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Tissue Uptake, Mortality, and Sublethal Effects of Monomethylarsonic Acid (MMA(V)) in Nestling Zebra Finches (*Taeniopygia guttata*)

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odel passerine, the zebra finch (***Taeniopygia***
es were orally dosed for 20 d from hatching to
24, 36, or 72 μg/g bw/d of monomethylarsonic
corresponds to MSMA at physiological pH).

10-d-old bobwhite yielded a 96-h LD_{50} of approximately 650 mg/kg/d (MMA Task Force Three, 1993). However, mallards tested on the same dosing scheme showed no effects or mortality even at the highest dose administered (1100 mg/kg/d; MMA Task Force Three, 1993). In addition to acute toxicity, various sublethal effects of MSMA were reported in several lab mammals, including decreased hematocrit and blood glucose levels (Judd, 1979), gastrointestinal irritation, frequent vomiting and diarrhea (MMA Task Force Three, 1993), and behavioral abnormalities and lowered reproductive capabilities (Prukop & Savage, 1986). Methylated arsenicals also produce adverse developmental effects including malformations, and abnormal and slowed development of offspring in mice (Rogers et al., 1981), embryonic resorption and decreased mean fetal weight in hamsters (Hood et al., 1982; Hood, 1998), and decreased growth in rats (Rodriguez, 2002). There have been few similar studies in birds, though Hoffman et al., (1991) reported some reduction in growth in mallard ducklings dosed with environmentally relevant concentrations of sodium arsenate. Studies on the fertility and hatching success in breeding birds, as well as sublethal effects in nestlings, exposed to MSMA are lacking in the literature.

In this study the tissue distribution, growth parameters and general health of nestling zebra finches (*Taeniopygia guttata*) orally dosed with monomethylarsonic acid (MMA(V)) was investigated as a model for cavity-nesting birds that might be affected as nontarget species by MSMA-treatment against bark beetle (e.g., woodpeckers, chickadee). Specifically, nestlings were dosed daily with MMA through the entire nestling period and were measured in terms of chick growth, mortality or fledging success, leukocrit, hematocrit, and a measure of cell-mediated immunity (using the phytohemagglutinin [PHA] test). MMA(V) was used as it corresponds to MSMA at gastrointestinal pH (Dost, 1995) and is the primary form of As detected in the bark beetle samples collected in MSMA-treated pine stands in British Columbia (Morrissey et al., 2007). Furthermore, ecologically relevant doses were used, which were calculated based on total As detected in bark beetle samples (Morrissey et al., 2007) and food-ingestion rates of free-living woodpeckers (Nagy, 2001), which were extrapolated to the zebra finch.

collected from 5 randomly chosen nestlings per dose group at d 21 to determine total blood As content, 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 1 h. Hematocrit and leukocrit were also measured as described earlier.

MMA Analysis

Dosing solutions, blood, and organ tissues were sent to Dr. William Cullen's Chemistry Laboratory at the University of British Columbia, Vancouver, BC, where they were measured for As content. Within the literature, monomethylarsonic acid is abbreviated as MMA, MAA, and MMAA. To avoid confusion, only MMA is used throughout this article. Based on the metabolic pathway of ingested MMA(V), there are two valences one might expect to detect; MMA(V) in its original form or MMA(III), a more acutely toxic, unstable form of methylated As. However, our chemical analysis did not distinguish between different valences. Therefore, MMA and DMA were reported to represent both valences (III) and (V).

Samples were analyzed for total As as described previously by Lai et al. (2004). Briefly, blood and tissue samples were weighed into glass test tubes and 2 ml nitric acid and 3 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 150ºC and then cooled overnight. Hydrogen peroxide (2 ml) was added to the samples on the next day and the samples were heated to 150ºC 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 4ºC until analysis. Digested blood and tissue samples were diluted appropriately with the rhodium–nitric acid solution and analyzed for total As using Inductively coupled plasma-mass spectrometry (ICP-MS). 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 purchased from a local food store in Vancouver, BC, and is used as an internal laboratory standard (Lai et al., 1997).

significantly higher levels of As in the blood compared to the control. In the main study, nestlings dosed with 8 and 12 µg/g had significantly higher mean total blood As levels. Control birds had trace amounts of As in the blood, which may be attributed to background levels resulting from “natural” exposure of metal in drinking water and food.

