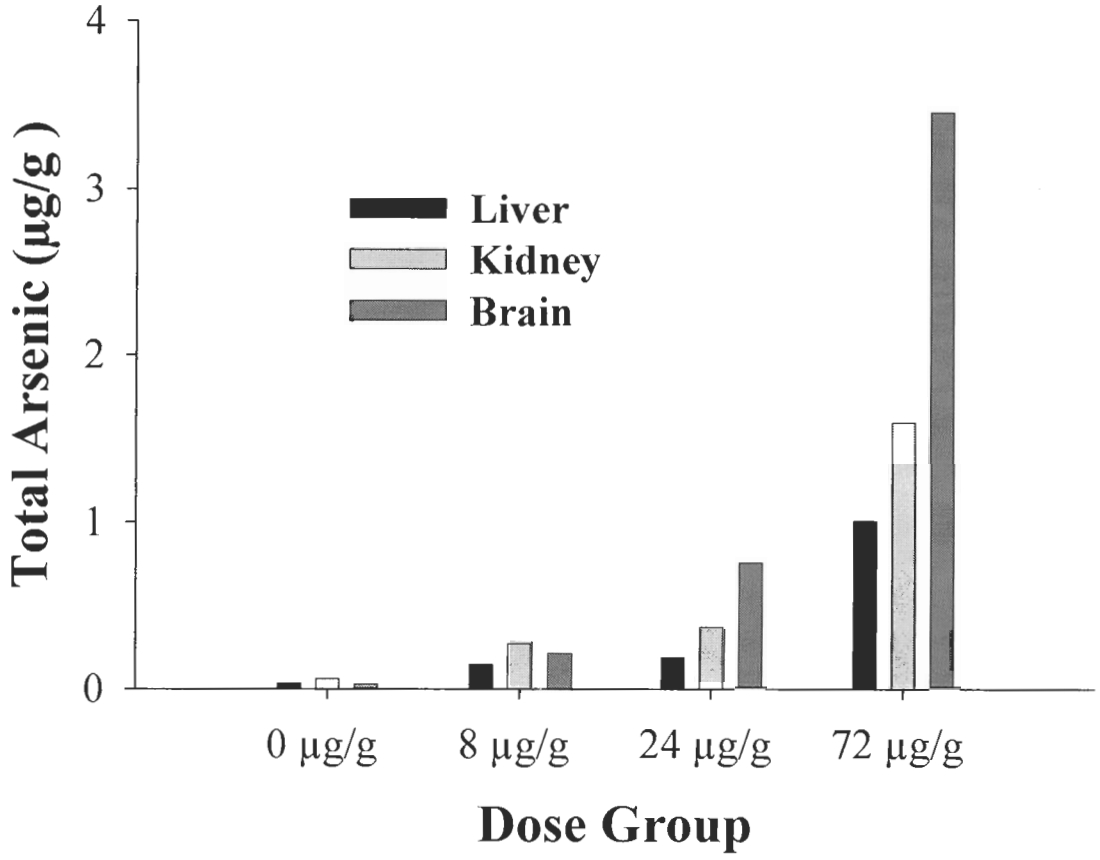

Figure 2.2 **Total arsenic detected in liver, kidney and brain samples (µg/g dry weight tissue) of Zebra Finches orally dosed with MMA (V) for 14 days.**

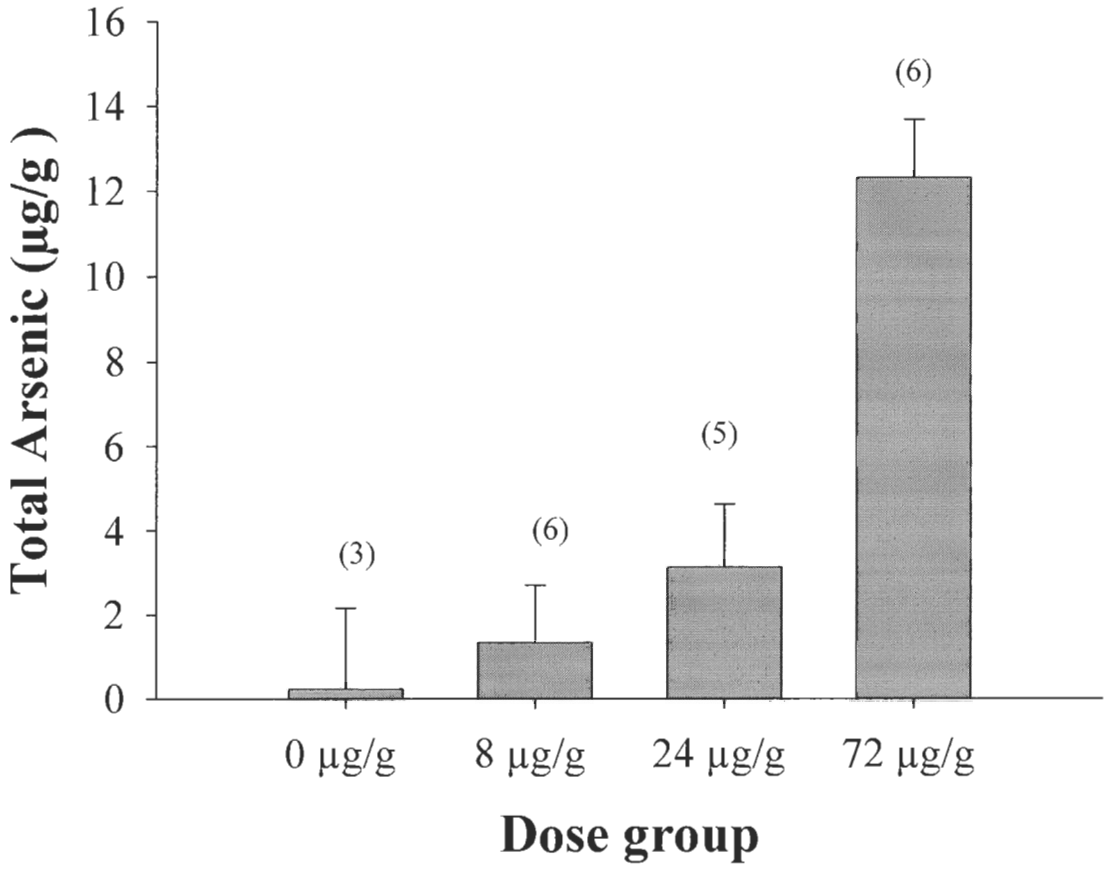

Figure 2.3 **Total arsenic in carcass tissues of adult Zebra Finches dosed with 8, 24 and 72 µg/g MMA (V) and a control. Bars represent the mean ± standard error with the sample size above each bar in parentheses.**

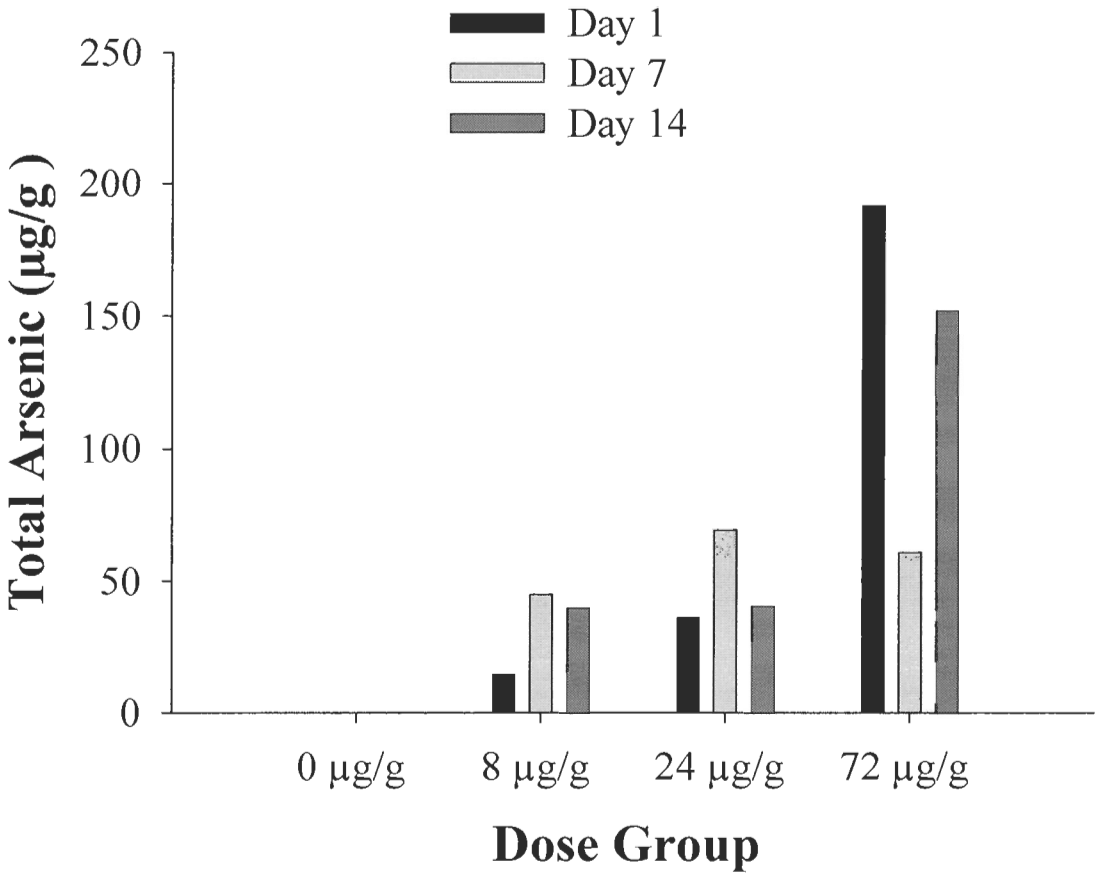

Figure 2.4 **Total arsenic detected in excreta samples of Zebra Finches dosed with MMA (V) on day 1, 7 and 14.**

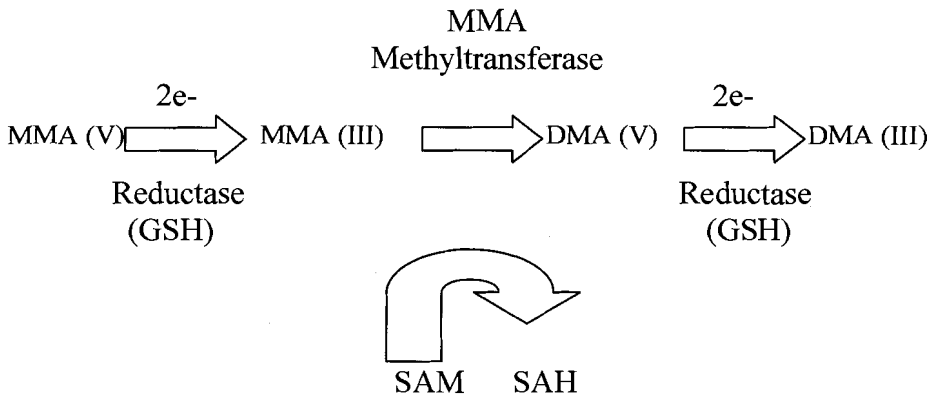

Figure 2.5 **Proposed metabolic pathway: MMA (V) is first reduced to MMA (III), followed by further methylation to DMA (V) and possible reduction to DMA (III). Glutathione (GSH) and other thiols serve as reducing agents, and S-adenosylmethionine (SAM) is the main methyl donor.**

CHAPTER 3:
Tissue distribution and toxicity of nestling Zebra Finches (*Taeniopygia guttata*) dosed with Monomethylarsonic acid (MMA (V))

3.1 Introduction

Monosodium methanearsonate (MSMA) is an arsenic-based pesticide that has recently been used in a suppression strategy to control the Mountain Pine Beetle (*Dendroctonus ponderosae*) in pine forests throughout British Columbia. The commercial formula of MSMA used in B.C. is called Glowon®, and up to 3000 litres, or 960 kg (6.6 lbs MSMA/gallon Glowon®) of this product has been used annually in British Columbia (Dost, 1995). In the Cascades Forest District alone, over 60,000 trees were treated between 2000 and 2004 (Morrissey *et al.*, 2006).

