EFFECTS OF SIX XENOBIOTICS ON SURVIVAL, HORMONE CONCENTRATIONS, AND MORPHOMETRIC ENDPOINTS OF NORTHERN BOBWHITE QUAIL (*COLINUS VIRGINIANUS*)

C. Sills McMurry, John M. Matter*, Richard L. Dickerson

The Institute of Environmental and Human Health, Box 41163, Texas Tech University, Lubbock, Texas 79409-1163, USA
*Juniata College, Department of Biology, 1700 Moore Street, Huntington, Pennsylvania, 166521, USA

Introduction

Bobwhite quail, (*Colinus virginianus*) a wild avian species, was selected to investigate the effects of six xenobiotics. The doses were chosen to encompass a range of environmental contaminant concentrations and toxic concentrations of several chemicals. The six xenobitoics selected were 2,3,7,8-TCDD (TC), ethynyl estradiol (EE), o,p'-DDE (OP), p,p'-DDE (PP), coumestrol (CO), and indole-3-carbinol (IN). TCDD is a dioxin that is considered anti-estrogenic (DeVito et al., 1991; Pohjanvirta and Tuomisto, 1994) and anti-androgenic (Mably et al., 1992; Pohjanvirta and Tuomisto, 1994). TC is considered approximately 1000 times more toxic than most substances with an LD_{50} equal to 1 ppb (Amdur et al., 1990). Ethynyl estradiol (EE) is a synthetic estrogen that has been used in birth control pills (Muechler and Kohler, 1980). In this study, EE is placed within the same potency range as TC. Coumestrol is a phytoestrogen (Whitten et al., 1992; Collins et al., 1997) found in clover species, alfalfa, and soy. Indole-3-carbinol (I) is a phyto anti-estrogen (Liu et al., 1994) found in broccoli, brussel sprouts, and cabbage. The next two compounds are isomeric degradation products of the insecticide DDT, OP and PP. OP is considered weakly estrogenic compound. PP is considered anti-androgenic (Kelce et al., 1995).

The purpose of this research was to perform a preliminary investigation of the effects of six xenobiotics used over a range of five doses to investigate dose-response relationships. The objectives were to determine dose-response relationships for EE, TC, CO, OP, and PP using the endpoints survival, estradiol and testosterone plasma concentrations, and morphometric endpoints (i.e., somatic indices of liver, kidney, brain, spleen, gonads, measurements of final weight and weight gain), and to determine the utility of these endpoints as biomarkers for these chemicals and in this species.

Materials and Methods

Six eggs per dose per treatment were injected under sterile conditions with a control (vehicle), 0.1, 0.3, 1.0, 3.0, 10.0 mg/kg for coumestrol, indole-3-carbinol, p,p'-DDE, o,p'-DDE or μg/kg for ethynyl estradiol or TCDD (approximately 2 μl). Chemicals were dissolved or suspended in absolute ethanol. The injection hole was covered with a small drop of melted paraffin mixed with petroleum jelly. An additional set of eggs were dosed with corn oil (vehicle) and injected below the air cell. Doses were 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0 μg/kg for ethynyl estradiol and 0, 0.003, 0.01, 0.03, 0.1, 0.3 μg/kg for TCDD. Eggs were placed into an incubator with rotating shelves at

37.5°C and 65% relative humidity for 21 days, placed in hatcher set at 37°C and 70% relative humidity, hatched, dried, weighed, measured, banded, and placed in a brooder with similarly dosed chicks. Chicks were given water and food *ad libitum*. Local heaters were set for 95 °C for the first week, 90 °C the second week, and 85 °C the third week. The room light cycle was 12 hours light and 12 hours dark. Ambient room temperature was 25-25.6°C.

Chicks (21 days old) were weighed then anesthetized with carbon dioxide (CO_2) gas. Blood was withdrawn from jugular veins or heart and placed into heparinized Eppendorf centrifuge tubes. Chicks were euthanized with CO_2 gas. Measurements were taken and tissues (liver, kidney, brain, gonads, oviduct, and spleen) were collected and weighed. Heparinized blood was kept chilled in a cooler until all necropsies for a given day were complete. Blood was centrifuged at 2500 rpm for 15 minutes. Plasma was removed to a clean Eppendorf tube and frozen at -20°C until analysis.

Hormone concentrations were determined by radioimmunoassay using ^{125}I labeled hormones (10,000 cpm). Duplicate samples (80 µl) were prepared by liquid-liquid extraction using ethyl acetate and hexane followed by solid phase extraction using isooctane, methanol, ethyl acetate with Alumina B columns. Samples for recovery determination were run with assay samples. Assays were incubated with antibody overnight. Charcoal was used to separate free from bound hormone. Samples were counted in a scintillation counter for 2 minutes.

Curve fit and sample concentration determination were performed by IMMUNOFIT® (Beckman) using a four parameter logistic or linear fit when applicable. Survival data for increasing doses of chemical were analyzed by Mantel-Haenzel Chi-square trend analysis and Likelihood Ratio Chi-Square.

Results and Discussion

Survival

Survival for quail eggs dosed with ethanol as vehicle was typically 67% survival (Table 1a) with a mean of 64% +/- 16. It was similar for eggs dosed with corn oil as vehicle (70% survival; Table 1b). Survival was significantly affected by increasing concentrations of TCDD as revealed by trend analysis. TCDD treatment produced a significant negative trend in survival with increasing doses (Table 1a). At the 0.3 dose, survival was 50%. There were no survivors above 0.3 µg/kg. TCDD is clearly lethal to these eggs over 0.3 µg/kg with ethanol as the vehicle. TCDD dosed in corn oil at concentrations of 0.003 to 0.3 µg/kg were expected to be below the 100% mortality range observed with the TCDD in ethanol (Table 1b). TCDD in corn oil did not cause significant dose effects (p=0.053) among these lower concentrations and did not indicate a trend affect with increasing dose. However, most of the treatment groups had a much greater mortality (at least 43% in 4 of 5 treatments) than the control.

