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Effects of Bis(tri-*n*-butyltin)oxide in Japanese Quail Exposed During Egg Laying Period: An Interlaboratory Comparison Study

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Abstract. The guideline no. 206 for testing of chemicals of the Organization for Economic Cooperation and Development (OECD) comprising an avian reproduction test using the Japanese quail *(Coturnix coturnix japonica;* Termminck and Schlegel 1849) as pair-hold test organisms has been applied in a version that reduced the treatment period to 6 weeks without any pretreatment. In the present study bis(tri-*n*-butyltin)oxide, C.A. No. 56-35-9 (tributyltin oxide, TBTO) was examined by five participants in an interlaboratory comparison test. A comparable regimen of dosing was performed by all participants starting either with 24 or 60 mg/kg TBTO in the feed and ending with 150 or 375 mg/kg. Within this dose range no signs of toxicity in adults were observed. Substance-related effects however were obvious with regard to egg production, fertility, hatching success, and survival of 14 day-old chicks. A clear dose dependency was given regarding effects on egg weight and on hatchability. The no-observed-effect concentrations for these two parameters was 60 mg/kg TBTO, characterizing these parameters as the most sensitive in this investigation. With the presented set of test parameters further aspects of subchronic toxicity in adults and chicks can be assessed as well as the validity of the performed test. Comparing the results for most test parameters consistency is obvious, thus confirming the applicability of the presented test guideline.

Causal relationships between reproductive lesions in fish-eating birds and exposure to certain substances have been reported (Gilbertson *et al.* 1991). The most important observations using such a conventional monitoring approach comprise the decline in the number of eggs laid per nest, the number of chicks hatched per nest and/or the number of chicks surviving a couple of weeks. Sometimes typical adverse effects like edema in embryos or hatchlings can be additionally noticed (Hart *et al.* 1991). Such adverse effects, however, are recognized only if either high exposure levels or substances with a high bioaccumulation potential are regarded.

There is a general agreement that the reproduction cycle of birds represents sensitive parameters that may be influenced by xenobiotic substances. Based on this ecologically important paradigm, various national environmental agencies and international organizations have designed avian test protocols for the evaluation of the potential hazard of pesticides and chemicals on the reproduction of birds. Protocols published by the U.S. Environmental Protection Agency (US EPA 1978) or the Organization for Economic Cooperation and Development (OECD 1984) recommend at least 10 weeks of dietary exposure prior to the onset of egg laying and at least 8 weeks exposure during egg production. The length of both treatment periods appears to have been chosen for detecting adverse effects of slowly bioaccumulating substances. Regarding the duration of the test period, in fact, no differentiation is made between bioaccumulating or non-accumulating substances. Results of concurrently conducted reproduction tests with rapidly acting substances designed either as a short-term exposure approach or a long-term exposure approach indicate that the length of the treatment period could be adapted to the chemical and toxic properties of the substance to be tested without any substantial loss of information (Bennett *et al.* 1990a, 1990b). Among the upland game birds the US EPA so far insists on the use of the bobwhite quail *(Colinus virginianus)* which begins egg production not before 6–7 months after hatch. The OECD, in addition, recommends the Japanese quail which reaches sexual maturity at an age of 5–6 weeks. This is one reason why a German group of toxicologists together with one participant from the Netherlands aimed to use the Japanese quail to reduce the test duration of 10 plus 8 weeks to a more realistic time span of 6 weeks dosing. Another objective of this study was to test the applicability of a test protocol with which agreement could be achieved between the members of a German working group of toxicologists (Draft

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1991). This modification of the OECD test guideline was to be evaluated by an interlaboratory comparison test which is reported in the present paper. Additionally, it should depend on the results of this study if this test protocol ought to be submitted as a proposal to international organizations like the OECD.

Tributyltin oxide (TBTO) has been selected as a test substance. It seemed to be promising due to its potential for bioaccumulation, its known toxic effects in mammals and in aquatic organisms. An increased annual use in the past in conjunction with its high persistance in aquatic compartments proves this chemical as an environmentally important substance. Regulations preventing the sale of TBTO paints with high leach rates, restrictions on their use for vessels in fresh water or generally for recreational vessels and the introduction of Aquatic Life Advisory Concentrations may lower concentrations of tributyltin compounds in aquatic environments.