Despite the heavy usage of MSMA, there is little knowledge of the toxicity of this product on exposed non-target avian wildlife. Woodpeckers and other cavity nesters are primary predators of the Mountain Pine Beetle with winter diets of up to 99% beetle adults and larvae (Crockett and Hansley, 1978). Three-toed (*Picoides tridactylus*) and Hairy (*Picoides villosus*) woodpeckers can ingest many thousands of beetles and larvae per day (Koplin, 1972), and have been shown to locally increase in numbers in response to increases in Mountain Pine Beetle infestations (Rust, 1929 and 1930; Baldwin, 1960; Koplin, 1972). Recent research has shown that arsenic can accumulate in beetles in MSMA treated stands of pine trees in the Merritt forest district of British Columbia, and that Three-toed and Hairy woodpeckers nest and forage in these pine stands (Morrissey *et*

al., 2006). Thus, it is important to understand the uptake and possible effects of this pesticide in both adult and nestling forest birds.

Acute toxicity of MSMA in terrestrial species has been reported in various laboratory studies, mainly for domesticated mammals. Treatments durations range from 96 hours to 21 days, and results suggest high species variation (Dickinson, 1972; Exon *et al.*, 1974; Judd, 1979; Jaghabir *et al.*, 1988). Acute toxicity tests using captive avian species are limited, and far less is known about sub-lethal behavioural or physiological effects of MSMA in birds. The 96 hour LD_{50} for a 17-week-old Bobwhite Quail (*Colinus virginianus*) exposed to MSMA in the diet was found to be 834 mg/kg MSMA, and 10-day-old bobwhite yielded a 96 hour LD_{50} of approximately 650 mg/kg/day (MAA Task Force Three, 1993). Mallards tested on the same dosing scheme showed no effects or mortality at any dose. The highest dose administered was 1100 mg/kg/day (MAA Task Force Three, 1993).

A suite of sublethal effects of MSMA have been reported in various laboratory mammals, ranging from decreased hematocrits and blood glucose levels (Judd, 1979) to behavioural abnormalities such as impaired female nest-building behaviour in white-footed mice (Lopez and Judd, 1979), to reproductive effects such as lowered reproductive capabilities of male mice and changes in reproductive behaviour of female mice (Prukop and Savage, 1986). In addition, clinical symptoms reported by studies using laboratory mammals dosed with monomethylarsonic acid (MMA), which is MSMA at physiological pH, include gastrointestinal irritation, frequent vomiting and diarrhea (MMA Task Force Three, 1993), as well as weakness, reduction of activity, depressed blood pressure and

altered electrocardiogram (ECG) results (Jaghabir *et al.*, 1988). Sublethal effects or chronic toxicity of MSMA to avian species are not well documented.

Developmental effects of methylated arsenicals include malformations and abnormal and slowed development of offspring in mice dosed with dimethylarsinic acid (DMA), a metabolite of MSMA, during gestation (Rogers *et al.*, 1981). Prenatal mortality, embryonic resorption and gross malformations have been reported by Hood *et al.* (1982) in hamsters dosed with the sodium salt of DMA, sodium cacodylate or SDMA. This same compound has also been shown to cause maternal deaths as well as decreased mean fetal weight in hamsters (Hood, 1998).

Studies of postnatal effects of arsenic such as growth and general health are limited. Rat pups exposed to sodium arsenite in drinking water had significantly lower weights from 1 month to 4 months. Pups also showed altered spontaneous locomotor activity (Rodriguez, 2002). Mallard ducklings dosed with environmentally relevant concentrations of sodium arsenate showed some reduction in growth (Hoffman *et al.*, 1991), as well as physiological and behavioural effects (Whitworth *et al.*, 1991). No studies have examined the possible effects on growth and health of nestling birds exposed to methylated arsenicals.

Among other factors modifying the expression of arsenic toxicity, the age of the animal exposed plays an important role, with the potential for increasing resistance with age (Harrisson *et al.*, 1958). The present study was designed to investigate the tissue distribution, growth parameters and general health of nestling Zebra Finches (*Taeniopygia guttata*) orally dosed with three levels of monomethylarsonic acid (MMA (V)) from day 1 to day 21. Differences in tissue distribution as well as general sensitivity

to MMA (V) in the nestlings compared to the adults in Chapter Two are also investigated. MMA (V) was used in this study as it corresponds to MSMA at gastrointestinal pH (Dost, 1995) and is the primary form of arsenic detected in the bark beetle samples collected in MSMA treated pine stands in British Columbia (Morrissey *et al.*, unpublished). Doses were calculated based on total arsenic detected in bark beetle samples (Morrissey *et al.*, unpublished) and beetle-ingestion rates of free-living woodpeckers (Nagy, 2001), and extrapolated to the Zebra Finch. Nestlings were used as they represent a sensitive stage of the life cycle and offer insight into the effects of contaminants on growth (Furness, 1993). In addition, developing birds are typically more sensitive to chronic metal exposure than adults (Scheuhammer, 1987).

Growth was measured by daily body mass and fledging tarsus length, and immune function was measured by the phytohaemagglutinin (PHA) cell mediated immune function test. The PHA immune function test is a useful tool to examine the cell-mediated immunity of animals (Smits *et al.*, 1996; Smits and Williams, 1999). Exposure of young, developing birds to contaminants may decrease their immunity and ability to ward off disease. Additionally, Gebhardt-Henrich and Richner (1998) suggest that building an immune response to these xenobiotics may be costly to young birds, and may affect their growth and ultimately their ability to reproduce in the future. Additional toxic effect end-points measured were hematocrit and leucocrit. Several studies have reported depressed hematocrit values as a result of metal exposure (Grue *et al*, 1986; Nyholm 1998).

3.2 Methods

3.2.1 Animals and Husbandry

This experiment was conducted using captive Zebra Finches in the Simon Fraser University Animal Care Facility located in Burnaby, British Columbia. Finches were maintained in CCAC (Canadian Committee on Animal Care) accredited facilities, with a constant 14 hr light: 10 hr dark cycle at 19-23 °C and 35-55% humidity. All birds were allowed free access to a mixed seed diet (Panicum and white millet, 50:50; approximately 12.0% protein, 4.7% lipid; Just For Birds, Surrey, BC) and water, grit and cuttlefish bone *ad lib*. For these experiments birds were held in cages (61 x 46 x 41 cm) with 3-6 birds per cage. Experiments and animal husbandry were conducted under a Simon Fraser University Animal Care Committee permit (666B-03). All work was done following CCAC guidelines.

3.2.2 Experiment #1 – Pilot studies to determine dosing levels and liver, kidney and brain tissue uptake of Monosodium Methanearsonate (MSMA) in nestlings.

This pilot study was designed to determine dosing levels for the main larger nestling study, as well as to determine if uptake and tissue distribution in nestlings dosed with MAA (V) was the same as in adult Zebra Finches. Technical grade MMA (V) was used in this study. The compound was dissolved in de-ionized distilled water. Solutions were analysed prior to administration, and were within 10 % of the target dose. Initially, nestlings were exposed to the same environmentally relevant doses of 0, 8, 24 and 72 μg/g MMA (V) used in a similar dosing study using adult Zebra Finches (See Chapter 2). These doses resulted in complete mortality of all nestlings dosed with medium and high

dose concentrations (see below). Doses were then lowered to 0, 4, 12 and 36 μg/g MMA (V) and a second study was carried out. Again, this resulted in complete mortality of both the medium and high dosed nestlings (see below). As it was not our aim to determine lethal doses of MSMA, but rather to investigate sublethal effects, doses were then lowered for the main, larger-scale study (see Experiment #2, section 3.2.3.), with concentrations of 0, 4, 8 and 12 μg/g MMA (V).

For each pilot study, 20 adult finches (10 males and 10 females) were randomly paired in individual breeding cages (61 x 46 x 41 cm), each with an external nest box (11.5 x 11.5 x 11.5 cm). In addition to the *ad lib* seed diet, breeding birds were offered egg food daily (approximately 6.0 g) until clutch completion (two days after the last egg was laid) and then again during the chick-rearing stage.

Nest boxes were monitored daily for egg laying and eggs were numbered in consecutive order and weighed (±0.001 g) to confirm normality of the reproductive cycle and nest productivity. Breeding pairs that did not produce eggs within two weeks following pairing were separated and classified as "non-breeders". Nest boxes were then checked again daily to determine hatching dates. Within 24 hours of hatching individual chicks within each nest were assigned to a control, low, medium or high solution dose group. Each nest therefore contained a control chick and chicks in 1-3 of the MSMA treatment groups (low, medium, high) depending on brood size. Some large broods had more than one control chick; in this situation we used the average value of the two chicks for subsequent analysis. Nestlings were orally dosed daily from day 1 to day 21, using a micropipette or via intubation. Doses were adjusted daily based on individual chick weight as chicks increased in mass. Volumes ranged from 10 ul to 130 ul. From day 1 to

day 21 all nestlings were weighed daily and all surviving nestlings were banded at ten days of age. Nestlings were put back into the nests immediately after handling.

For the second pilot study which used 0, 4, 12 36 µg/g MMA (V), all surviving nestlings were anesthetized on day 21 via an intramuscular injection of 50 ul ketamine and xylazine solution (50:50 by volume; Associated Veterinary Products, Abbotsford, British Columbia) followed by exsanguination. Blood samples were collected to determine total arsenic content, and were taken via both the jugular and brachial veins using heparinized pipettes or capillary tubes and transferred to heparinized centrifuge tubes. Within 1 hour of being collected, blood samples were frozen at –20 C. Liver, kidney and brain tissues were obtained through dissection and were frozen prior to being freeze dried and sent to the University of British Columbia for total arsenic analysis.

