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Posthatch Methoxychlor Exposure Adversely Affects Reproduction of Adult Zebra Finches, *Taeniopygia guttata*

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Several naturally occurring and human-produced chemicals have estrogenic-like effects on wildlife that may affect their survival and reproductive success. Methoxychlor (MXC; 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane) is a widely used organochlorine pesticide that readily accumulates in wildlife (e.g., Wu et al. 2000). It is a known endocrine disruptor in many vertebrate taxa but has been most extensively studied in female rats and mice (Cummings 1997). We investigated the effects of MXC exposure in a songbird, the zebra finch (*Taeniopygia guttata*). Specifically, we examined reproductive function of zebra finch pairs that had been orally-treated with MXC as chicks, a time of vulnerability of altricial songbirds to adverse effects of oral estrogens on adult reproduction (Millam et al. 2001). We gauged the effects of MXC by measuring latency to begin egg laying, egg production, and egg viability (candled fertility, cracked or broken eggs, embryo viability, and hatching success).

Among precocial avian species, such as the chicken (*Gallus domesticus)* and Japanese quail (*Coturnix coturnix japonica*), MXC exposure *in ovo* alters normal sexual differentiation, namely male sexual behavior (Eroschenko et al. 2002; Ottinger et al. 2001). In altricial birds, including the zebra finch, the period of sensitivity to steroid hormone exposure extends into the posthatch period (Millam et al. 2001). Thus, in addition to MXC exposure to embryos via maternal deposition, altricial nestlings may be directly affected by MXC in their diet, a naturalistic route of exposure in the wild, at a time when they may still be subject to organizational effects of estrogenic compounds.

Although parenteral treatment of female zebra finch chicks with estradiol masculinizes song control nuclei in the female brain (e.g., Gurney and Konishi 1980), dietary MXC does not (Quaglino et al. 2002), even at doses that are potently estrogenic (Millam et al. 2002). In the present study, we tested the possibility that dietary exposure to MXC may alter reproductive success of zebra finch pairs through mechanisms other than changes in brain song nuclei. We found that individuals that were orally dosed with MXC as chicks experienced an increased number of cracked and/or missing eggs, a decreased number of hatched eggs and a borderline effect on fertility of eggs, but that post-hatch MXC-treatment did not affect the latency to lay eggs, or the number of eggs produced.

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MATERIALS AND METHODS

Zebra finches were produced by the Department of Animal Science's random-bred-colony. The colony was founded from local avicultural stock of wild-type phenotype. Finches were provided with *ad libitum* tap water and Finch Super mixed bird seed (Volkman Seed Co., Ceres, CA). As a supplement to the diet, we fed the finches ground-up boiled egg twice per week. We provided wooden dowels as perches, and once a week, we set out shallow dishes of water as baths. The photoperiod was 16 h light: 8 h dark; room temperature was approximately 21°C.

Chicks were identified by the color of their down, which we dyed on the day of hatch, and at 13-15 days of age by a uniquely numbered leg band. Chicks were weighed daily and dosed orally, according to body mass, once per day, on days of age 5-11 with 100 nmol/g (low dose) or 1000 nmol/g (high dose) of methoxychlor (95% purity; Sigma Chem. Co., St. Louis, MO) in canola oil (1 ul/g body weight), or canola oil alone as a control. Chicks were reared by their parents in individual pair breeding cages (46 X 46 X 46 cm) until approximately 45 days of age and then transferred to mixed-sex cages containing from 6-20 birds each. Between 130 and 180 days of age, five males and five females receiving either canola oil (control), 100 nmol/g or 1000 nmol/g methoxychlor, were introduced into communal cages (0.9 X 0.9 X 1.8 m) for experimental trials (10 individuals per cage [5 potential pairs], each receiving the same dose treatment). This was repeated, so an N of 10 potential pairs per treatment was achieved. None of the finches were more closely related than as cousins.

Birds were induced to breed by providing eight sheet metal nest boxes (15 X 15 X 13 cm) per communal breeding cage. Supplying more nest boxes in communal caging than is minimally necessary (for one pair per nest box) is common avicultural practice to reduce competition for nesting sites. Shredded burlap was provided in nest boxes for nest construction. An experimentally blind observer checked nests daily for number of eggs and chicks, and eggs were candled at weekly intervals during the incubation period to determine fertility. Data were collected over the following 7-week period. Protocols were approved by the U.C. Davis Institutional Animal Use and Care Committee.

