Bull. Environ. Contam. Toxicol. (2001) 67:807–814

DOI: 10.1007/s00128-001-0194-8

Bulletin of Environmental Contamination and Toxicology

Dietary Administration of Dimethoate to the Japanese Quail: Reproductive Effects and Successful Hatchability of Eggs

R. Solecki,[1] L. Niemann,[1] C. Gericke,[2] I. Chahoud[2]

[1] Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Thielallee 88-92, 14195 Berlin, Germany
[2] Freie Universität Berlin, Institut für Klinische Pharmakologie und Toxikologie, Garystrasse 5, 14195 Berlin, Germany

Received: 10 January 2001/Accepted: 4 August 2001

Dimethoate [0,0-dimethyl-S-(2-methylamino-2-oxoethyl)dithiophosphate] is a widely used systemic organophosphate insecticide and acaricide which is registered for application to various food and feed crops. The compound is used to control a wide variety of pests including insects, mollusks, fouling organisms, and miscellaneous invertebrates. Some examples of the pests that dimethoate is intended to control are aphids, citrus thrips, grasshoppers, leafminers, spider mites, and whiteflies. Contamination can occur following application of dimethoate to fruit, vegetables, grain, fiber, feed, ornamental, and other crops and, thus, exposure of birds is not unlikely.

Dimethoate is considered to exhibit a moderate chronic risk to birds and mammals. The systemic toxicity of dimethoate is mainly the result of cholinesterase inhibition leading to excessive stimulation of cholinergic nerves by excess acetylcholine at the postganglionic membrane of the synapses. Accordingly, the toxicity endpoints selected for risk assessment are primarily based on neurotoxic effects, in particular cholinesterase (ChE) inhibition in red blood cells (RBC), and brain.

Dimethoate was tested for reproductive toxicity in two studies on bobwhite quail resulting in no effect concentrations of 6 and 10 ppm for both effects on reproduction as well as systemic effects in parental birds (Munk 1986; Gallagher et al. 1996 unpublished, kindly provided by the Dimethoate Task Force member company Cheminova Agro A/S). However, cholinesterase inhibition and transfer into the eggs was not measured in these trials.

The goal of this study further validation of a new OECD guideline for the testing of reproductive toxicity in birds proposed by a working group of the Organisation for Economic Cooperation and Development (OECD 1996, 1999, 2000). Therefore, the main objectives of our work were to elucidate which reproductive endpoints were affected in the Japanese quail during a 6-week exposure period, to establish a NOEC and to facilitate a comparison between Bobwhite and Japanese quail with regard to the effects observed. Such an analysis of available data on the relative sensitivity of bobwhite and Japanese quail to reproductive toxicants was recommended by an OECD working group.

A further goal of this study was to investigate cholinesterase inhibition in brain and to establish the transfer into the eggs after dietary exposure with dimethoate.

Correspondence to: R. Solecki

MATERIALS AND METHODS

In the described experiment the insecticide was administered to Japanese quail *(Coturnix coturnix japonica)* in the diet over a period of six weeks to investigate in particular a possible impact on reproduction. Japanese quail (breeder: Küberich, Geesdorf/Wiesentheid, Bavaria, FRG) were brought into our animal quarter at five weeks of age. Immediately after arrival, the male and female birds were assigned to 4 groups of 22 pairs each. The animals were singly housed in wire pens with sloped floors and egg catchers at a room temperature of 22°C ± 2°C and 55 ± 10% relative humidity. Air changes per hour were kept constant (15x). The daily photoperiod was 16 h light : 8 h dark. Light intensity in the cages was in the range of 120 to not less than 65 lux. The animals received a standard feed ("CLUB-Wachtelfutter", obtained from the breeder) and tap water ad libitum. Diets were mixed with dimethoate manufactured and provided by Cheminova (Lemvig/Denmark; batch 20522-00; purity 99.1%).
After a 7-week acclimatization (adaptation) period, the birds were randomized according to body weight and definitely allocated to the control and the three dose groups. The number of animals was reduced to 18 pairs per group. Only proven breeders were included. Then, all test parameters were monitored in a 2-week pretreatment period during which all animals were maintained on untreated diet. During the following 6-week treatment period, the exposure groups received test diets containing 10, 35 or 70 ppm dimethoate whereas the control group continued on standard diet. The birds were kept in single cages and mated once a day (5 times per week) by introducing the drake into the cage of the allocated hen for not more than 20 minutes.