Additional toxicity end-points measured in the pilot study were percent hematocrit and leucocrit. Hematocrit is a measure of the total red blood cells in a blood sample and may indicate the amount and efficiency of oxygen uptake and transfer to tissues (Ots *et al*, 1998). Leucocrit is a measure of total white blood cells in a blood sample, and indicates humoral immune function (Wardlaw and Levine 1983).

3.2.3 Experiment #2 – Effects of Monosodium methanearsonate (MSMA) on the growth and general health of nestling Zebra Finches (Taeniopygia guttata)

Zebra Finches (20 males and 20 females) were randomly paired into separate cages (61 x 46 x 41 cm) with a nest box (11.5 x 11.5 x 11.5 cm). Birds were bred and nestlings were dosed as described above (see section 3.3.2). Nestling growth was measured by body mass on days 1, 5, 10, 15 and 21, and right tarsus length at fledging. Immune function was measured by conducting a phytohaemagglutinin (PHA) cell mediated immune

function test on all nestlings at 21 days of age. Nestlings received a wing web injection of 30 ul lectin (kidney bean extract) in the left wing web. The right wing web received an injection of 30 ul of phosphate buffered saline (PBS) which serves as a control. Prior to injection, the feathers in the injection area were plucked and injections were given using an insulin syringe. Thickness of wing webs were measured 24 hours later using calipers to determine swelling. Blood samples were collected from 5 nestlings per dose group at day 21 to determine total arsenic content, and were taken via both the jugular and brachial veins using heparinized pipettes and capillary tubes and transferred to heparinized centrifuge tubes. Blood samples were frozen at –20 °C within one hour.

MMA (V) analysis

Solutions, blood and organ tissues were sent to Dr. William Cullen's Chemistry Laboratory at the University of British Columbia, BC where they were measured for arsenic content by Vivian W. M. Lai.

Freeze-dried blood and organ tissue samples were weighed into centrifuge tubes (either 15 mL or 50 mL) and 5 mL of a methanol/water mixture (1:1, v/v) was added to each tube. Tubes were then sonicated for 10 min and centrifuged (3,000 rpm) for 10 min, and the supernatant was removed by means of a Pasteur pipette and placed in a round bottom flask. This extraction procedure was repeated four times for each sample. The combined supernatants were evaporated to dryness and dissolved in 10 mL of deionized water prior to further analysis. Extracts were stored at -20°C and transferred to the cold room (~ 4°C) on the day of analysis (Lai *et al.*, 2004).

Digested (see Chapter 2 MAA (V) analysis for digestion procedures) blood and tissue samples were diluted appropriately with the rhodium-nitric acid solution and analysed for total arsenic using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Arsenic compounds in the samples were identified by matching the retention times of the peaks in the chromatograms with those of known standards. Quantification was done by comparing peaks with those of matching standards. All reagents used were of high purity suitable for ICP-MS analysis. Quality assurance included the analysis of standard reference materials: dogfish muscle (DORM-2) from National Research Council Canada and *fucus* sample (IAEA-140) from International Atomic Energy Agency. Kelp powder was also used as a laboratory standard (Lai *et al.*, 2004).

Throughout the literature, monomethylarsonic acid is abbreviated as MMA, MAA and MMAA. To avoid confusion we will use MMA throughout this paper. In addition, our chemical analysis cannot distinguish between different valences. Therefore, we report MMA (V) and DMA (V) to represent both valences (III) and (V).

Statistical Analyses

All statistical analyses were conducted using JMP (Version 5.0, SAS Institute Inc 2003). A Shapiro-Wilks W test (Version 5.0, JMP, SAS Institute Inc 2003) showed that the blood arsenic data deviated significantly from normality, and therefore data were $\log_{10}$ transformed prior to analysis to meet assumptions of normality for subsequent parameteric statistical analyses. For pilot study #2, analysis of variance (ANOVA) was applied to determine if a difference existed in mean total arsenic in blood and tissues, as well as percent hematocrit in surviving nestlings exposed to 0 or 4 μg/g MMA (V). Where differences existed, Tukey's Honestly Significant Difference (HSD) tests were

used to identify which treatment levels differed significantly in their response to MMA (V) exposure. To determine if there was a difference in weight gain among dose groups during the dosing period, a repeated measures ANOVA was conducted for both the pilot and main studies. For the main study, paired t-tests were used to determine significant differences in body mass, cell-mediated immunity and tarsus length at day 21 between treatment and control groups among nests. This ruled out any confounding factors such as brood size and parental quality.

3.3 Results

3.3.1 Experiment #1-Pilot Studies: mortality, growth, general health and total blood arsenic residues.

Mortality in both pilot studies was high (Table 3.1). All nestlings in the 72 μg/g (n = 7) dose group died between days 2 – 10. All nestlings in the 24 μg/g (n = 5) dose groups died within the same time frame. All but one nestling in the 12 μg/g (n = 5) dose group died between days 2-4, with the remaining medium-dosed nestling surviving until day 17. One nestling in the 4 μg/g (n = 5) dose group died on day 6. No nestlings in 8 μg/g (n = 4) or 0 μg/g (n = 10) dose groups died.

For pilot study #1, the surviving sample size was too low for statistical analyses. Therefore, we will not refer to pilot study #1 for the remainder of the results. For pilot study #2, surviving nestlings dosed with low or control concentrations of MMA (V) showed no signs of ill health. Weight gain did not differ significantly between treatment groups (repeated measures ANOVA, $F_{1,7} = 1.10$, $P > 0.05$) and hematocrit and leucocrit percentages were not correlated with dose group (ANOVA, $F_{1,8} = 1.85$, $P > 0.05$).

Total concentrations of arsenic detected in the blood and organ tissues from surviving nestling Zebra Finches from pilot study #2 dosed with control and low concentrations of MMA (V) are listed in Table 3.2. As expected, total arsenic detected in blood was significantly higher in the low dose group compared to the control group (one-way ANOVA, $F_{1,8} = 42.69$, $P < 0.001$; Figure 3.1). Control birds had trace amounts of arsenic in the blood, which can be attributed to background levels resulting from "natural" exposure of arsenic in drinking water and food.

All organ tissues contained total arsenic residues that increased in low-dosed nestlings compared to control nestlings (ANOVA, $F_{1,8}$, $P < 0.01$ in all cases; Table 3.3) In the low dosed birds, the liver contained the highest amount of arsenic, ranging from 1.13 to 3.6 μg/g, followed by the kidney with a range of 1.04 to 2.59 μg/g. The brain tissue had the lowest total arsenic, ranging from 0.52 to 1.34 μg/g.

3.3.2 Experiment #2-Main study: Mortality, growth, general health and total blood arsenic residues

In the main study, there was very little mortality, and this was restricted to on or after day 2, and occurred in all dose groups. A total of three nestlings dosed with a high concentration (12 μg/g, n = 23) died between days 2 – 12. A single nestling from the medium dose group (8 μg/g, n = 20) died on day 2, and two nestlings from the low dose group (4 μg/g, n = 21) died between days 2 – 10. One nestling from the control group (0 μg/g, n = 25) died on day 11 of dosing.

Cell mediated immune response to PHA was not reduced by MMA (V) treatment. (paired t-test, $P > 0.05$ in all cases).

Body mass throughout the 21 day period did not differ between treatment groups (repeated measures ANOVA, $F_{3,85} = 0.28$, $P > 0.05$). Body mass on day 21 did not differ between control and treatment groups (paired t-test, $P > 0.05$ in all cases). Tarsus length did not differ between control and low groups (paired t-test, $P > 0.05$, $df = 12$) but differed between control and medium dose groups (paired t-test, $t = 2.0$, $P < 0.05$, $df = 13$) and control and high dose groups (paired t-test, $t = 2.29$, $P > 0.05$, $df = 12$), with medium and high dosed nestlings having shorter tarsi than control dosed nestlings.

Mean total blood arsenic varied with dose group, with medium (8 μg/g) and high (12 μg/g) dosed birds had significantly more mean total blood arsenic than controls (ANOVA, $F_{3,20} = 4.83$, $p < 0.05$; Fig.3.2).

3.4 Discussion

Preliminary studies showed that dosing nestling Zebra Finches with MMA (V) at levels detected in bark beetles from MSMA treated stands of trees results in high mortality. When compared to recent dosing studies using adult Zebra Finches, the same environmentally relevant concentrations resulted in very low mortality, illustrating that nestlings are a much more sensitive life stage. Preliminary studies also demonstrated a dose dependent relationship in blood and tissue concentrations of surviving nestlings. Arsenic concentrations in the blood and tissues of nestlings were measured to determine if uptake and tissue distribution of MMA (V) is the same as in adult Zebra Finches.

3.4.1 Survival, growth and general health

Our preliminary studies showed that nestlings dosed with concentrations of 12, 24, 36 and 72 μg/g MMA (V) did not survive (Table 3.1). The high mortality of nestlings as compared to adults on the same dosing scheme suggests that nestlings are far more sensitive to MMA (V). This appears to hold true for most heavy metals, with young developing birds exhibiting increased sensitivity to metal exposure than adults (Scheuhammer, 1987). Nestlings likely lack the ability of adults to rapidly methylate and excrete the compound. This could be due to underdeveloped tissues in the nestlings, which may be unable to cope with exposure to a potentially toxic compound while developing. For example, nestling organs may not be able to properly metabolize a compound in the early stages of development. Administration of high concentrations of MMA (V) may cause the nestlings to rapidly absorb the compound, and the biotransformation capabilities of the nestling tissues may become quickly saturated. Nestlings are likely to become exposed to MMA (III) during biotransformation, which may be binding to critical thiol groups, inhibiting crucial biochemical reactions and leading to cytotoxicity (Hughes, 2002). This would ultimately lead to failure of specific organs and tissues and result in death of the nestlings. Conversely, nestlings dosed with much lower concentrations of 4 and 8 μg/g MMA (V) experienced a much higher survival rate, with no apparent sublethal toxicity. In this case, nestlings appear to have the ability to cope with low levels of MMA (V), and are perhaps able to methylate and excrete the compound more efficiently and subsequently experience less toxicity.

Although there was complete mortality of nestlings dosed with 12 μg/g in pilot study #2, nestlings in the main study that received the same dosage had a much higher

survival rate. These results may be due to sample size, with a very low sample size in the pilot studies. Other variables that may have influenced nestling survival are the reproductive experience of parent Zebra Finches, or lack thereof, as well as the general quality and fitness of the parents. Parents were selected from the colony based on body mass and general appearance, with unhealthy looking birds or those with low body mass excluded. However, birds were of limited supply and quality of parents was sure to vary.