We measured reproductive performance with the following: latency in days between the start of trial and production of the first egg, number of eggs (clutch size), and number of hatched chicks. We also calculated the number of candled fertile eggs, broken eggs, and eggs that could not be found after once having been identified (missing eggs), and dead embryos (shells intact). A clutch was defined as a sequence of eggs with no more than 4 days passing between the laying of successive eggs. Generally, a particular pair of finches was observed to occupy a nest box and incubate a clutch of eggs. However, because of the possible incidence of brood parasitism, the analysis was restricted to the top five producing nest boxes in each trial and the analysis was restricted to first clutches. Top producing nest boxes were identified on the basis of the presence and number of eggs, candled fertility and hatching success of eggs. We employed one-way ANOVAs to compare means of each parameter across treatment groups (control, low-dose, and high-dose) and used Bonferroni's test for multiple means comparisons when main effects were significant ($P<0.05$). A part of the same experiment was previously published (Millam et al. 2001).

RESULTS AND DISCUSSION

The overall finding of this study provides evidence that orally-administered MXC decreases avian reproductive success by directly inhibiting reproductive performance. Results indicated that zebra finches orally dosed with MXC had significantly decreased reproductive performance due to impaired egg and gamete quality. Though latency to lay (Fig. 1A) and number of eggs (Fig. 1B) did not vary between MXC-dosed and control groups, the high-dose group (1000 nmol/g) had fewer hatchlings relative to those treated with vehicle alone (Fig. 1F, $P<0.05$, Bonferroni). The number of eggs candled fertile was only borderline in significance at $P=0.0585$ (Fig. 1C), so multiple overall means tests were not done. Lowered fertility, if present, may have been either due to male or female infertility. The incidence of reproductive failure due to missing eggs and egg breakage was greater in the high dose group, compared to controls (Fig. 1D, $P<0.01$, Bonferroni). No other differences were significant.

Metabolites of MXC have potent estrogenic effects, bind to androgen receptors and act as anti-androgens by blocking androgen-induced transcription (Gray et al. 1999). However, doses used in many toxicological studies are selected to reflect maximum pesticide concentrations expected on plant material and other wildlife food items following chemical applications, and these concentrations often exceed those to which wildlife might realistically be exposed (Fletcher et al. 1994). Lower, chronic doses may be more realistic to actual exposure levels, which may be more indicative of actual exposure levels and may produce no effect or even opposite effects from those produced by acute doses in the laboratory (Palanza et al. 2001). With these caveats in mind, the low dose treatment had no effects on the parameters measured. However, our results suggested that higher MXC dose treatment may have had effects similar to other pesticides on eggshell thinning (e.g., DDT and its metabolite DDE, Cooke 1973). Our results suggest that the decreased hatching success of zebra finches was due to an increased incidence of cracked/broken and missing eggs and a possible effect on fertility or embryo viability.

These results support previous findings suggesting MXC may have broad detrimental effects on the reproductive function of various vertebrates. Methoxychlor exposure during both the embryonic and adult phases of the mammalian life cycle affects normal functioning of the reproductive axis (Eroschenko et al. 1995). Specifically, reproductive abnormalities may occur due to the acceleration of puberty and loss of fertility, as well as developmentally-

