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Effects of Methyl Parathion on Reproduction in the Japanese Quail

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Organophosphorus insecticides, including methyl parathion, degrade rapidly in the environment but may pose lethal and sublethal effects to birds immediately after application in the field (White et al. 1982). Methyl parathion is used widely because of its broad spectrum insecticide properties. It affects the brood rearing phase of reproduction in birds by inducing behavioral change and mortality in mallard ducks (Fairbrother et al. 1988). Adult mortality and mallard brood loss were reported after an aerial field spray of methyl parathion (Brewer et al. 1988). Exposing high concentrations of methyl parathion to northern bobwhite (Colinus virginianus) in their natural habitats may increase susceptibility to predation (Buerger et al. 1991). Test diets containing 0, 14, 20, 28 or 40 ppm of methyl parathion exposed to bobwhite during the egg producing period caused a dose related reduction in body weight, egg production, and egg weight as well as eggshell strength and thickness (Bennett and Bennett 1990).

The purpose of the present paper was to investigate the reproductive effects of methyl parathion on Japanese quail. The study was carried out under the conditions of the draft test guideline, prepared by a working group of German toxicologists and intended to replace the existing OECD guideline 206 (OECD, 1994). The results obtained were compared to that of Bennett et al. (1990) on bobwhite. An analysis of available data on the relative sensitivity of bobwhite and Japanese quail to reproductive toxicants was recommended by a Working group of the SETAC/OECD Workshop Avian Toxicity Testing (OECD 1995).

MATERIALS AND METHODS

Six week old male and female Japanese quail (*Coturnix coturnix japonica* were purchased from Zentrale Versuchstierfarm des BGA, Berlin-Marienfelde) and brought to our institute. Immediately after delivery, the quail (n = 48 per sex) were randomly divided into 8 groups of 12 birds. They were adapted to the conditions of our animal quarters for two weeks before beginning the experiment.

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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). Lighting was 16 h: 8 h, light: dark. Light intensity in the cages was 300 lux. They received standard pellet feed (Altromin® GmbH, D-4937 Lage FRG) and tap water ad libitum. Diets were prepared by dissolving methyl parathion (93.1% purity) in olive oil and thoroughly mixing with layer feed (7 ml/kg feed) for 20 minutes.
The study was started at the age of 9 weeks. At this time, the average body weight of the birds was : (male: 181 ± 15g; female: 229 ± 16g). The treated groups received test diets containing 3, 12 and 48 ppm of methyl parathion (Thiophosphourus acid-o,o- dimethyl-o-p-nitrophenyl ester) ad libitum for 6 weeks throughout the laying period. The control group was fed standard diet mixed with olive oil (7 ml/kg feed). The concentration of the test substance in the diet was maintained at a minimum of 80% of the nominal concentration during the whole experimental period. The birds were kept in single cages and mated (1 male : 1 female) for 1 hour per day for 6 weeks. Six birds (3 males and 3 females) from each group were further observed for 3 weeks to detect the potential of recovery from the effect of the treatment.

Each day, the birds of both sexes were monitored for clinical and toxic symptoms. Food intake as well as body weight was registered weekly.
Eggs were removed from adults the day they were laid, weighed and stored in a refrigerator (16° ± 1° C and 60 - 70% relative humidity) for a maximum of 10 days. They were set weekly in an automatic incubator at 38.7° C and 60% relative humidity. Eggs were turned 6 times per day at approximately 180° over the egg equator until day 16 of incubation. Hatched chicks were not removed from the incubator until they were completely dry. After 5 and 6 weeks of exposure, all eggs from this two-week period were used for the measurement of eggshell thickness. The thickness (µm) of the egg was measured at different points around the egg girth. The mean value thickness for each egg was calculated from four thickness measurements. This was done after the eggs were cut around the equator with scissors, the contents except the membrane, removed and the shells dried for 2 days at room temperature according to Bennett et al. (1988). Fertility rate and hatchability also were calculated.

For each dose level, 12 male and female quail were used to determine brain and plasma cholinesterase activity as well as clinical chemistry parameters.
The whole brain was homogenized in tris buffer (35 mh/ml), pH 7.4 and stored in -20°C. The brain cholinesterase activity was determined in accordance with the method of Ellman et al. (1961) as modified by Dieter and Ludke (1975). Plasma cholinesterase activity was determined following the calorimetric method of Ellman et al. (1961).