None of the surviving nestlings in the 4, 8 and 12 μg/g MMA (V) dose groups exhibited characteristic signs of sublethal arsenic toxicity such as weakness, frequent vomiting, diarrhea or reduction of activity seen in other arsenic dosing studies using laboratory mammals (Jaghabir *et al.*, 1988). However, it is risky to extrapolate mammalian results to those in an avian system. Body mass, cell-mediated immune response and hematological parameters of nestlings at day 21 were not affected by MMA (V) dosing. Comparable studies examining the effects of arsenic on the health of birds are lacking, however our results are in accord with those of Fair and Myers (2002) who reported no effects of increased metal exposure on developing Western Bluebird (*Sailia mexicana*) hematocrit. However, this study looked the effects of combinations of metals in a polluted site. Thus, it is difficult to compare these results to those seen in the Zebra Finches dosed with MMA (V) in the present study.

Tarsi length of nestlings Zebra Finches in the 8 and 12 μg/g MMA (V) dose groups were significantly shorter than control nestlings. Similarily, wild nestling pied fly-catchers (*Ficedula hypoleuca*) exposed to a variety of heavy metals exhibited growth abnormalities of the wings and legs with increased metal exposure, with no effect on growth rate (Eeva and Lehikoinen, 1996). However, flycatchers with leg and wing

growth abnormalities were unable to fledge, while nestlings in the current study all fledged successfully. Shorter tarsi length in the Zebra Finches may be due to a possible interaction between arsenic and the mineral fraction of the bone. Kretschmer *et al.* (2002) have suggested that arsenic in bone may be associated with the mineralized calcium phosphate, or hydroxyapatite, with arsenic likely replacing the phosphate. Hydroxyapatite is an important part of the framework in bones, making them strong and rigid (Heaney, 1999). The possible interaction between arsenic and phosphate in the hydroxyapatite may have consequences in developing birds by interfering with normal growth. Shorter tarsi length in the nestlings appeared to have no effect on the general health and success of the fledglings. However, Zebra Finch nestlings are held in a controlled laboratory setting. Studies determining the effects of shorter tarsi in wild passerine fledglings on parameters such as anti-predatory responses require further study.

Of the sub-lethal end-points measured, there were relatively few observed effects. This is likely due to the relatively low MMA (V) concentrations. In similar studies using higher dose concentrations, several sub-lethal effects have been shown. Duckling mallards (*Anas platyrynchos*) receiving 200-300 ppm sodium arsenate in the diet had decreased growth by over 30% by the end of 4 weeks (Hoffman *et al.*, 1991), and mice receiving 447 ppm MSMA in the drinking water had significantly decreased hematocrits and blood glucose levels after 30 days (Judd 1979). Future studies measuring additional toxicity end-points in nestling passerines exposed to MMA (V) are required.

3.4.2 Bioaccumulation and distribution of arsenic in blood and tissues

Since the purpose of this study was to investigate the effects of MMA (V) on nestling growth and general health, total blood arsenic residues are needed to verify exposure. Blood samples from the pilot study indicated a significant increase in total blood arsenic from control to low (4 μg/g) dosed birds. However, these effects may have been due to low sample sizes and lack of power, as the main study showed that there was no significant dose-dependent accumulation of arsenic in the blood. However, all dose groups had more total blood arsenic than the control group (Table 3.2). Tissue distribution was varied, with up to 3.6 μg/g arsenic in the liver, 2.59 μg/g in the kidney and 1.34 μg/g in the brain of low (4 μg/g) dosed nestlings. Future studies are necessary to determine any histopathological effects of this compound.

When compared to adult Zebra Finches dosed with the same concentrations of MMA (V) (Chapter 2), it is clear that nestling Zebra Finches are more susceptible to accumulation in organ tissues than adults (Figure 3.3), but not in the blood. Nestlings dosed with 8 μg/g MMA (V) had an average of 0.19 μg/g arsenic in the blood, while adults dosed with 8 μg/g MMA (V) had an average of 0.45 μg/g arsenic in the blood. However, nestlings dosed with 4 μg/g MMA (V) had up to 3.60 μg/g arsenic in the liver, 2.59 μg/g in the kidney and 1.34 μg/g in the brain. Adults dosed with twice as much MMA (V) (8 μg/g) had up to only 0.19 μg/g arsenic in the liver, 0.3 μg/g in the kidney, and 0.2 μg/g in the brain. Since nestlings were dosed for approximately one week longer than adults, the differences in accumulation may be due to duration of treatment, with birds accumulating more with longer dosing periods. However, our results are similar to other studies using wild bird populations, examining age differences in accumulation of

arsenic, selenium and manganese which report a decrease in tissue and blood concentrations with age (Burger and Gochfeld, 1999). Since our study was done in a controlled laboratory setting, we can rule out any possible factors that could be contributing to age differences such as differences in colony/habitat, or seasonal effects. In our study, age differences in accumulation of arsenic are most likely related to differences in the ability to rid their bodies of this metal. Another possibility is age differences in intestinal absorption of metals. It has been shown that adult mammals absorb an average of 2% of ingested inorganic mercury, while young mammals absorb an average of 35% (Kostial *et al,* 1978).

As with adult Zebra Finches, nestlings had considerable amounts of arsenic in the brain tissue. Arsenic has the ability to cross the blood-brain barrier, and has been to shown to accumulate in the brain and cause neurotoxic effects in rats by altering ATP metabolism and influencing neurons and glia (Nagaraju and Desiraju, 1993; Valkonen *et al*, 1983). Nagaraju and Desiraju (1993) showed that developing rats exposed to low doses of 5 μg/g inorganic arsenic for 60 days had delayed eye opening, reduced weight gain and irreversible body and brain weight deficits. The authors suggest that the chronic exposure to arsenic may have induced irreversible reorganization of the developing brain. Future studies examining possible neurological and behavioural effects of MMA (V), as well as developmental effects on these systems in passerines are warranted.

3.4.3 Environmental Relevance

Blood sample results from woodpeckers exposed to MSMA via ingestion of contaminated bark beetles in the Merritt forest district in British Columbia ranged from 0.03 to 3.73 μg arsenic/g dw blood (Morrissey et al, unpublished). Nestling Mountain Chickadees (*Poecile gambeli*) in the same forest district had total blood arsenic values ranging from 0.07 to 1.88 μg arsenic/g dw blood just prior to fledging (Morrissey *et al.* 2006, unpublished data). Zebra Finch nestlings dosed with presumably the same environmentally relevant concentrations of MMA (V) (24, 36 and 72 μg/g) did not survive. Nestlings dosed with lowered concentrations of MMA (V) (0, 4, 8 and 12 μg/g) had total blood arsenic values ranging from 0 to 0.56 μg/g at fledging. Although the precise exposure of nestling Mountain Chickadees is unknown, the fledging period of chickadees and Zebra Finches are approximately equal, so it is informative to compare the two. Based on the total blood arsenic results, it is possible that cavity nesting insectivorous birds exposed to MMA (V) would accumulate similar tissue concentrations, which could cause significant nestling mortality. Although growth (in terms of daily weight gain) of nestling Zebra Finches was not affected by the lowered dose concentrations, these results cannot necessarily be extrapolated to wild populations. Chickadee nestlings in the Merritt forest district contained much higher concentrations of arsenic in the blood than our laboratory nestlings. Therefore, it is possible that these wild nestlings may be exposed to higher concentrations of MMA (V), and may be experiencing reduction in body mass and general health. Nestling Zebra Finches experienced reduced tarsi length in the 8 and 12 μg/g MMA (V) dose groups. Wild nestlings exposed to the same concentrations MMA (V) may be experiencing similar skeletal growth impairments which may effect their fledging success as well as

predisposing them to increased predation risks. When assessing these possible effects at the population level, it has been shown that reduced fledgling weights in great tits can affect survival (Gebhardt-Henrich and Richner 1998) and recruitment rates (Gebhardt-Henrich and van Noordwijk 1991), thus having an effect on the whole exposed population.

3.4.4 Conclusions

In conclusion, we found that dosing nestling Zebra Finches with high environmentally relevant concentrations of 24 and 72 and possibly 12 μg/g MMA (V) results in high mortality. We found no evidence that dosing of nestlings with 4 or 8 μg/g MMA (V) had significant negative effects on growth or cell-mediated immune function in nestling Zebra Finches. A significant effect of MMA (V) on tarsi length at fledging was detected at 8 and 12 μg/g MMA (V) in the main study. However, this did not seem to affect fledging success. Liver, kidney and brain residues were high compared to adults dosed with similar concentrations. Future studies are necessary to any histopathological, as well as any neurological or behavioural effects of this compound on passerines.

Table 3.1 Nestling Zebra Finch mortality observed in pilot studies 1 and 2. Dose groups are in µg/g MMA(V).

Dose Group (µg/g)	0	4	8	12	24	72
Sample size	10	5	4	5	5	7
Mortality (%)	0%	80%	0%	100%	100%	100%

Table 3.2 Total arsenic detected in whole blood of nestling Zebra Finches dosed with 0, 4, 8 and 12 µg/g MMA (V). Pilot and main study data combined. Total arsenic values are means ± standard error with sample sizes in parentheses.

Dose Group (µg/g)	Total arsenic (µg/g)	Range (ug/g)
0	0.055 ± 0.048 (10)	0 – 0.18
4	0.26 ± 0.048 (10)	0.02 – 0.56
8	0.19 ± 0.068 (5)	0.08 - 0.38
12	0.2 ± 0.068 (5)	0.1 – 0.41

Table 3.3 Total arsenic detected in organ tissues of nestling Zebra Finches dosed with control and low (4 µg/g) concentrations of MMA (V). Total arsenic values are means ± standard error with sample sizes in parentheses.

