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Dietary TBTO Exposure to the Japanese Quail: Relation Between Exposure Period and Appearance of Reproductive Effects

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Tributyltinoxide (TBTO) is used as wood preservative, as an antifouling agent in marine paints and as a molluscicide for the control of parasites (Cardarelli 1976; Duncan 1980). The teratogenicity of TBTO in NMRI mice after oral dosing indicates that cleft palate is the main malformation, but this occurs at dosages overtly toxic to the mother (Davis et al. 1987; Faqi et al. 1997 a), whereas in an in *vitro* study, a pronounced teratogenic effect of TBTO was reported (Krowke et al. 1989). Due to its persistence in aquatic compartments, there is cause for concern that TBTO accumulates into the food chain and thus may pose a hazard for terrestrial life forms. The toxic effects of TBTO on aquatic and terrestrial organisms has been summarized (Hall & Pickney 1985; BUA 1989; WHO 1990; Schweinfurth et al. 1987). Exposure of Japanese quail *(Coturnix coturnix japonica)* to 60 or 150 mg/kg TBTO led to a significant decrease in hatchability and increased number of dead chicks found in the shell (Coenen et al. 1992). Significant reductions in egg production, fertility, hatching success and survival of 14-day old chicks were reported after dietary TBTO exposure of 375 ppm (Schlatterer et al. 1993).

The aim of this paper was to reevaluate the reproductive effects of TBTO on the Japanese quail and elucidate which reproductive endpoints (mean number of eggs laid/hen/week, egg weights, percent of cracked eggs, egg shell thickness and fertility rate) are affected at 6 and 13 weeks. Also, our aim was to find out whether these effects vary qualitatively depending on the time when the Japanese quail are exposed to TBTO for 13 weeks.

MATERIALS AND METHODS

Japanese quail *(Coturnix coturnix japonica;* from the breeder Gudrun Pottiez, D-75192 Eppingen, Germany) were brought into our animal quarter at the age of six weeks. Immediately upon arrival, the quail were randomly assigned to 3 groups of 12 animals/sex. The birds were housed individually in wire pens with sloped floors and egg catchers. The quail were kept at a room temperature of 22 ± 2°C and 55 ± 10 % relative humidity. Air changes per hour were kept constant (15x).

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The photoperiod for all birds was 16 h: 8 h, light: dark. Light intensity in the cages was in the range of 120 to not less than 65 lux. Quail had free access to tap water and to standard pellet feed (Altromin® GmbH, Lage, FRG) and mated (1 male: 1 female). Diets were mixed with TBTO (Schering AG, 97.4% purity, batch No. 0830) in 0.5% olive oil by weight. The concentration of TBTO in the diet was analytically determined by gas chromatography using flame ionization detection, after derivatization of the extracted active compound with ethylmagnesium bromide. By this procedure, a recovery of at least 80% was achieved. The substance proved to be stable in the diet for at least three weeks. The concentration of the test substance in the diet was maintained at a minimum of 80% of the nominal concentration during the whole dietary exposure period.
After a two-week adaptation period, all test parameters measured during the treatment period were registered in a one-week pre-treatment period (hence referred to as week 0) during which the control diet was fed. Subsequently, the treated groups received test diets containing 150 or 375 ppm of TBTO *ad libidum* for 13 weeks throughout the laying period, while the control group was fed standard diet mixed with olive oil (7 ml/kg feed). The animals were monitored daily for abnormal behavior, general signs of toxicity and mortality. Body weights of male and female birds were measured weekly from the beginning of the acclimatization period to the end of the treatment period. Food consumption was monitored weekly by weighing leftovers.

Eggs laid during the study period were stored in a refrigerator at 16±1°C and 60-70 % humidity. Storage was limited to less than 10 days. Eggs were incubated on a weekly basis in an automatic brooder at 37.8°C and 60% relative humidity. Eggs were turned several times a day at approximately 180° over the egg equator up to day 16 of incubation. Hatched chicks were not removed from the incubator unless they were completely dry. Eggshell thickness was measured at several points around the egg equator after the eggs had been opened, washed out and dried for at least 48 hr at room temperature according to Bennett et al. (1988). Only the shell thickness of the eggs collected during week 6 was evaluated. Stored eggs were used for the measurement of eggshell thickness. The number and weights of eggs were determined to the respective week of the treatment period and were expressed as number of eggs and mean egg weight per hen and week. The egg quality was characterized by recording the percentage of broken/cracked eggs and eggshell thickness. Fertility as well as hatching success and hatchling survival was determined for eggs laid in the 6th and 13th week. The number and weight of hatchlings and 14-day old survivors were analyzed as well. In this paper only effects observed at week 6 and week 13 are presented.