In the present study, the Japanese quail was selected as a model organism because any test procedure on waterfowl is far away from standardization. Studies with TBTO or its degradation product dibutyltin oxide in rats revealed adverse effects on the immune system (Vos *et al.* 1990). Such effects, however, could not be observed in Japanese quail. Adult quail were very resistant to tributyltin compounds when dosed up to 450 mg per kg feed and administered for 2 weeks. Neither body weight nor lymphoid organs were influenced adversely (Seinen *et al.* 1977).

The aim of this interlaboratory comparison study was to test if reproduction of Japanese quail would be affected at a dose range which was not acutely and subchronically toxic for the adult birds. Based on a range-finding reproduction test, TBTO concentrations ranging from 24 to 375 mg per kg feed have been used by the participants of this investigation. Five different laboratories participated, three industrial, one governmental and one of a commercial contract laboratory.

Materials and Methods

All participants obtained Japanese quail from the same breeder (Gudrun Pottiez, W-7519 Eppingen 2, Germany). The birds' age at the start of the acclimatization period varied among the laboratories between 8 and 12 weeks. Pairs of males and females were randomly combined and housed in wire pens with sloping floors and egg catchers. Either a pair housing or an individual housing with at least 30 min pair housing each day was performed. Twelve pairs were assigned to each of at least three treatment groups in a stratified random scheme based on body weight.

Diets were amended with tributyltin oxide (Schering AG, 97.4% purity, batch No 0830) in 0.5% olive oil by weight. Twelve pairs were assigned to control diet (0% TBTO), and 12 pairs each to either 24, 60, 150, or 375 mg/kg TBTO amended diets. The concentration of TBTO in the various diets was analytically determined either by atomic absorption spectrometry or by gas chromatography after treatment of the extracted compound with ethylmagnesium bromide, using flame ionisation detection.

The duration of the test substance feeding period was limited to 6 weeks. The photoperiod for all birds was 16 h:8 h, light:dark. The light intensity in the cages was in the range of 65–200 lux.

Food consumption per pair was monitored weekly. Individual body weights were determined at the beginning of the acclimatization period and at the beginning and end of the treatment period. Behavior and general health condition were monitored daily on working days, Monday through Friday.

Among the reproductive parameters egg production was recorded daily. The number of eggs and their weights however were assigned to

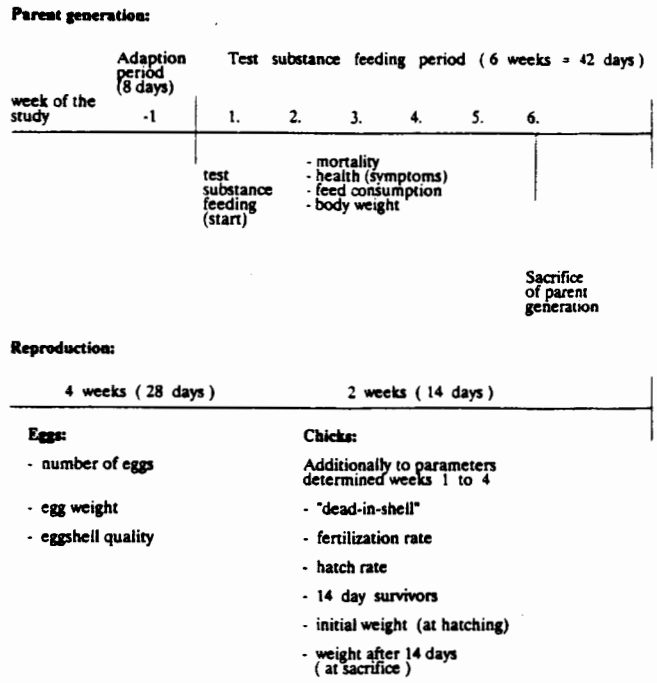

Fig. 1. Diagram of test layout

the respective week of the treatment period and were expressed as numbers of eggs and mean egg weight per pair and week. The egg quality was characterized by recording the percentage of broken or cracked eggs and in some laboratories by recording the eggshell thickness. Fertile eggs and viable embryos as well as hatching success and survivability of hatchlings were determined for eggs laid in the 5th and 6th week of treatment.