In quail, both the TCDD corn oil dosed and the TCDD ethanol dosed eggs showed an increase in survival at the 0.1 µg/kg concentrations when compared to controls. This effect has also been observed in the ethynyl estradiol dosed quail (ethanol) at the 0.1 µg/kg and 0.3 µg/kg concentrations. Further, effects like these have been observed in TCDD dosed alligators in a parallel study (unpublished).

Ethynyl estradiol appears to cause a downward trend in survival with increasing doses. Ethynyl estradiol dosed in corn oil at concentrations of 0.3 to 10 µg/kg caused a reduction in survival but

not in a trend with increasing dose (Table 1b). The effects of ethynyl estradiol appear more toxic when dosed in corn oil below the air cell than when dosed with ethanol in the air cell. Perhaps the observed differences are a function of the injection site. Also, where the survival of eggs dosed with ethynyl estradiol appeared to decrease with increasing dose in ethanol as vehicle, that relationship was not apparent with the corn oil based treatments.

Ethynyl estradiol had low control survival, however very high survival at the low doses compared to average treatment control values (Table 1a). These results suggest that low concentrations of ethynyl estradiol may promote survival. When the control value for ethynyl estradiol is set to the mode of 67%, the survival trend analysis is significantly different and negative with increasing concentrations ($p=0.028$). Ethynyl estradiol curve needs to be repeated to verify the control results for that treatment set.

Table 1a. Percent survival of quail of doses of all treatments, where n=6.

Treatment	0	0.1	0.3	1	3	10
Coumestrol	67	50	50	17	33	33
Ethynyl Estradiol	33	100	83	67	67	33
Indole-3-Carbinol	67	50	67	67	67	33
o,p'-DDE	67	33	0	33	0	33
p,p'-DDE	67	17	50	0	50	33
TCDD*	83	100	50	0	0	0

* significant for trend analysis ($p<0.05$).

Table 1b. Percent survival of quail dosed with corn oil based treatments of ethynyl estradiol and TCDD below the air cell (n=10).

Treatment	0	0.003	0.01	0.03	0.1	0.3	1	3	10
Ethynyl Estradiol	70	na	na	30	30	10	30	60	10
TCDD	70	40	10	30	90	40	na	na	na

na = not applicable, concentration not used.

All treatment chemicals appeared to have a negative effect on quail embryo survival. o,p'-DDE had a significant effect, however it was not by trend (Table 1a). Coumestrol appeared to decrease survival especially at the three highest doses (1.0, 3.0, 10.0 mg/kg) (Table 1a). Indole-3-carbinol did not have a significant trend in survival with increasing doses (Table 1a). p,p'-DDE did not produce a significant trend (Table 1a). However, all doses were lower than controls.

Tissues, Measurements, and Hormone Concentrations

Because of initial small sample sizes compounded with poor survival and the inability to control for sex, resulting sample sizes were too small for any statistical analysis with strength. However, trends were observed for some of the variables of some treatments.

Ethynyl estradiol appeared to increase female hatchling weight with increasing concentration especially at the two higher doses. An increase in human female body weight has been reported. Weight gain appears to be strongly correlated with blood pressure in women administered oral contraceptives and is a result of the renin-aldosterone system (Oelkers, 1996). The estradiol

appears to stimulate fluid retention, increasing body weight. At lower doses, deer mice (unpublished) increase in weight gain, an effect that diminishes at higher doses.

Indole-3-carbinol injected eggs that were male showed an increase in weight gain with increasing dose. This is most apparent after the 0.3 mg/kg dose. No literature was found that discussed effects of indole-3-carbinol on weight.

Males of o,p'-DDE dosed eggs, showed a decline in weight gain with increasing dose. A similar but significant affect was observed in male alligators dosed *in ovo* with the same concentrations of o,p'-DDE. At the lowest doses there was a significant reduction in weight gain (unpublished). Male liver somatic index increased with increasing concentration of o,p'-DDE. This needs to be repeated with larger sample sizes. No effects of o,p'-DDE on liver weight have been found in the literature.

Female quail that had been exposed *in ovo* to TCDD appear to increase in weight gain with increasing concentrations. This is contrary to the literature where TCDD has been found to cause decreases in food consumption, decreases in weight, and wasting syndrome (Umbreit and Gallo, 1988; Pohjanvirta and Tuomisto, 1994; Henshel et al., 1995; Janz and Bellward, 1996; Henshel et al., 1997). Male quail exposed to TCDD showed an increase in spleen somatic index. This is contrary to results found in male C57BL/6J mice where there were dose-dependent decreases in spleen weights (Birnbaum et al., 1990). Female quail exposed to TCDD showed an increase in gonad somatic index and a decrease in egg gland somatic index with increasing concentrations. These effects of TCDD on egg gland weight are consistent with results found in mouse uteri where it decreases estradiol-dependent uterine wet weight (Goldstein and Safe, 1989). The plasma estradiol concentrations do not reflect a similar trend. Further investigation is needed with larger samples sizes.

Estradiol and testosterone plasma concentrations were not significantly different, nor did they show trends. As a result, these plasma hormone concentrations may not be effective biomarkers in these species. Larger sample sizes are needed to confirm this.

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