All organ tissues analyzed from surviving nestlings in the second pilot study contained total As residues that were higher in nestlings dosed with 4 µg/g bw/d MMA(V) compared to control nestlings (Figure 2). In the 4-µg/g dosed birds, the liver contained the highest amount of arsenic, ranging from 1.13 to 3.6 µg/g, followed by kidney with a range of 1.04 to 2.59 µg/g (mean: 2.01 ±oth-3 b atiilnts of A0(sed)-5.9(wir3 13.142]6 ysyT* .5(tis)d b)is(nbutnt ford)-e ssed.9(ver)]TJ -

on measures of skeletal or body size: tarsus and wing cord length at fledging at MMA(V) concentrations as low as 8 µg/g, which is equivalent to consuming a diet of beetles with a concentration of 50 µg/g As.

In a previous study using adult zebra finches and orally administering doses of MMA(V) at 0, 8, 24, or 72 µg/g, Albert (2006) reported no mortality even at the highest dose levels. Although there was a high level of excretion (>90%) of As in all dose groups, there was a dose-dependent accumulation of As in the blood and other tissues (kidneys, liver, brain). MMA(V) treatment was also associated with reduced body mass in the 24- and 72-µg/g dose groups (Albert, 2006). The high mortality of nestlings in the current study as compared with adults on the same dosing scheme suggests that nestlings are much more sensitive to MMA(V). Young developing birds frequently exhibit increased sensitivity to metal exposure compared with adults (Scheuhammer, 1987). Nestlings likely lack the ability of adults to rapidly methylate and excrete the compound. This might be due to underdeveloped metabolic processes in the nestlings, as these are altricial birds and their tissues are still undergoing functional differentiation (Hoffman 2003); thus, their organs may be unable to cope with exposure to a potentially toxic compound while developing. Administration of high concentrations of MMA(V) may cause the nestlings to rapidly absorb the compound, and 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 may be experiencing a threshold effect, whereby metabolism and excretory mechanisms are able to cope with the compound at lower doses, and subsequently experience less toxicity.

Exposure to MMA(V) was verified with analysis of blood and tissue As residues. From the combined data it was noted that there is accumulation of As in blood, and in all experiments, all dose groups had more total blood As than the control. Tissue distribution results showed up to 3.6 µg/g As in liver, 2.59 µg/g in kidneys, and 1.34 µg/g in brain of 4 µg/g dosed nestlings. Histopathological effects of this tissue uptake and accumulation were not assessed. However, based on the finding of Jaghabir et al. (1989), who observed lesions in several tissues of New Zealand White rabbits (*Oryctalagus cuniculus*) dosed with MSMA, clearly further investigation of potential pathological effects is warranted. In addition, studies on the possible effects produced by accumulation of As in the liver and kidneys would be useful in determining the overall risk of exposure to MMA(V). When compared to adult zebra finches dosed with the same concentrations of MMA(V) (Albert, 2006), it is clear that nestling zebra finches are more susceptible to accumulation in organ tissues than adults, but not in the blood. Nestlings dosed with 8 µg/g MMA(V) had an average of 0.19 µg/g As in blood, while adults dosed with 8 µg/g MMA(V) had an average of 0.075

cord length in the zebra finches may be due to a possible interaction between As and the mineral fraction of the bone. Kretschmer et al. (2002) suggested that As in bone may be associated with the mineralized calcium phosphate, or hydroxyapatite, with As likely replacing the phosphate. Hydroxyapatite is an important part of the framework in bones, making them strong and rigid (Heaney, 1999). A possible interaction between As and phosphate in the hydroxyapatite may have consequences in developing birds by interfering with normal bone deposition. However, it is likely As in the form of inorganic arsenate (As(V)) would be substituting the phosphate in this interaction, and demethylation of MMA(V) to inorganic As is highly unlikely (Yamauchi et al., 1988). Shorter tarsi and wing cord length in the nestlings appeared to exert no effect on the general health and fledging success of the nestling zebra finches. However, zebra finch nestlings were held in a controlled lab setting, and determining the effects of shorter tarsi and wing cord length in wild passerine fledglings on future fitness (e.g. anti-predatory responses) requires further study. In addition, it remains unknown whether As accumulation in nestlings has longer term effects in terms of post-fledging sus hs 143he]TJhe]TJtwruc, ie065 Tw[248 Tcty5(ood sam.5(y5(p))5.4lr)5.romldadp5-5.4(eckers.6(ngot.5(y5()

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