At the end of the study, the birds were then sacrificed by decapitation and the organs (liver, heart, spleen, testis, ovary and kidney) removed. The organs were fixed with 4% formaldehyde, embedded in paraffin, cut, stained with hematoxylin eosine and examined under a light microscope.

For hematological study, blood was collected through heart puncture using heparin as an anticoagulant. The clinical chemistry parameters were assessed according to the method described by Jain (1986). The erythrocyte count was obtained by means of an automated blood cell counter (Channelyzer® 256, Coulter, Hialeah, FL, USA); the hematocrit was estimated by use of a microhematocrit centrifuge and the hemoglobin concentration by the conventional cyanmethemoglobin method. Serum enzyme activities (gamma glutamyltransferase [γ-GT], alkaline phosphatase, GPT, GOT); glucose level, uric acid, total cholesterol, bilirubin, creatinine, protein, albumin and blood electrolytes (phosphate, calcium, magnesium, iron) were determined using autoanalyzer (Technicon® Autoanalyze™, Evergreen Scientific, Vernon, USA). The plasma was kept at -20°C for at least 4 weeks before analysis.

Statistical evaluation was performed using the single factor analysis of variance followed by the Tukey test for multiple means comparison with equal or unequal sample sizes at a significance level of $p < 0.05$ (Gad and Weil 1982). The data were compared on a dose and week basis. For each group, the data obtained at treatment weeks 6 and 13 were compared with data of week 0 as well as with control data for each time point of investigation. Both comparisons revealed similar results. Furthermore, the percentage of cracked eggs was analyzed by contingency tables.

RESULTS AND DISCUSSION

No treatment related effects on food consumption or body weight were observed during the entire exposure period. The number of eggs and the mean egg weight were significantly decreased at a concentration of 375 ppm at weeks 6 and 13 of study when compared with week 0 or with the control group (Figs. 1 and 2). Similarly, the percentage of cracked eggs was significantly increased at 375 ppm dose at weeks 6 and 13 of treatment (Fig. 3). Moreover, at 375 ppm dose, reduced eggshell thickness was observed at week 6 when compared to week 0 or control eggs (Fig. 4). The fertility rate (i.e., the ratio of fertile eggs to incubated eggs) was significantly decreased at a dose of 375 ppm on weeks 6 and 13 of study (Fig. 5). Hatchability was also decreased, but no abnormalities were reported in the hatchlings (data not shown). The number of 14-day old survivors hatched from eggs that had been collected during weeks 6 or 13 was not reduced at either dose.

Assessment of hematological and clinical chemistry data obtained at weeks 6 and 13 of the study showed no differences between control and treatment groups. Likewise, the histological preparations of the organs showed no morphological changes.

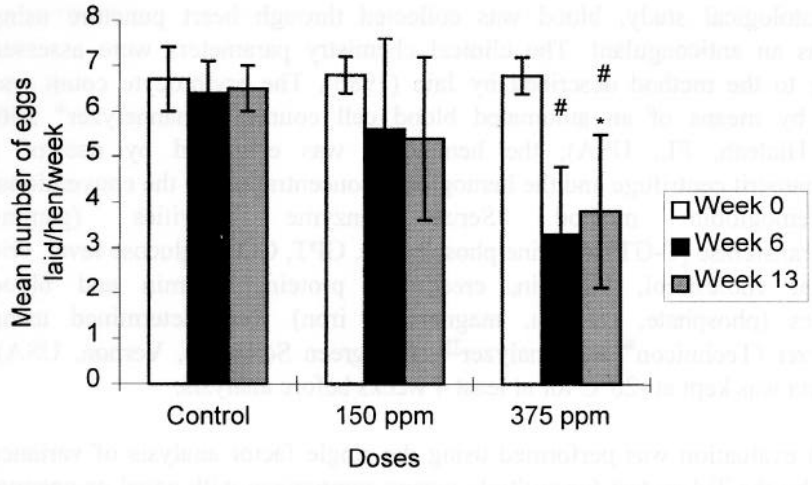

Figure 1. Mean number (X ± SD) of eggs laid/hen/week. * = significant differences when compared to week 0; # = significant difference when compared to the control group