Tissue	Dose Group (µg/g)	Total Arsenic (µg/g)	Range (µg/g)
Liver	0	0.1175 ± 0.8498 (4)	0.05 – 0.31
Kidney	0	0.0950 ± 0.5795 (4)	0.05 – 0.2
Brain	0	0.0475 ± 0.3012 (4)	0.05 – 0.11
Liver	4	2.1340 ± 0.7601 (5)	1.13 – 3.6
Kidney	4	2.0140 ± 0.5183 (5)	1.04 – 2.59
Brain	4	1.0860 ± 0.2694 (5)	0.52 – 1.34

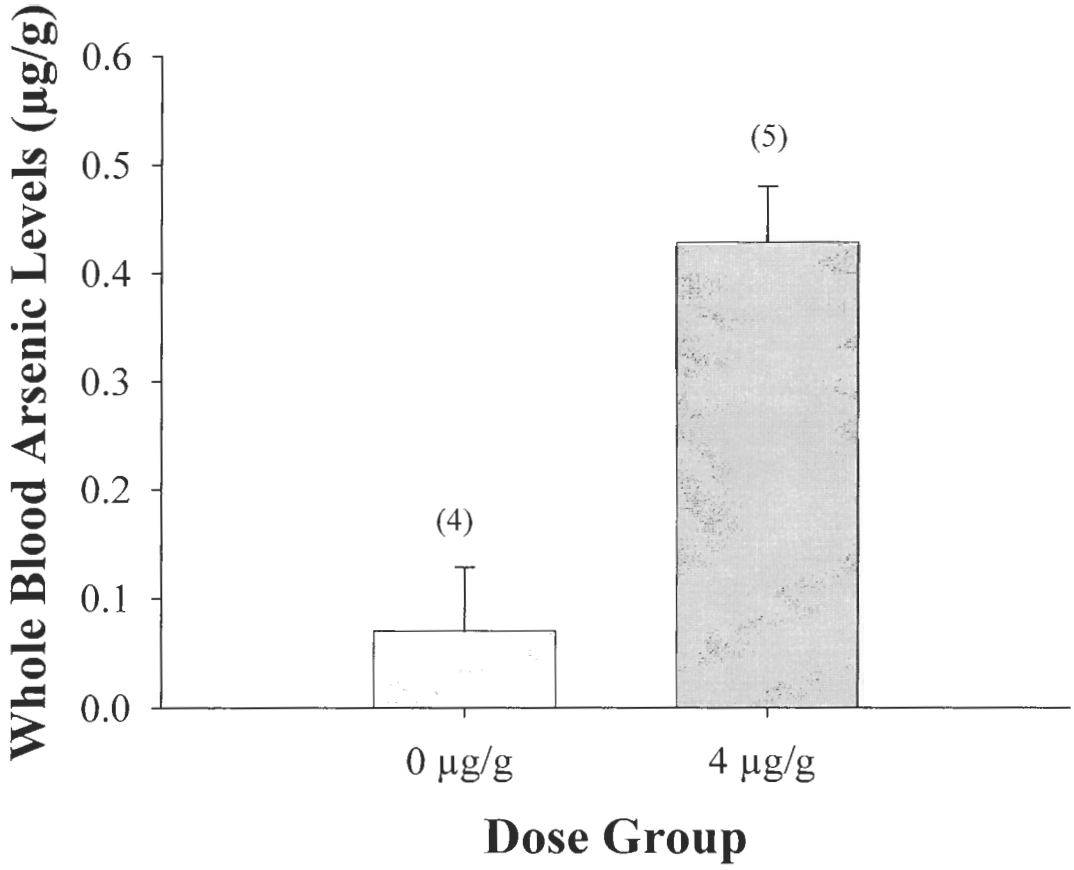

Figure 3.1 **Total blood arsenic in nestlings dosed with control and low (4 µg/g MMA(V)) in pilot study #2. Data are expressed as the mean ± standard error.**

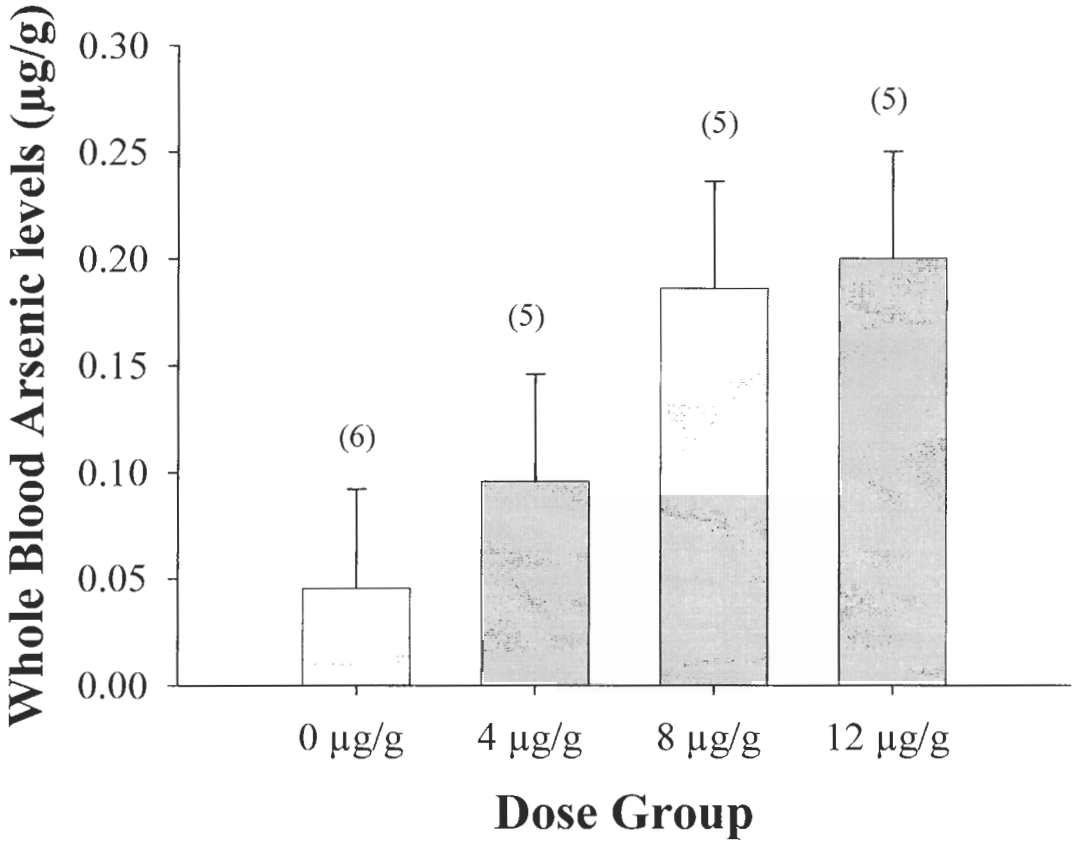

Figure 3.2 **Total blood arsenic detected in nestling Zebra Finches dosed with 4, 8 and 12 µg/g MMA (V) and a control. Pilot study data not included. Total arsenic expressed in µg/g dry weight. Results are shown as the mean ± standard deviation with the sample size above each point in parentheses. $p > 0.05$**

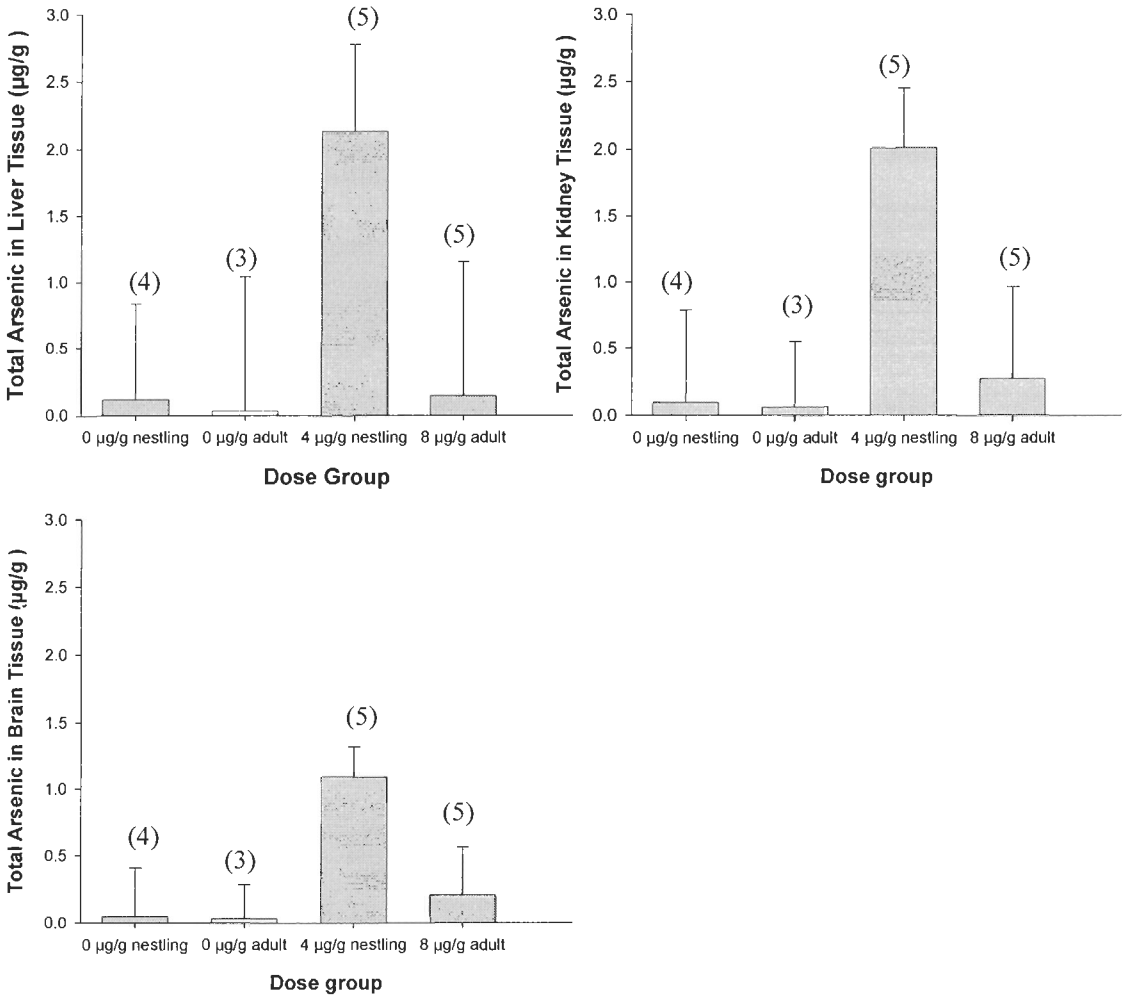

Figure 3.3 **A comparison of total arsenic uptake in nestling and adult Zebra Finch blood, liver, kidney and brain tissues. Total arsenic expressed in µg/g dry weight tissue. Sample sizes are in parentheses.**

CHAPTER 4:
Conclusions

The primary objective of the research described in this thesis was to determine how MSMA may be affecting wild cavity nesting forest birds exposed to this compound through ingestion of contaminated bark beetles using a model laboratory species, the Zebra Finch. The uptake, elimination and sub-lethal toxic effects of MMA (V), which corresponds to MSMA at physiological pH, on adult and nestling Zebra Finches was examined. This research resulted in four major findings. Firstly, both adult and nestling Zebra Finches orally dosed with environmentally relevant concentrations of MMA (V) accumulated arsenic in the blood, liver, kidney, brain and carcass tissues in a dose-dependent manner. Low tissues residues and high excretion of this compound in adult finches indicate low absorption or rapid excretion, or a combination of the two processes. These results are typical of methylated arsenical exposure in other laboratory species (Chapter 2). Secondly, the species of arsenic detected in the blood plasma of adult finches was almost exclusively in the original MMA (V) form. Similarly, the species of arsenic excreted by the finches was primarily in the MMA (V) form. Arsenic species detected in tissue samples from adult finches included both the original species (MMA (V)) and the metabolite DMA (V). The liver and kidney contained primarily DMA (V) while the brain contained slightly more MMA (V) than DMA (V). These results indicate that the administered compound is being metabolized *in vivo,* and that finches are exposed to the toxic trivalent intermediate MMA (III) through this process. The extent of this exposure is currently unknown. Thirdly, the oral dosing and subsequent accumulation of arsenic

resulted in significant body mass loss in adult finches in the two highest dose groups. No other negative effects were observed in the adult finches. And finally, complete mortality occurred in nestling Zebra Finches dosed with 24 and 72 μg/g MMA (V), and shorter tarsi were observed when dosed with 4 and 12 μg/g dose groups (Chapter 3). These results indicate a greater sensitivity to this compound in developing life stages. No additional clinical effects were observed during exposure.