The rate of viability was expressed as percent viable embryos of eggs set as observed between day 7 and day 10 of incubation. Number and weight of hatchlings as well as those of 14 day-old survivors were registered as well. Chicks were observed for abnormal behavior, severe birth defects, abnormal reflexes, general state of health and mortality.

All birds that died during the test as well as all survivors asphyxiated with CO_2 were submitted to a complete necropsy and a macroscopic-pathological assessment. Necropsies involved examination for injuries, characterization of reproductive condition and the collection of weights of whole body, liver, heart, spleen, testis and oviduct. A scheme of the test layout comprising the test parameters is shown in Figure 1.

Handling of Eggs and Eggshell Quality Measurements

Eggs laid during the TBTO treatment period were stored in a refrigerator at 16 ± 1°C and 60–70% relative humidity. Storage was limited generally to 7 days. The eggs were placed in an automatic incubator at about 37.8°C and 60% relative humidity. Eggs were turned several times a day by approximately 180° over the egg equator up to day 16 of incubation. They were candled for fertility between day 8 and 10 of incubation. Only those laid in weeks 5 and 6 were left in the incubator to hatching. Hatched chicks were not removed from the incubator unless they were completely dry. Eggshell thickness was measured at three to four points around the egg equator after the eggs have been opened, washed out and dried for at least 48 h at room temperature. Eggs laid during the whole treatment period were used for this measurement.

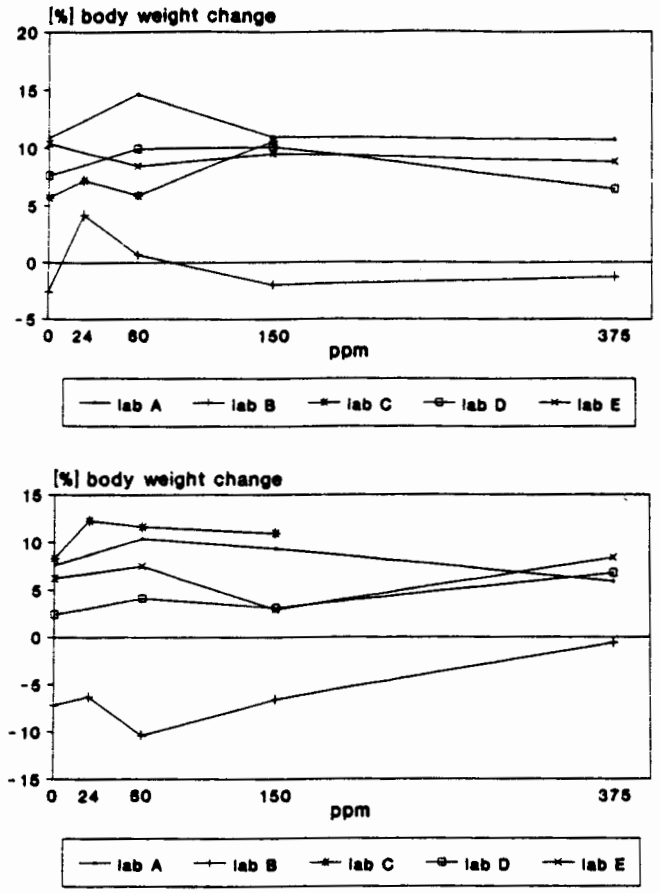

Fig. 2. Body weight was measured at the start of the application of TBTO (day 1) and at its end (day 42). Body weight change is given as percent deviation of the final weights from starting weights for males (top) and for females (bottom) as means of the respective dosage groups