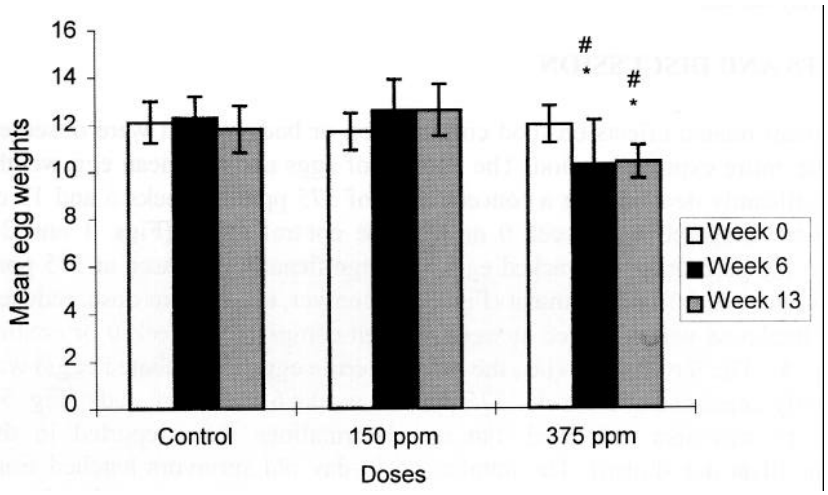

Figure 2. Mean egg weights (X ± SD) of TBTO-exposed Japanese quail.
* = significant differences when compared to week 0; # = significant differences when compared to the control group

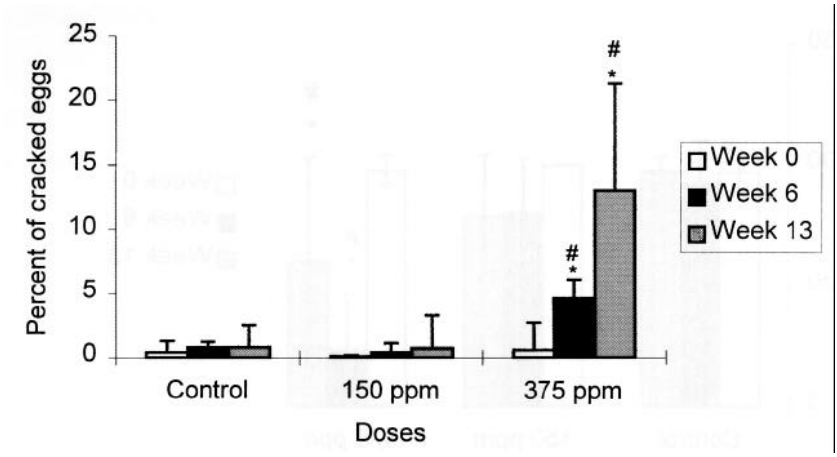

Figure 3. Percent of cracked eggs (X± SD) in control and TBTO-exposed Japanese quail. * = significant differences when compared to week 0; # = significant differences when compared to the control group

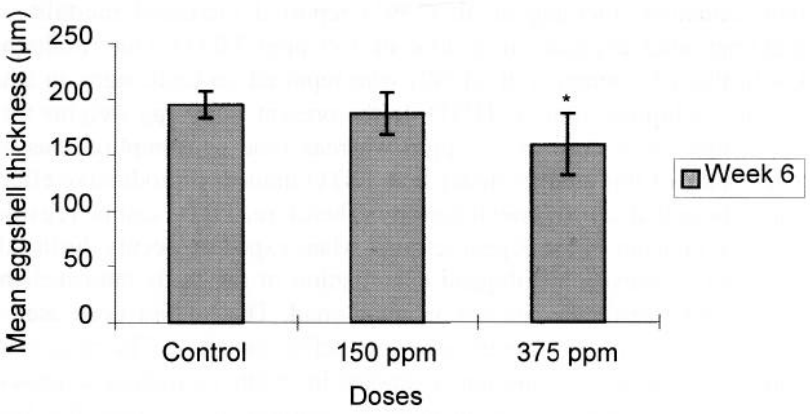

Figure 4. Mean eggshell thickness (X± SD) of TBTO-exposed quail. * = significant differences when compared to the control group