Several studies have reported acute effects of methylated arsenicals on rats, mice, hamsters and rabbits, as well as some avian species such as bobwhite and mallards (Chapter 2 and 3). However, few studies exist that examined the sub-lethal effects of these compounds on exposed songbirds and passerines. Currently, cavity nesting forest birds such as woodpeckers and chickadees nest in areas of high MSMA use, and forage on bark beetles containing high concentrations of this pesticide. Blood samples from woodpeckers and chickadees sampled in the Merritt Forest District, an area of high MSMA use, show total arsenic levels of up to 3.73 μg/g and 2.20 μg/g, respectively. Interpreting these tissue concentrations, in terms of toxic effects, is difficult in wild birds due to confounding factors (Chapter 1). Our laboratory study mimicked the field exposure of cavity nesting forest birds using Zebra Finches as a model species, and providing a better understanding of the effects of these compounds on exposed wild populations.

Based on the findings of this study, it is clear that the Zebra Finches primarily excrete MMA (V). However, accumulation occurred at all dose concentrations in all tissues examined. In the adult Zebra Finches, significant amounts of arsenic accumulated in the brain tissue. Arsenic has the ability to cross the blood-brain barrier, and cause

neurological damage. Longer-term studies examining the effects of arsenic-based pesticides on the neurological effects and subsequent behavioural changes are warranted, as this could have potentially detrimental effects on wild populations of passerines exposed to these compounds.

A clear understanding of these toxicological effects is critical when developing and implementing forest management strategies. Based on this study, there is evidence that exposure to MMA (V) for 14 days results in mass loss at the highest environmentally relevant concentrations. Other than significant mass loss, there were no additional observed acute or sub-acute effects. However, it is possible that 14 days is not adequate enough time for many sub-lethal effects of this compound to become apparent. Wild populations of forest birds in areas of high MSMA use are likely experiencing longer-term exposure to this pesticide, and possibly over more than one generation. Once trees are treated with MSMA, they are often left standing, allowing birds to continuously forage on them. Future studies examining the long-term effects of MSMA to exposed populations of birds is necessary for developing management options for pesticide use in bark beetle control in British Columbia.

While few sub-lethal effects were observed in adults, nestling Zebra Finches exhibited high mortality when exposed to the same concentrations of MMA (V). Additionally, nestlings accumulated significantly more arsenic in all organs compared to adults. The implications of these high tissue residues are currently unknown. Furthermore, nestlings in the two highest dose groups exhibited shorter tarsi length at fledging than controls. The effects of arsenic exposure during the developmental stage could have consequences that may surface as decreased reproductive success and

recruitment rate as adults. Further studies examining the reproductive capabilities of fledglings when they reach maturity are necessary. In addition, our study examined the postnatal effects of MMA (V) dosing on nestlings during development. In order to gain a complete understanding of the reproductive effects of MMA (V) on Zebra Finches, dosing studies examining the effects MMA (V) on reproductive success in adults are critical. Such studies should include end points such as total arsenic detected in eggs (to determine possible transfer of arsenic to eggs), clutch size, hatching and fledging success.

The results reported in this study suggest that Zebra Finches exposed to MSMA or MMA (V), as used for bark beetle control in British Columbia, are at risk of decreased health and survival. Cavity nesting forest birds breeding and foraging in areas of high MSMA use, such as the Merritt forest district, are likely exposed to high concentrations of this compound, for long periods of time. Zebra Finches experienced weight loss as well as mortality and growth impairments. The obvious sensitivity of nestlings to MMA (V) must be taken into account when considering the possible implications of the use of arsenic-based pesticides. The significant findings from this study warrant further studies of long-term effects of these pesticides on health, reproduction and behaviour in cavity nesting forest birds.

Reference List

Albores A, Koropatnick J, Cherian MG, and Zelazowski AJ. 1992. Arsenic induces and enhances rat hepatic metallothionein production in vivo. *Chem. Biol. Interactions* 85: 127–140.

Aposhian HV. 1989. Biochemical toxicology of arsenic. In: Hodgson E, Bend JR, Philpot RM (Eds). *Reviews in Biochemical Toxicology*, Elsevier, New York, pp 265-299.

Aposhian HV, Zakharyan RA, Wu Y, Healy S, Aposhian MM. 1997. Enzymatic methylation of arsenic compounds. II. An overview. In *Arsenic Exposure and Human Health,* Vol 2. C Abernethy, R Calderon, W Chappell (Eds). London: Chapman and Hall.

Baldwin OH. 1960. Overwintering of woodpeckers in bark-beetle infested spruce-fir forests in Colorado. *12th International Ornithological Congress Proceedings, Helsinki, 1958* : 71-84.

Birkhead M. 1982. Causes of mortality in the Mute Swan *Cygnus olor* in the river Thames. *J. Zool.* 198: 15-25.

Buchet JP, Lauwerys R, Roels H. 1981. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. *International Archives of Occupational and Environmental Health* 48: 71-79.

Blus LJ, Henny CJ, Stafford CJ, Grove RA. 1987. Persistence of DDT and metabolites in wildlife from Washington state orchards. *Archives of Environmental Contamination and Toxicology* 16(4):467-476.

Brown JL, Kitchin KT, George M. 1998. Dimethylarsinic acid treatment alters six different rat biochemical parameters: Relevance to arsenic carcinogenesis. *Teratogenesis, Carcinogenesis, Mutagenesis* 17(2): 71-84.

Buchet JP, Lauwerys R. 1985. Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man. *Archives of Toxicology* 57: 125-129.

Buchet JP and Lauwerys R. 1988. Role of thiols in the in vitro methylation of inorganic arsenic by rat liver cytosol. *Biochemical Pharmacology* 37: 149-153.

Burger J, Gochfeld M. 1997. Lead and neurobehavioral development in gulls: a model for understanding effects in the laboratory and the field. *Neurotoxicology* 18(2):495-506.

Burger J, Gochfeld M. 1999. Heavy metals in Franklin's Gull tissues: age and tissue differences. *Environmental Toxicology and Chemistry* 18(4): 673–678.

Campbell KR. 1994. Concentrations of heavy metals associated with urban runoff in fish living in stormwater treatment ponds. *Archives of Environmental Contamination and Toxicology* 27 (3):352-356.

Cherian MG. 1995. Metallothionein and its interaction with metals. In: Toxicology of Metals: Biochemical Aspects/ Contributors. Goyer RA, Cherian MG (Eds). Springer-Verlag, New York.

Clotfelter ED, Bell AM, Levering KR. 2004. The role of animal behaviour in the study of endocrine-disrupting chemicals. *Animal Behaviour* 68: 465-476.

Cole WE, Amman GD, Jensen CE. 1985. Mountain pine beetle dynamics in lodgepole pine forests. Part III: Sampling and modeling of mountain pine beetle populations. USDA Forestry Service Gen. Tech. Rep. INT 188.

Cory-Sletcha DA, Weiss B, Cox C. 1985. Performance and exposure indices of rats exposed to low concentrations of lead. *Toxicology and Applied Pharmacology* 71: 342-352.

Crockett AB, Hansley PL. 1978. Apparent response of *Picoides* woodpeckers to outbreaks of the pine bark beetle. *Western Birds* 9: 67-70.

Cullen WR, McBride BC, Reglinski J. 1984. The reaction of methylarsenicals with thiols: some biological implications. *Journal of Inorganic Biochemistry* 21: 179-194.

Cullen WR and Reimer KJ. 1989. Arsenic speciation in the environment. *Chemical Reviews* 89: 713-764.

Dauwe T, Bervoets L, Blust R, Eens M. 2002. Tissue levels of lead in experimentally exposed Zebra Finches (*Taeniopygia guttata*) with particular attention on the use of feathers as biomonitors. *Archives of Environmental Toxicology and Chemistry* 42 (1): 88-92.

Dearing DM, Foley WJ, McLean S. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annual Review in Ecology, Evolution, and Systematics* 36: 169-189

De Flora S, Bennicelli C, Bagnasco M. 1994. Genotoxicity of mercury compounds. A review. *Mutatation Research* 317: 57–79.

Dickinson JO. 1972. Toxicity of the arsenical herbicide monosodium acid methanearsonate in cattle. *American Journal of Veterinary Research* 33: 1889-1892.

Dieter MP, Ludke JL. 1975. Studies on combined effects of organophosphates and heavy metals in birds. I. Plasma and brain cholinesterase in coturnix quail fed methyl mercury and orally dosed with parathion. *Bulletin of Environmental Contamination and Toxicology* 13(3): 257-262.

Dixon HBF. 1997. The biochemical action of arsonic acids especially as phosphate analogues. *Advanced Inorganic Chemistry* 44: 191-227.

Dost, FN. 1995. Public Health and Environmental Impacts of Monosodium Methanearsonate as used in Bark Beetle Control in British Columbia. Ministry of Forests.

Dunn MA, Blalock TL, Cousins RJ. 1987. Metallothionien. *Proceedings of the Society for Experimental Biology and Medicine* 185 (3): 107-119.

Dunning JB Jr. 1993. Handbook of Avian Body Masses. CRC Press, London.

Eguchi N, Kuroda K, Endo G. 1997. Metabolites of arsenic induced tetraploid and mitotic arrest in cultured cells. *Archives of Environmental Contamination and Toxicology* 32(2): 141-145.

Eeva T, Lehikoinen E. 1996. Growth and mortality of nestling great tits *(Parus major)* and pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. *Oecologia* 108 (4): 631-639.

Exon JH, Harr JR, Claeys RR. 1974. The effects of long term feeding of monosodium methanearsonate (MSMA) to rabbits. *Nutrition Reports International* 9: 351-357.

Fry DM. 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environmental Health Perspectives* 7:165-171.

Furness RW. 1993. *Birds as Monitors of Pollutants* In: Furness RW, Greenwood JJD (Eds). Birds as Monitors of Environmental Change. Chapman and Hall, London.

Furniss RL, Carolin VM. 1980. Western Forest Insects. USDA For. Serv. Misc. Pub. No. 1339.

Fyfe WS. 1998. Toward 2050: the past is not the key to the future – challenges for the science of geochemistry. *Environmental Geology* 33(2-3): 92-95.

Garrett RG. 2000. Natural sources of metals to the environment. *Human and Ecological Risk Assessment* 6(6): 945-963.

Gebhardt-Henrich SG, Richner H. 1998. Causes of growth variation and its consequences on fitness. In: Starck JM, Ricklefs RE (Eds). Avian Growth and Development. Oxford University Press, Oxford, pp 324-339.