The dietary concentration of the test substance was subject to regular examinations. Furthermore, stability of dimethoate in the diet was investigated. Food samples were taken once in the adaptation period and then weekly during the 6-week treatment period. The samples were kept frozen (-20°C) until qualitative and quantitative analyses were carried out by gas chromatography and mass spectrometry (Hewlett-Packard 5890 GC, 5971 A MSD, Dos-Chemstation). Examination of the eggs for dimethoate was performed on pooled samples from the adaptation phase and from eggs laid during the 3rd and 6th week of substance administration.

The animals were monitored daily for abnormal behaviour, signs of toxicity and mortality. Body weights of male and female birds were determined at the start and the end of the acclimatization period and at scheduled termination. Food consumption was estimated weekly based on replicates of three pairs each.
Eggs laid during the pretreatment and administration periods were stored in a refrigerator at 16 ± 1°C and 60 - 70% humidity before incubation started. Storage was limited to less than 10 days. Eggs were incubated on a weekly basis in an automatic breeder 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 until they became completely dry. Eggshell thickness was measured weekly in one "intact" egg per

pen at five sites 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). Additionally, the shell thickness of cracked eggs collected during the pretreatment and treatment periods was evaluated separately.
The number of eggs and their weights were assigned to the respective week of the pretreatment and treatment periods and expressed as number of eggs and mean egg weight per hen and week. The percentage of broken/cracked eggs, fertile and viable eggs, death in shell as well as number and weight of hatchlings were determined for eggs laid for the whole pretreatment and treatment period. The weight and number of survivors at the end of the 14-day growing periods were registered in the 2nd week of pretreatment and in the 2nd, 4th and 6th week of the treatment period. Additionally, the sex ratio was determined in the 14 days old chicks upon necropsy.

At the end of the 6-week treatment period, the adult birds were sacrificed and examined macroscopically. Organ weight of liver and testes were determined. From nearly one half of the animals, the brain was removed for cholinesterase measurement. The brain cholinesterase activity was determined in accordance with the method of Ellman et al. (1961) as modified by Dieter and Ludke (1975). Samples of the right testis and liver were taken from 5 animals per sex and group, fixed with 4% formaldehyde and embedded in paraffin for subsequent histopathological examination.

Body weights, brain cholinesterase activities and the reproduction data were subject to statistical analysis using the SAS standard software package. The reproduction data were collected on a weekly basis and a comparison was made between the control and the treatment groups on the one hand and between pretreatment and treatment period within each study group, on the other hand. For the latter purpose, data from two weeks each were summarized to enable comparison of data from the initial, intermediate and terminal phases of the treatment period with those obtained during the 2-week pretreatment interval.

Student's t-test, the Chi-square test and Fishers exact test were used for testing of the differences obtained for statistical significance. When, as was frequently the case, distributions were not clearly Gaussian, the Wilcoxon rank sum test was applied. The multiple analysis of variance (ANOVA) was performed including the parameters dose level, age, and hen. The coefficient of determination was calculated. Testing for statistical significance of differences was conducted by means of the Dunnett test.

RESULTS AND DISCUSSION

In all dose groups and weeks, the analytically determined dietary concentrations were similar to the nominal concentrations. Stability of dimethoate in the diet was analytically verified for at least 14 days. This is considered sufficient since test diets were prepared fresh once a week.

No residues of dimethoate exceeding the limit of detection of 0.3 µg/l were found in the egg samples. Thus, there is no evidence of a significant transfer of the test substance into the eggs.

There were no substance-related mortalities and no clinical signs of intoxication occurring during the course of the study.

The body weight development was significantly impaired at the two upper dose levels of 35 and 70 ppm in both male and female quail (Table 1). Food consumption was reduced at the highest dose only.