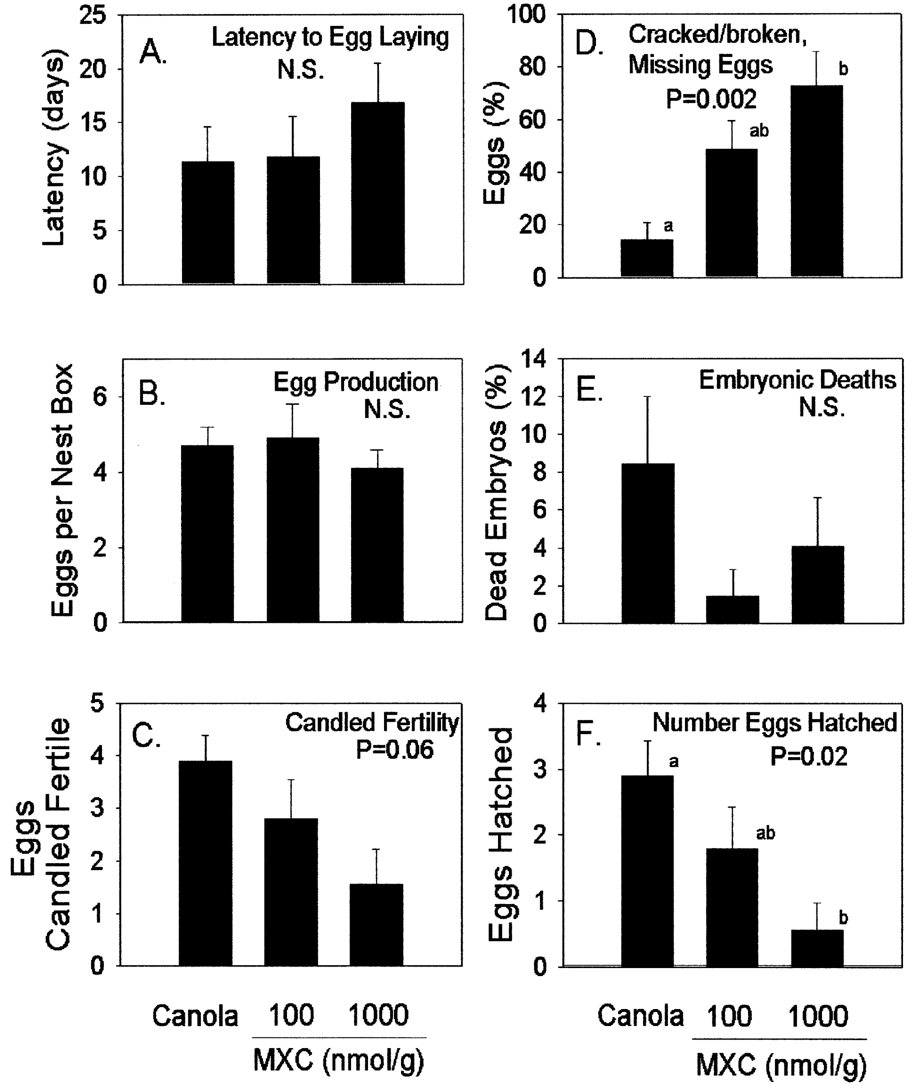

Figure 1. Reproductive performance of zebra finch pairs treated orally as chicks with canola oil (control; "Canola") or 100 or 1000 nmol/g methoxychlor (MXC): A. Latency to egg laying after nest box presentation; B. Number of eggs per nest box; C. Number of eggs candled fertile in each nest box; D. Percent cracked/broken and missing eggs per nest box; E. Percent dead embryos; and F. Number of eggs hatched. Unlike superscripts denote significant differences at indicated levels using Bonferroni's multiple means test, when main effect is significant (ANOVA).

induced damage to brain and organ development (Palanza et al. 2001). Methoxychlor also causes infertility by impairing implantation and embryonic development in mice (*Mus musculus*). In Japanese quail, low doses of dietary

MXC alter normal sexual behavior, as well as steroid hormone levels (Ottinger et al. 2001).

Methoxychlor has been licensed at up to 3 pounds per acre on some crops, e.g., dry beans in Colorado (Insecticide, Herbicide, Fungicide Committees 1996). At this application rate, Kenaga's nomogram, as modified by Fletcher et al. (1994), estimates that residue on plants and plant parts ranges from approximately 21 to 720 ppm. Obligate granivores, such as zebra finches, fed 1 to 8 g plant material per day would therefore likely ingest from 0.02 to 0.72 mg MXC per day at low feeding rates (1 g per day) and from 0.17 to 5.76 mg MXC per day at high feeding rates (8 g per day). The estimated ranges of exposure are large because it is not clear which of Kenaga's plants/plant parts categories (i.e., short rangegrass, long grass, forage [alfalfa, clover], leaves and leafy crops, pod-containing seeds [legumes], fruit [cherries, peaches] - none of which include grass seeds), best approximates grass seeds, the preferred food of estrildid finches. The 100 nmol/g dose of MXC used in this study would result in daily intakes of approximately 0.12 mg MXC per day for younger chicks (approximately 3.5 g body weight) and 0.36 mg per day for older chicks (approximately 10.5 g body weight); the higher dose would result in ten-fold greater intakes. Thus, the MXC doses used in this study are within the upper exposure range of doses encountered in field conditions.