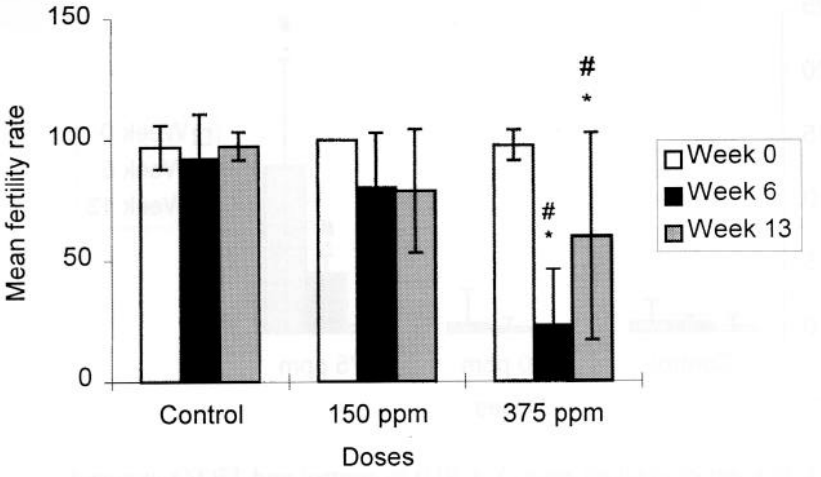

Figure 5. Mean fertility rate (X ± SD) of TBTO-exposed Japanese quail. * = significant differences when compared to week 0; # = significant differences when compared to the control group

Exposure to TBTO for 13 weeks did not induce signs of general toxicity such as body weight reduction, Fleming et al. (1991) reported increased mortality in mallard ducklings after exposure to a dose of 150 ppm TBTO. Our results are consistent with that of Coenen et al. (1992) who reported no toxic signs in adult quail after dietary administration of TBTO. In the present study egg weights were significantly reduced at a dose of 375 ppm whereas food consumption was not reduced. The results of this study indicate that TBTO induces reproductive effects at doses below those that cause general toxicity. Therefore, TBTO can be regarded as a reproductive toxicant in the Japanese quail when exposure occurs during the egg laying period. However, histological examination of the testis indicated that TBTO does not elicit testicular toxicity in male quail. The highest dose used in this study reduced fertility and, therefore, reproductive outcome. The production of viable chicks is the most important endpoint in avian reproductive toxicity testing (OECD 1995). Moreover, a decrease in reproductive success has been associated with a decrease of eggshell thickness of about 20 % (Anderson and Hickey 1972). Eggshell thickness has been used as a biomarker for monitoring some environmental contaminants. Bennett et al. (1988) argued that obtaining information on eggshell thickness and shell strength is the best way of evaluating effects of chemicals on eggshell quality. However, our data suggest that egg quality may better assessed by investigating eggshell thickness and percentage of cracked eggs. While effect on egg fertility was not shown by Coenen et al. (1992) after dietary TBTO exposure for 6 weeks, Schlatterer et al. (1993) showed an embryotoxic effect of TBTO at dietary dose of 375 ppm. Our finding that exposure of Japanese quail to TBTO for 13 weeks did not induce effects on blood

effects on blood parameters is supported by the study of Coenen et al. (1992) that similarly showed no relevant effects on blood parameters.

We have previously shown that a dietary exposure period of 6 weeks was sufficient to detect reproductive effects of a non-bioaccumulating substance (methyl parathion) in Japanese quail (Solecki et al. 1996). In this study, the reproductive effects of TBTO observed at week 6 were similar to that seen at week 13. Japanese quail reach peak egg production at 8-9 weeks of age and maintain this production at least for the following 12 weeks (Solecki et al. 1994). This is probably the reason why - even in the case of bioaccumulating compounds (like TBTO) –reproductive effects are manifested after a relatively short exposure period.

The United State Environmental Protection Agency (U.S. EPA 1978) and the Organization of the Economic Cooperation and Development (OECD 1984) recommend at least 10 weeks of dietary exposure before the onset of egg laying and at least 8 weeks exposure during egg production for detecting adverse effects of chemicals in birds. The Japanese quail exhibit several advantage when compared with Bobwhite quail and Mallard ducks and has been suggested for use in the study of reproductive effects of chemicals in birds (Faqi et al. 1997 b).

In conclusion, the present study indicate that: a) short term dietary exposure period is sufficient enough to detect the reproductive effects of TBTO in the Japanese quail and b) this is supported by the fact that all the endpoints examined were affected similarly in terms of magnitude at week 6 and at week 13. Therefore, reduction of the dietary exposure period to 6 weeks is recommendable. This may help decrease the cost and time required for carrying out the reproductive effects of pesticides on birds.

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