Table 1. Body weight gain of male and female Japanese quail from the start of pretreatment to the end of the 6-week treatment period

	0 ppm	10 ppm	35 ppm	70 ppm
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Males (g)	17.7 ± 9.0	15.9 ± 7.9	5.4 ± 7.3 *	2.9 ± 7.2 *
Females (g)	17.5 ± 12.8	14.1 ± 6.3	8.6 ± 11.6 *	-6.4 ± 16.7 *

* $p<0.05$ t-test

At necropsy, no pathological changes were seen in the adult birds which could be attributed to treatment although special attention had been given to the possible occurrence of ovarian and gizzard findings as reported by Gallagher et al. (1996) for the Bobwhite quail. Furthermore, no absolute or relative organ weight changes were to be noted up to the highest dose of 70 ppm.

A statistically significant and dose-related inhibition of brain cholinesterase activity was detected at the two upper dose levels. Inhibition was 21% at 35 ppm and 40% at 70 ppm in males and 17% and 41%, respectively in females (Table 2). The extent of the inhibition of brain cholinesterase is regarded as a useful indicator of organophosphate and carbamate exposure in both mammals and birds (Hill and Fleming 1982; Fossi et al. 1992). However, no clinical effects were observed during the 6-week treatment period.

Table 2. Brain cholinesterase activity at study termination

	0 ppm	10 ppm	35 ppm	70 ppm
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Males (10^{-3} U/g)	6.14 ± 1.04	6.18 ± 0.90	4.87 ± 0.70*	3.65 ± 0.83*
Females (10^{-3} U/g)	5.90 ± 0.62	5.80 ± 0.71	4.90 ± 0.54*	3.46 ± 0.56*

$p< 0,05$ t-test and ANOVA with subsequent Dunnett test

Neither the number of eggs laid per hen nor the absolute number of eggs per week were affected by test substance administration.

The mean egg weight was diminished prior to and during treatment at the top dose level. For clarification, a comparison between the three treatment phases and the pretreatment period was performed within the groups (Table 3).

Table 3. Mean weight of eggs

	0 ppm	10 ppm	35 ppm	70 ppm
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Pretreatment (g)	12.5 ± 0.85	12.4 ± 0.85	12.7 ± 1.30	12.2 ± 0.94
1st and 2nd wk (g)	12.4 ± 0.83	12.5 ± 0.91	12.6 ± 1.22	11.9 ± 0.96*
3rd and 4th wk (g)	12.4 ± 0.74	12.5 ± 0.94	12.7 ± 1.28	11.9 ± 1.01*
5th and 6th wk (g)	12.4 ± 0.76	12.5 ± 0.95	12.6 ± 1.44	12.0 ± 1.03*

* $p < 0,05$ t-test

Only in the highest dose group was the egg weight significantly lower in all three treatment intervals than during the pretreatment period, whereas this parameter was not significantly increased prior to and during treatment at the 35 ppm dose level. The analysis of variance (ANOVA) revealed effects for all three parameters (dose level, age, and hen) reaching a high coefficient of determination of 83%. The Dunnett test confirmed a significant reduction in egg weight only at the dose level of 70 ppm.

The absolute and relative number of cracked eggs among the whole number laid was increased at 70 ppm from the 2nd week of treatment onwards. Since this parameter already varied considerably throughout the pretreatment period and since there was an increase in cracked eggs also in the mid dose group before and during treatment, an intra-group comparison between the three treatment phases and the pretreatment interval was made (Table 4) revealing a statistically significant difference only for the high dose group in the intermediate and terminal phase (3rd to 6th week).

Table 4. Percentage of cracked eggs

	0 ppm	10 ppm	35 ppm	70 ppm
Pretreatment (%)	14	15	17	14
1st and 2nd wk (%)	14	18	21	22
3rd and 4th wk (%)	16	19	19	31*
5th and 6th wk (%)	16	18	21	30*

* $p < 0,05$ χ^2-Test.

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 additionally influenced by disturbances of the housing conditions (Bennett et al. 1988). The increase in frequency of cracked eggs is apparently not attributable to a reduced eggshell thickness (Table 5). In contrast, we even observed an increase in eggshell thickness in the treated groups occasionally reaching statistical significance. However, this is not considered an adverse or dose-related effect of biological relevance.