Gebhardt-Henrich SG, van Noordwijk AJ. 1991. Nestling growth in the great tit I. Heritability estimates under different environmental conditions. *Journal of Evolutionary Biology* 4(3): 341-362.

Gill H. 2003. The Effects of p,p'-DDE and Current Use Pesticides on Reproduction and Health in Zebra Finches. University of British Columbia, Vancouver, Canada.

Gill H, Williams TD, Bishop CA, Cheng KM, Elliott JE. 2004. Effects of Azinphos-methyl on cholinergic responses and general health in Zebra Finches (*Taeniopygia guttata*) after previous treatment with *p,p'*-DDE. *Archives of Environmental Toxicology and Chemistry* 48(1): 118-126.

Grue CE, Hoffman DJ, Beyer WN, Franson LP.1986. Lead concentrations and reproductive success in European starlings (*Sturnus vulgaris*) nesting within highway roadside verges. *Environmental Pollution* 42: 157-182.

Harrison JWE, Packman EW, Abbott DD. 1958 Acute oral toxicity and chemical and physical properties of arsenic trioxides. *Archives of Industrial Health* 17: 118-23.

Healy SM, Wildfang E, Zakharyan RA, Aposhian HV. 1999. Diversity of inorganic arsenite biotransformation. *Biol. Trace Elem. Research* 68: 249-266.

Heany RP. Bone Biology in Health and Disease. In: Modern Nutrition in Health and Disease, 9th Edition. Shils ME. Eds. 1999. Williams and Wilkins.

Hei TK, Liu SX, Walden C. 1998. Mutagenicity of arsenic in mammalian cells: Role of reactive oxygen species. *Cell Biology* 95(14): 8103-8107.

Heinz GH. 1974. Effects of low dietary levels of methylmercury on mallard reproduction. *Bulletin of Environmental Contamination and Toxicology* 11: 386-392.

Heinz GH. 1979. Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. *Journal of Wildlife Management* 43(2): 394-401.

Heinz GH, Hill E, Contrera J. 1980. Dopamine and norepinephrine depletion in ring doves fed DDE, dieldrin, and Aroclor 1254. *Toxicology and Applied Pharmacology* 53: 75–82.

Heinz GH, Fitzgerald MA. 1993. Reproduction of mallards following overwinter exposure to selenium. *Environmental Pollution* 81 (2): 117-22.

Heinz GH, Hoffman DJ. 1998. Methylmercury chloride and selenomethionine interactions on health and reproduction in mallards. *Environmental Toxicology and Chemistry* 17(2): 139-145.

Hoffman DJ, Heinz GH, LeCaptain LJ, Bunck CM, Green DE. 1991. Subchronic hepatotoxicity of selenomethionine ingestion in mallard ducks. *Journal of Toxicology and Environmental Health* 3(4): 449-64.

Honda K, Byung YM, Tatsukawa1R. 1986. Distribution of heavy metals and their age-related changes in the eastern great white egret, *Egretta alba modesta*, in Korea. *Archives of Environmental Contamination and Toxicology* 15(2): 185-197.

Honda K, Lee DP, Tasukawa R. 1990. Lead poisoning in Swans in Japan. *Environmental Pollution* 65: 209-218.

Hood RD, Harrison WP, Vedel GC. 1982. Evaluation of arsenic metabolites for prenatal effects in the hamster. *Bulletin of Environmental Contamination and Toxicology* 29: 679-687.

Hood RD. 1998. Developmental effects of methylated arsenic metabolites in mice. *Bulletin of Environmental Contamination and Toxicology* 61: 231-238.

Huang H, Huang CF, Wu DR, Jinn CM, Jan KY. 1993. Glutathione as cellular defence against arsenite toxicity in cultured Chinese hamster ovary cells. *Toxicology* 79 (3): 195-204.

Hughes MF, Kenyon BM. 1998. Dose-dependent effects on the disposition of monomethylarsonic acid and dimethylarsinic acid in the mouse after intravenous administration. *Journal of Toxicology and Environmental Health Part A* 53: 95-112.

Hughes MF. 2002. Arsenic toxicity and potential mechanisms of action. *Toxicology Letters* 133 (1): 1-16.

Hulse M, Mahoney JS, Schroder GD, Hacker CS, Pier SM. 1980. Environmentally acquired lead, cadmium, and manganese in the cattle egret, *Bubulcus ibis*, and the laughing gull, *Larus atricilla. Archives of Environmental Contamination and Toxicology* 9(1): 65-77.

Hutchinson TC, Meema KM (Eds). 1987. Lead, mercury, cadmium, and arsenic in the environment. Published on behalf of the Scientific Committee on Problems of the Environment (Scope) of the International Council of Scientific Unions by (ICSU) Wiley, Chichester, New York.

Ilback N-G, Sundberg J, Oskarsson A. 1991. Methyl mercury exposure via placenta and milk impairs natural killer (NK) cell function in newborn rats. *Toxicology Letters* 58:149–158.

Jaghabir MTW, Abdelghani A, Anderson AC. 1988. Oral and dermal toxicity of MSMA to New Zealand white rabbits, *Oryctalagus cuniculus. Bulletin of Environmental Contamination and Toxicology* 40: 119-122.

Jones JC, Reynolds JD. 1997. Effects of pollution on reproductive behaviour of fishes. *Reviews in Fish Biology and Fisheries* 7(4): 463-491.

Judd F. 1979. Acute toxicity and effects of sublethal dietary exposure of monosodium methanearsonate herbicide to *Perotnyscus leucopus* (Rodentia: Cricetidae). *Bulletin of Environmental Contamination and Toxicology* 22: 143-150.

Kislalioglu M, Scherer E, McNicol RE. 1996. Effects of cadmium on foraging behaviour of lake charr, *Salvelinus namaycush. Environmental Biology of Fishes* 46(1): 75-82.

Klaasen CD (ed). 2001. Casarett and Doull's Toxicology; The Basic Science of Poisons. McGraw-Hill Companies Inc.

Kreppel H, Liu J, Liu Y, Reichl FX, Klaassen CD. 1994. Zinc-induced arsenite tolerance in mice. *Fundamental and Applied Toxicology* 23:32–37

Kretshmer X, Pingitore NE, Cruz-Jimenez G. 2002. Incorporation of arsenic in mammal bone: X-ray absorption spectroscopy. American Geophysical Union, Fall Meeting 2002. Abstract # B51B-0728.

Koplin JR. 1972. Measuring predator impact of woodpeckers on spruce beetles. *Journal of Wildlife Management* 36: 308-320.

Kostial K, Kello D, Jugo S, Rabar I, Maljkovic T. 1978. Influence of age on metal metabolism and toxicity. *Environmental Health Perspectives* 25: 81-86.

Kuroda K, Yoshida K, Yoshimura M, Endo Y, Wanibuchi H, Fukushima S, Endo G. 2004. Genotoxicity of dimethylarsinous acid: high induction of tetraploids. *Applied Organometallic Chemistry* 19(2): 221-225.

Lai V, Yongmei S, Ting E, Cullen WR, Reimer KJ. 2004. Arsenic speciation in human urine: are we all the same? *Toxicology and Applied Pharmacology* 198:297-306.

Lee DP, Honda K, Tatsukawa R, Pyong-Oh Won. 1989. Distribution and residue level of mercury, cadmium and lead in Korean birds. *Bulletin of Environmental Contamination and Toxicology* 43(4): 550-555.

Little EE, Archeski RD, Flerov BA, Kozlovskaya VI. 1990. Behavioural indicators of sublethal toxicity in rainbow trout. *Archives of Environmental Contamination and Toxicology* 19(3): 380-385.

Lopez JF, Judd FW. 1979. Effect of sublethal dietary exposure of monosodium methanearsonate herbicide on the nest building behaviour of the white-footed mouse Peromyscus leucopus. *Bulletin of Environmental Contamination and Toxicology* 23:32.

Maclauchlan E, Borden H, D'Auria M. 1988. Distribution of arsenic in lodgepole pines treated with MSMA. *Western Journal of Applied Forestry* 3(2): 37-40.

Maedgen JL, Hacker CS, Schroder GD, Weir FW. 1982. Bioaccumulation of lead and cadmium in the Royal Tern and Sandwich Tern. *Archives of Environmental Contamination and Toxicology* 11(1): 99-102.

Marafante E, Vahter M, Envall J. 1985. The role of the methylation in the detoxification of arsenate in the rabbit. *Chemico-biological Interactions* 56: 225-238.

Marafante E, Vahter M, Norin H, Envall J, Sandstrom A, Christakopoulos A, Ryhage R. 1987. Biotransformation of dimethylarsinic acid in mouse, hamster and man. *Journal of Applied Toxicology* 7:111-117.

Marafante E, Vahter M. 1987. Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. *Environmental Research* 42(1): 72.

Mass MJ, Tennant A, Roop BC, Cullen WR, Styblo M, Thomas DJ, Kligerman AD. 2001. Methylated trivalent arsenic species are genotoxic. *Chemical Research in Toxicology.* 14(4): 355-361.

Mathews NE, Porter WF. 1989. Acute arsenic intoxication of a free-ranging white-tailed deer in New York. *Journal of Wildlife Disease* 25:132-135.

MMA Task Force Three. 1993. SARA Title III Section 313 Delisting petition for monosodium methanearsonate (MSMA) and disodium methanearsonate (DSMA). Prepared by OHM Corporation, Walnut Creek CA, for ISK Biotech Corporation, Mentor, OH.

Morrissey C, Elliott JE, Dods P, Albert CA, Wilson L, Lai V, Cullen WR. 2006. Assessing forest bird exposure and effects from Monosodium Methanearsonate (MSMA) during the Mountain Pine Beetle epidemic in British Columbia. Canadian Wildlife Service Technical Report No. 460.

Nagaraju TN, Desiraju T, 1993. Regional alterations in the levels of brain biogenic amines, glutamate, GABA, and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. *Bulletin of Environmental Contamination and Toxicology* 50 (1): 100-107.

Nagy KA. 2001. Food requirements of wild animals: predictive equations for free-living mammals, reptiles, and birds. *Nutrition Abstracts and Reviews, Series B* 71:21r-31r.

NAS. 1977. Medical and Biological Effects of Environmental Pollutants: Arsenic. National Academy of Sciences, Washington D.C.

NRCC .1978. Effects of arsenic in the Canadian environment. National Research Council. Canada Publication No. NRCC 15391. 349 pp.

Nyholm NEI. 1998. Influence of heavy metal exposure during different phases of the ontogeny on the development of Pied Flycatchers, *Ficedula hypoleuca,* in natural populations. *Archives of Environmental Contamination and Toxicology* 35: 632-637.

Odanaka Y, Matano O and Goto S. 1980. Biomethylation of inorganic arsenic by the rat and some laboratory animals. *Bulletin of Environmental Contamination and Toxicology* 24: 452-459.