The data presented here support earlier results showing that exposure to estrogens may adversely influence reproduction in songbirds. In prior studies, short-term, posthatch exposure of zebra finches to estradiol benzoate (EB) caused reproductive impairments that were similar to the effect of our higher dose of MXC (1000 nmol/g), including reduced egg fertility, reduced hatching success, and increased egg breakage (Millam et al. 2001). The reduced fertility is likely due to impaired male copulatory behavior (Adkins-Regan and Ascenzi, 1987). However, the lower dose of MXC (100 nmol/g) used in the present study and an equimolar dose of the industrial surfactant, octylphenol (100 nmol/g OP; 4 octylphenol), used by Millam et al. (2001), did not significantly affect these reproductive measures, though more subtle effects cannot be ruled out. More sensitive assays are necessary to reveal possible behavioral effects of MXC; experiments should include longer durations of exposure, especially at low doses, and further experiments are needed to understand the factors that result in increased egg breakage, decreased fertility and fewer hatched young.

REFERENCES

Adkins-Regan E, Ascenzi M (1987) Social and sexual behavior of male and female zebra finches treated with estradiol during the nestling period. Anim Behav 35: 1100-1112

Cooke, AS (1973) Shell thinning in avian eggs by environmental pollutants. Environ Pollut 4:58-157

Cummings AM. (1997) Methoxychlor as a model for environmental estrogens. Crit Rev Toxicol 27:367-79

Eroschenko VP, Abuel-Atta AA, Grober MS (1995) Neonatal exposure to methoxychlor alters ovaries in adult mice. Reprod Toxicol 9:379–387

Eroschenko, VP Amstaslavsky SYY, Schwabel H, Ingermall RL (2002) Altered behaviors in male mice, male quail, and salamander larvae following early exposures to the estrogenic pesticide methoxychlor. Neurotoxicol Teratol 24:29-36

Fletcher JS, Nellessen JE, Pfleeger TG (1994) Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, an instrument for estimating pesticide residues on plants. Environ Toxicol Chem 13:1383-1391

Gray LE, Ostby J, Cooper RL, Kelce WR (1999) The estrogenic and antiandrogenic pesticide methoxychlor alters the reproductive tract and behavior without affecting pituitary size or LH and prolactin secretion in male rats. Toxicol Ind Health 15:37-47

Gurney ME, Konishi M (1980) Hormone-induced sexual differentiation of brain and behavior in zebra finches. Science 208:1380-1382

Insecticide, Herbicide, Fungicide Committees (1996) Colorado Pesticide Guide – Field Crops. Colorado State University Cooperative Extension, Fort Collins, Colorado

Millam JR, Craig-Veit CB, Batchelder ME, Viant MR, Herbeck TM, Woods LW (2002) An avian bioassay for environmental estrogens: the growth response of zebra finch (*Taeniopygia guttata*) chick oviduct to oral estrogens. Environ Toxicol Chem 21: 2663-68

Millam JR, Craig-Veit CB, Quaglino AE, Erichsen AL, Famula TR, Fry DM (2001) Posthatch oral estrogen exposure impairs adult reproductive performance of zebra finch in a sex-specific manner. Horm Behav 40:542-9

Ottinger MA, Abdelnabi MA, Henry P, McGary S, Thompson N, Wu JM (2001) Neuroendocrine and behavioral implications of endocrine disrupting chemicals in quail. Horm Behav 40:234-47

Palanza P, Parmigiani S, vom Sal FS (2001) Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'DDT, and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. Horm Behav 40:252-265

Quaglino AE, Craig-Veit CB, Viant MR, Erichsen AL, Fry DM, Millam JR (2002) Oral estrogen masculinizes female zebra finch song system. Horm Behav 42:236-41

Wu TH, Rainwater TR, Platt SG, McMurry ST, Anderson TA (2000) Organochlorine contaminants in Morelet's crocodile eggs from Belize. Chemosphere 40:671-678