Fertility (as indicated by the percentage of fertile eggs), hatchling rate and viability of the hatchlings as well as the incidence of malformations were not affected.

Table 5. Mean eggshell thickness of unbroken eggs and cracked eggs

	0 ppm	10 ppm	35 ppm	70 ppm
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Unbroken (mm)	0.209±0.016	0.211±0.017	0.211±0.019	0.214±0.015*
Cracked (mm)	0.200±0.012	0.205±0.019*	0.194±0.020*	0.205±0.015*

* p< 0,05 t-test

During the pretreatment period, the body weight of the hatchlings in the three dose groups was lower than in the control (Table 6). Therefore, a comparison between treatment and pretreatment period within the groups was conducted revealing a significant decrease at the 70 ppm level. In contrast, chicken weight was increased in the low and intermediate dose groups. There was a close correlation between substance-related reduction in egg weight and hatchling weight as also indicated by a high coefficient of determination. However, the lower chicken weight at hatch did not result in a diminished terminal body weight at the end of the 14-day growing period.

Table 6. Body weight of hatchlings

	0 ppm	10 ppm	35 ppm	70 ppm
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Pretreatment (g)	9.5 ± 0.57	9.2 ± 0.72	9.3 ± 1.00	8.9 ± 0.72
1st and 2nd wk (g)	9.3 ± 0.61*	9.5 ± 0.75*	9.2 ± 1.04	8.6 ± 0.71*
3rd and 4th wk (g)	9.3 ± 0.70*	9.3 ± 0.71	9.6 ± 0.94*	8.9 ± 0.74
5th and 6th wk (g)	9.3 ± 0.62*	9.3 ± 0.80	9.4 ± 1.10	8.6 ± 0.69*

* p< 0,05 t-test.

During and after the growth period of 14 days, the survival rate of the chicks was not impaired by treatment and the sex ratio in the survivors was not affected.

Thus, reproductive toxicity of dimethoate in the Japanese quail was characterized by a significant decrease in egg weight and hatchling body weight and an increase in the amount and percentage of cracked eggs at the highest dose level of 70 ppm. The test substance had no adverse impact on the overall reproductive success (fitness) as would be evidenced by a lower number of surviving chicks per hen. The toxic effects on reproduction were confined to the highest dose level of 70 ppm at which parental toxicity also was apparent. An impaired body weight development and inhibition of brain cholinesterase in the adults were already noted at the intermediate dose level of 35 ppm. On this basis, a NOEC of 35 ppm for reproductive toxicity and of 10 ppm for systemic effects on the adult birds can be established.

Further data on reproductive toxicity of dimethoate in the Japanese quail are not available to us. However, a comparison with the results obtained in the Bobwhite quail *(Colinus virginianus)* can be made. The more recent study (Gallagher et al. 1996) revealed a NOEC at the intermediate dose of 10.1 ppm. Administration of the high dose of 35.4 ppm caused body weight decrease in female animals and

reduced food consumption in both sexes. Furthermore, pathological changes in the ovaries and the gizzard were described which were not observed in the Japanese quail. Reproductive effects comprised a marked decrease in the laying rate by nearly 50%, and a higher mortality and lower body weight gain of the chicks. Eggshell thickness was also reduced. A previous study in the Bobwhite (Munk 1986) also elicited impaired food consumption and reduced body weight gain in the adults at the highest dose level of 30 ppm. The number of eggs laid and the eggshell thickness were also diminished. Furthermore, the percentage of infertile eggs was marginally increased and hatch rate and hatchling weight were lower than in the control group. The NOEC in this study was 6 ppm, based on parental and reproductive effects at 10.1 ppm.