Ogle MC, Scanlon PF, Kirkpatrick RL, Gwynn JV. 1985. Heavy metal concentrations in tissues of mink in Virginia. *Bulletin of Environmental Contamination and Toxicology* 35(1): 29-37.

Ots I, Murumagi A, Horak P. 1998. Haematological health state indices of reproducing Great Tits: Methodology and sources of natural variation. *Functional Ecology* 12: 700-707.

Patriquin M, Heckbert S, Nickerson C, Spence M, White B. 2005. Regional implications of the Mountain Pine Beetle infestation in the Northern Interior Forest Region of British Columbia. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Center, Victoria B.C. Mountain Pine Beetle Initiative Working Paper 2005-3.

Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Vasken Aposhian H. 2000. Monomethylarsonous acid (MMA III) is more toxic than arsenite in Chang Human Hepatocytes. *Toxicology and Applied Pharmacology* 163(2): 203-207.

Prukop JA, Savage NL. 1986. Some effects of multiple sublethal doses of monosodium methanearsonate (MSMA) on hematology, growth and reproduction of laboratory mice. *Bulletin of Environmental Contamination and Toxicology* 36:337-341.

Raffa KF, Berryman AA. 1987. Interacting Selective Pressures in Conifer-Bark Beetle Systems: A Basis for Reciprocal Adaptations? *American Naturalist* 129(2): 234-262.

Ramos O, Carrizales L, Yanez L, Mejia J, Batres L, Ortiz D, Diaz Berriga F. 1995. Arsenic Increased Lipid Peroxidation in Rat Tissues by a Mechanism Independent of Glutathione Levels. *Environmental Health Perspectives* 103 Suppl 1: 85-88.

Riviere JL, Grolleau G, Bach J. 1985. Hepatic biotransformation in the buzzard (*Buteo buteo)* and the Japanese quail (*Coturnix coturnix*): effect of PCBs. *Comparative biochemistry and physiology. Part C* 82(2):439-43.

Rodriguez VM. 2002. Effects of sodium arsenite exposure on development and behaviour in the rat. *Neurotoxicology and Teratology* 24(6): 743-750.

Rogers EH, Chernoff N, Kavlock RJ. 1981. The teratogenic potential of cacodylic acid in the rat and mouse. *Drug and Chemical Toxicology* 4: 49-61.

Rust HJ. 1929. Relation of insectivorous birds to the mortality of the mountain pine beetle during flight period. *U.S.D.A. Bur. Entomol. For. Insect Field Stn., Couer d'Alene, Idaho. Unpub. Rep.* 5 p.

Rust HJ. 1930. Relation of insectivorous birds to the mortality of the mountain pine beetle during flight period. *U.S.D.A. Bur. Entomol. For. Insect Field Stn., Couer d'Alene, Idaho. Unpub. Rep.* 11 p.

Scheuhammer AM. 1987. The chronic toxicity of aluminum, cadmium, mercury, and lead in birds; a review. *Environmental Pollution* 46: 263-295.

Scheuhammer AM. 1996. Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. *Environmental Pollution* 94 (3): 337-343.

Schulz H, Nagymajtenyi L, Institoris L, Papp A, Siroki O. 2002. A study on behavioural, neurotoxicological, and immunotoxicological effects of subchronic arsenic treatment in rats. *Journal of Toxicology and Environmental Health Part A* 65(16): 1181-1193.

Scott N, Hatlelid KM, MacKenzie NE, Carter DE. 1993. Reactions of arsenic (III) and arsenic (V) species with glutathione. *Chemical Research in Toxicology* 6: 102-106.

Shariatpanahi M, Anderson AC. 1984. Distribution and toxicity of monosodium methanearsonate following oral administration of the herbicide to dairy sheep and goats. *Journal of Environmental Science and Health part B* 19: 427-439.

Smits JE, Blakely BR, Wobeser GA. 1996. Immunotoxicity studies in mink (*Mustela vison*) chronically exposed to dietary bleached kraft pulp mill effluent. *Journal of Wildlife Disease* 32:199-208.

Smits JE, Williams TD. 1999. Validation of immunotoxicology techniques in passerine chicks exposed to oil sands tailings water. *Ecotoxicology and Environmental Safety* 44:105-112.

Sorensen JS, Dearing MD. 2003. Elimination of plant toxins by herbivorous woodrats: revisiting an explanation for dietary specialization in mammalian herbivores. *Oecologia* 134:88–94

Stanley TR, Spann JW, Smith GJ, Rosscoe R. 1994. Main and interactive effects of arsenic and selenium on mallard reproduction and duckling growth and survival. *Archives of Environmental Contamination and Toxicology* 26 (4): 444-451.

Stevens JT, Hall LL, Farmer JD, DiPasquale LC, Chernoff N, Durham WF. 1977. Disposition of ^{14}C and/or ^{74}As-cacodylic acid in rats after intravenous, intratracheal or peroral administration. *Environmental Health Perspectives* 19: 151-157.

Styblo M, Yamauchi H, Thomas DJ. 1995. Comparative in vitro methylation of trivalent and pentavalent arsenicals. *Toxicology and Applied Pharmacology* 135: 172-178.

Styblo M, Delnomdedieu M, Thomas DJ. 1996. Mono and dimethylation of arsenic in rat liver cytosol in vitro. *Chemical Biological Interactions* 99: 147-164.

Styblo M, Serves SV, Cullen WR, Thomas DJ. 1997. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chemical Research in Toxicology* 10(1): 27-33.

Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas D. 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Archives of Toxicology* 74: 289-299.

Suzuki KT, Tomita T, Ogra, Y, Ohmichi M. 2003. Arsenic metabolism in hyperbilirubinemic rats: Distribution and excretion in relation to transformation. Arsenic Exposure and Health Effects V, Chappell, W., Abernathy, CO, Calderon RL, Thomas DJ (Eds). Elsevier B.V., Amsterdam:355-368.

Suzuki KT, Katagiri A, Sakuma Y, Ogra Y, Ohmichi M. 2004. Distributions and chemical forms of arsenic after intravenous administration of dimethylarsinic and monomethylarsonic acids to rats. *Toxicology and Applied Pharmacology* 198 (3): 336-344.

Thies M, Gregory D. 1993. Residues of lead, cadmium, and arsenic in livers of Mexican free-tailed bats. *Bulletin of Environmental Contamination and Toxicology* 52(5):641.

Vahter M. 1981. Biotransformation of trivalent and pentavalent inorganic asenic in mice and rats. *Environmental Research* 25:286-293.

Vahter M, Marafante E, Lindgren A, Dencker L. 1982 Tissue distribution and subcellular binding of arsenic in marmoset monkeys after injection of ^{74}As-arsenite. *Archives of Toxicology* 51:65-77.

Valkonen S, Savolainen H, Järvisalo J. 1983. Arsenic distribution and neurochemical effects in peroral sodium arsenite exposure of rats. *Bulletin of Environmental Contamination and Toxicology* 30 (1): 303-308.

VanderKop and MacNeil.1989. Effects of arsanilic acid and monensin when given simultaneously in the diet of broiler chicks. *Veterinary and Human Toxicology* 31 (3): 209-213.

Vega L, Styblo M, Patterson R, Cullen W, Wong C, Germolec D. 2001. Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. *Toxicology and Applied Parmacology* 172(3): 225-232.

Walker CH, Hopkin SP, Sibly RM, Peakall DB. 1996. Principles of Ecotoxicology. Taylor and Francis Ltd, London.

Wanibuchi H, Yamamoto S, Chen H, Yoshida K, Endo G, Hori T, Fukushima S. 1996. Promoting effects of dimethylarsinic acid on N-butyl-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in rats. *Carcinogenesis* 17(11): 2435-4239.

Wardlaw SC, Levine RA. 1983. Quantitative buffy coat analysis: A new laboratory tool functioning as a screening complete blood count. *Journal of the American Medical Association* 249:617-620.

Wei M, Wanibuchi H, Yamamoto S, Li W, Fukushima S. 1999. Urinary bladder carcinogenicity of dimethylarsinic acid in male F344 rats. *Carcinogenesis* 20(9): 1873-1876.

Whitworth MR, Pendleton GW, Hoffman DJ, Camasdese MB. 1991. Effects of dietary boron and arsenic on the behaviour of mallard ducklings. *Environmental Toxicology and Chemistry* 10: 911-916.

Woolson EA (ed). 1975. Arsenical pesticides : a symposium sponsored by the Division of Pesticide Chemistry at the 168th meeting of the American Chemical Society, Atlantic City, N.J., Sept. 9, 1974

Wren CD. 1986. A review of metal accumulation and toxicity in wild mammals. I. Mercury. *Environmental Research* 40(1): 210-244.

Wren CD, Fischer KL, Stokes PM. 1988. Levels of lead, cadmium and other elements in mink and otter from Ontario, Canada. *Environmental Pollution* 52(3): 193-202.

Yamamoto S, Konishi Y, Matsuda T, Murai T, Shibata MA, Matsui-Yuasa I, Otani S, Kuroda K, Endo G, Fukushima S. 1995. Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pre-treatment with five carcinogens. *Cancer Research* 55: 1271-1276.

Yamauchi H, Fowler BA. *Toxicity and metabolism of inorganic methylated arsenicals.* In: Arsenic in the Environment, Part II: Human Health and Ecosystem Effects, Nriagu J.O. (Ed). John Wiley and Sons, Ltd, New York, 1994, pp 35-53.

Yamauchi H and Yamamura Y. 1985. Metabolism and excretion of orally administered arsenic trioxide in the hamster. *Toxicology* 34:113-121.

Yamauchi H, Yamato N and Yamamura Y. 1988. Metabolism and excretion of orally and intraperitoneally administered methylarsonic acid in the hamster. *Bulletin of Environmental Contamination and Toxicology* 40: 280-286.

Yoshida K, Chen H, Inoue Y, Wanibuchi H, Fukushima S, Kuroda K, Endo G. 1997. The urinary excretion of arsenic metabolites after a single oral administration of dimethylarsinic acid to rats. *Archives of Environmental Contamination and Toxicology* 32 (4): 416- 421.

Yoshida K, Inoue Y, Kuroda K, Chen H, Wanibuchi H, Fukoshima S, Endo G. 1998. Urinary excretion of arsenic metabolites after long term oral administration of various arsenic compounds to rats. *Journal of Toxicology and Environmental Health Part A* 54(3): 179-192.

Zakharyan RA, Wu Y, Bogdan GM, Aposhian HV. 1995. Enzymatic methylation of arsenic compounds. I. Assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. *Chemical Research in Toxicology* 8: 1029-38.

Zakharyan RA, Wildfang E, Aposhian HV. 1996. Enzymatic methylation of arsenic compounds. III. The marmoset and tamarind, but not the rhesus, monkey are deficient in methyltransferses that methylate inorganic arsenic. *Toxicology and Applied Pharmacology* 140: 77-84.

Zann RA. 1996. *The Zebra Finch; A Synthesis of Field Studies and Laboratory Studies.* Oxford University Press, Oxford.