For the clinical chemistry parameters, blood was collected through heart puncture. The erythrocyte count was obtained by means of an automated blood cell counter

(Coulter Counter ZF); the hematocrit by use of a microhematocrit centrifuge and the hemoglobin concentration was estimated by the cyanmethemoglobin method. Heparin was used as anticoagulant. The blood chemistry parameters (glucose, uric acid, total chlolesterol, bilirubin, alanine aminotransferase [ASAT], aspartate aminotransferase [ALAT], gamma glutamyltransferase [γ-GT], alkaline phosphatase, cholinesterase, creatinine, protein, albumin, phosphate, calcium, magnesium, iron) were performed on plasma by using an autoanalyzer (Technicon SMAC). The plasma was kept in -20 °C for 4 weeks before analysis. The clinical chemistry parameters were determined according to the methods described by Jain (1986).

The birds were then sacrificed and the organs (liver, heart, spleen, testis, ovary and kidney) removed. The organs were fixed with 4% formalin, embedded in paraffin, cut, stained with hematoxylin eosin and examined under a light microscope.

For the statistical analysis, the software package used was Statgraphics (1987). Data were tested for homogeneity of variance using the Bartlet-test (Gad and Weil, 1982). They were then analyzed by using single analysis factor followed by Tukey's multiple comparison procedure with equal or unequal sample size (Zar, 1984). Furthermore, food intake, body weight gain and egg parameters were analyzed by fitting linear step-wise regression models. All tests were conducted at a significance level of $\alpha < 0.05$.

RESULTS AND DISCUSSION

No clinical, toxic symptoms or behavioral changes were observed during the exposure period. Daily food intake was decreased at dose level of 48 ppm with subsequent reduction of body weight. Heart and liver weight of birds receiving 48 ppm were reduced in both sexes.

The histological preparations of the organs showed no morphological changes. Results of blood analyses indicated low levels of plasma protein at the highest dose in female birds. At the same dose level, plasma albumin in the male quail was reduced.

Brain cholinesterase activity was reduced at dose levels of 3, 12 and 48 ppm first after 4 weeks of treatment and remain constant until week 6 of exposure (Fig 1). Plasma cholinesterase decreased only at 48 ppm. All other clinical chemistry parameters were not affected. Fig. 1 presents only the concentration of brain cholinesterase determined at week 6 of exposure.

At the dosage of 48 ppm, the number of eggs laid (Fig. 2) as well as their mean weight (Fig 3) was decreased.

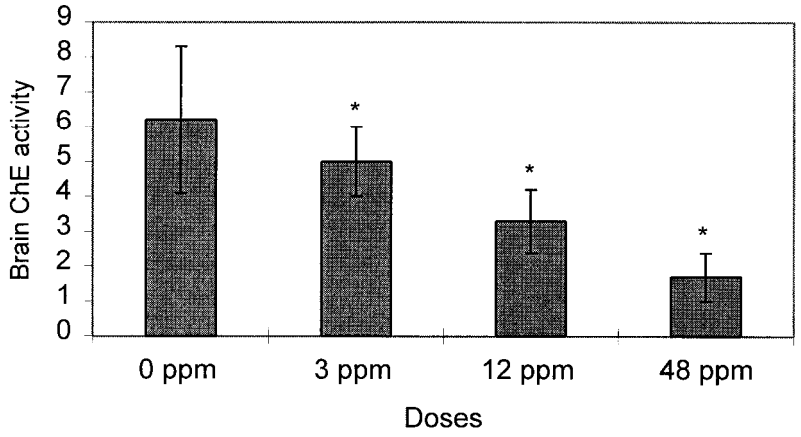

Figure 1. Brain cholinesterase activity (M ± SD) in control and treated quail at week 6 of exposure. Groups that are significantly different from the control are indicated by an asterisk (*$\alpha < 0.05$).

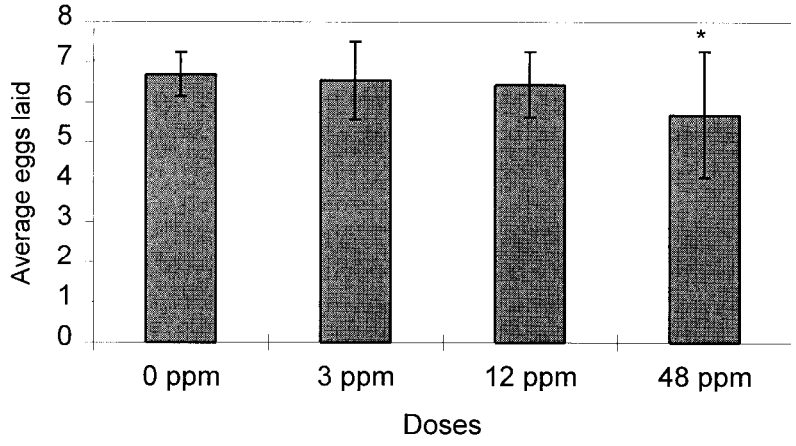

Figure 2. Average number (M ± SD) of eggs laid per hen/week during the exposure period. The asterisk * indicates that the group is significantly different from the control ($\alpha < 0.05$).