Thus, findings in the adult animals were similar in both species at least with regard to effects of dimethoate on body weight and food consumption. In contrast, there were considerable qualitative differences in reproductive effects. The marked reduction in laying rate and number of hatched chicks as observed in both studies on the Bobwhite quail was not confirmed in the Japanese quail up to the highest dose tested. This difference could be related to the markedly higher overall reproduction rate in the Japanese quail as compared to the Bobwhite. Similarly, the marginally decreased eggshell thickness in the bobwhite was not confirmed in the Japanese quail. Chick survival and body weight gain of the hatchlings were not impaired although the much higher dose of 70 ppm was included in our study. These effects were reported in the Bobwhite quail at 35 ppm. In contrast, in both species, dietary administration of dimethoate caused a lower body weight at hatch. No increase in malformations was observed in this study confirming the lack of teratogenicity in birds following dietary application of other organophosphates (Bennett et al. 1988, Munk 1986; Gallagher et al. 1996, Solecki et al. 1996). This is in contrast to the well known developmental toxicity of organophosphate after incubation of bird eggs, which leads to micromelia, parrot break and abnormal feathering; or involves defects in axial skeleton (Hoffman 1990). It seems, that at least in quail the teratogenic effects observed after in ovo exposure to organophosphates do not occur following dietary exposure of proven breeders.

Summarizing the systemic toxicity of dimethoate, the Japanese quail is of comparable sensitivity as the Bobwhite quail. The difference in the expression of reproductive toxicity might be due to a different species sensitivity to dimethoate. A comparative review on the toxicity of pesticides on birds showed that the bobwhite is slightly more sensitive than Japanese quail (Joermann 1991). For another organophosphate compound (methyl parathion) the same parental and reprotoxic NOEC could be derived for Bobwhite and Japanese quail but the qualitative expression of reprotoxic effects was at least partially different (Solecki et al. 1996). These slight species differences show that the Bobwhite and the Japanese quail are equally appropriate models for assessment of avian reproductive toxicity.

Acknowledgments. This work was supported by the Bundesminister für Umwelt, Naturschutz und Reaktorsicherheit (Umweltforschungsplan - Forschungsvorhaben 20606128). We are indebted to Mr. Wittfoht (Freie Universität of Berlin) for the

residue analysis and last but not least to Mrs. Barac, Mrs. Bürkle, Mrs. Ladwig, Mrs. Schwarck and Mrs. Zarnecke for animal care and for their reliable technical assistance.

REFERENCES

Anderson D, Hickey J (1972) Eggshell changes in certain North American birds. Proc XV Int Ornithol Congress, 514-540

Bennett JK, Ringer RK, Bennett RS, Williams BA, Humphrey PE (1988) Comparison of breaking strength and shell thickness as evaluators of eggshell quality. Environ Toxicol Chem 7: 351-357

Dieter MP, Ludke JL (1975) Studies on combined effects of organophosphates and heavy metals in birds. i. plasma and brain cholinesterase in coturnix quail fed methyl mercury and orally dosed with parathion. Bull. Environ Contam Toxicol 13: 257-262

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid calorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88-95

Fossi MC, Leonzio C, Massi A, Lari L, Casini S (1992) Serum inhibition in birds: A non destructive biomarker to assess organophosphorus and carbamate contamination. Arch Environ Contam Toxicol 23: 99-104

Hill EF and Fleming WJ (1982) Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. Environ Toxicol Chem 1: 27-38

Hoffman DJ (1990) Embryotoxicity and teratogenicity of environmental contaminants to birds. Rev Environ Contam Toxicol 115: 40-89

Joermann G (1991) Vergleich der Empfindlichkeit verschiedener Vogelarten in akuten and subakuten Toxizitätstests. Nachrichtenbl Deutscher Pflanzenschutzd 43: 275-279

OECD (1996) Report of the SETAC/OECD Workshop on Avian Toxicity testing. (1996). Environment Health and Safety Publication - Series on Testing and Assessment: No. 5. OECD/GD(96)166, Paris. p 32-50

OECD (1999) Report from the meeting of the expert group on avian reproductive toxicity testing, 23rd-24th May 1999, Leipzig, Germany

OECD (2000) Revised draft proposal for a new OECD Test Guideline: "Avian Reproduction Toxicity in the Japanese Quail or Northern Bobwhite". April 2000 draft. (available on the OECD Internet homepage: http://oecd.org/ehs/test/biotic.htm)

Solecki R, Faqi AS, Pfeil R, Hilbig V (1996) Effects of methyl parathion on reproduction in the Japanese quail. Bull Environ Contam Toxicol 57: 902-908