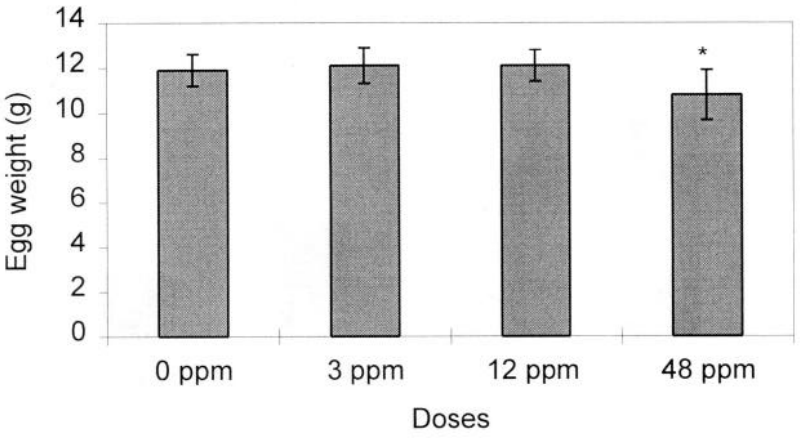

Figure 3. Egg weight (M ± SD) of quail exposed to methyl parathion for six weeks. The asterisk * indicates that the group is significantly different from the control ($\alpha < 0.05$).

At the highest dose level, the number of cracked eggs increased from 60 % (49 cracked out of a total of 81) in the first week to 74.5 % (35 cracked out of a total of 47) after 6 weeks of exposure compared to the control group. Similarly, after 5 and 6 weeks of exposure, eggshell thickness was statistically reduced (7-10% less than the control eggs). Fertility and hatchability were not affected.
The number of 14-day-old survivors per pen was significantly reduced only at a dose level of 48 ppm (0.8 survivors per pen) compared to the control group (2.3 survivors per pen). All the effects, including plasma albumin level, were reversed three weeks after the exposure ceased.

Significant depression of brain cholinesterase activity occurred in all the doses tested, whereas the reproductive parameters were only affected at the dose level of 48 ppm. The extent of the inhibition of brain and plasma cholinesterase is regarded as a useful indicator of organophosphate and carbamate exposure in both humans and birds (Hill and Fleming 1982; Fossi et al. 1992). In addition, effects on eggshell thickness are useful biomarkers for monitoring environmental contaminants. A decrease in eggshell thickness of about 20% has been associated with low reproductive success (Anderson and Hickey 1972). In the laboratory tests eggshell quality also may be influenced by disturbances of the housing conditions (unpublished data). Obtaining both shell thickness and shell strength information may provide better means to evaluate effects of chemicals on eggshell quality (Bennett et al. 1988).

In the present study, the highest dose of methyl parathion decreased the laying performance of hens and therefore the reproductive outcome. In avian reproductive testing the production of viable chicks is the most important endpoint (OECD 1995).

Concentrations of methyl parathion greater than 28 ppm produced unacceptably high levels of mortality in bobwhite after five weeks of treatment (Bennett et al. 1990). In the Japanese quail, 48 ppm of methyl parathion administered for six weeks did not increased the mortality rate. Comparative toxicity study of pesticides on birds showed that the bobwhite is relatively more sensitive than Japanese quail (Joermann, 1991). But almost all toxic effects observed in bobwhite were also reported in Japanese quail.

Maintaining the birds under surveillance for three weeks after methyl parathion feeding resulted in complete reversion of all the effects reported. An investigation of the reversibility of pesticide effects currently is not required. It could be helpful for interpreting the information during an ecotoxicological risk assessment and, therefore, it may be useful for the avian reproductive test to include a withdrawal period.

It is difficult to incorporate into the test procedures a more realistic exposure scenarios, such as would occur in the field. However, the results obtained showed that the exposure period proposed in the present test is long enough to demonstrate the effects of chemicals on avian reproduction.

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