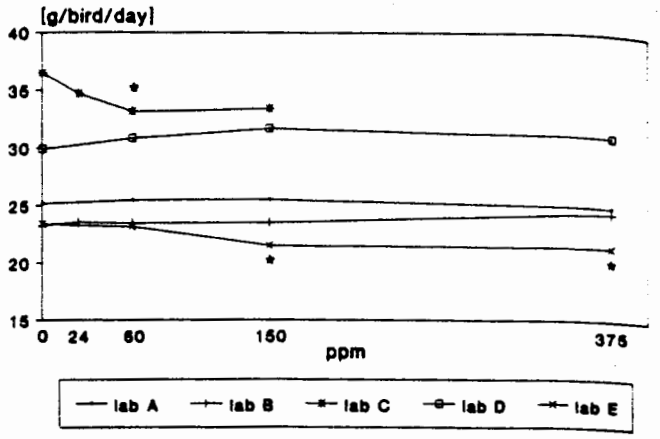

Fig. 3. Mean weekly food consumption is measured by difference between offered and left feed for the respective dosage groups. *$p < 0.05$ (ANOVA/Tukey-test)

Data Analysis

Data were analyzed using the single factor analysis of variance followed by a multiple comparison procedure as exemplified by the Tukey test with equal or unequal sample sizes at a significance level of alpha, 0.05 (Zar 1984). Egg data were analyzed by fitting linear regression models enclosing parameters like numbers or mass of eggs laid per week. Regression lines from distinct dose groups were evaluated by multiple comparison either among slopes or among elevations.

Results

The dosing of TBTO via the diet was justified by its chemical stability for at least three weeks and its homogenous distribution.

Dietary concentrations of TBTO up to 375 mg/kg had no effect on body weight gain of the parent generation in both sexes during the entire exposure period (Figures 2a, 2b). Weights of parent birds in laboratories A, C, D, E increased in a comparable manner during the 6-week treatment period at all dose levels. Those in laboratory B lost weight in all experimental groups; the females lost relatively more weight than the males (Figure 2). Weight losses did not, however, coincide with a decreased food consumption as compared to the respective results in all other laboratories. Generally no dose-related depression of food consumption was observed except for animals in laboratory E at the concentration levels of 150 and 375 ppm. Birds in laboratory C and D consumed more food per day than those in the remaining laboratories (Figure 3).

Clinically, the birds shows no substance-related symptoms. Mortalities occurred in a irregular and isolated manner and could not be attributed to the administered test substance. Moreover, no dose-related impairment of the investigated organ weights was observed.

A dose-related decrease in the number of eggs laid was observed at TBTO concentrations >60 mg/kg in laboratory A and >150 mg/kg in laboratories B, D, and E (Table 1). The respective reduction in laboratory C as observed already at 60 mg/kg, seems to be an anomaly, since at the next higher concentration the egg laying rate was not affected at all.

Starting at the 150 mg/kg treatment, egg mass decreased in a dose-dependent manner in all laboratories except laboratory B where the decrease started at 375 mg/kg (Table 2).

Eggshell thickness was measured by two of the participants. Laboratory C noticed a significant decrease of shell thickness beyond 24 mg/kg. Laboratory A, however, did not find any substance related effect on this parameter (Table 3).

Eggshell strength was evaluated indirectly by recording the percentage of cracked eggs (Table 4). A significant increase of this parameter was observed in those laboratories which dosed up to 375 mg/kg (A and E).

Decreases in the rate of fertile eggs of eggs incubated were observed by four of the five participants. Laboratory E first detected this effect at 150 mg/kg and laboratories B an D at 375 mg/kg (Table 5). In laboratory A no substance-related decrease of the fertility rate has been observed. A respective decrease at week 5 in the 60 mg/kg treatment group in laboratory C, however, could not be confirmed at week 6 and in the next higher treatment group.

The hatch rate of fertile eggs was clearly affected by TBTO (Table 6). In laboratories A, B and E, reductions were first noted at 150 mg/kg, and a decline was first observed at 375 mg/kg in laboratory D. No apparent effect on this parameter has been found by participant C.

There was no comparable effect of TBTO at 60 mg/kg diet on the rate of chicks "dead-in-shell" related to fertile eggs as

Table 1. Response of the Japanese quail to tributyltinoxide in the diet during weeks 1–6. Number of eggs laid per week†

TBTO (mg/kg) in the diet	Laboratory A	B	C	D	E
0	$y = 0.19x + 5.85$ $n = 6$; $R^2 = 0.52$	$y = 0.09x + 4.35$ $n = 6$; $R^2 = 0.44$	$y = -0.02x + 6.15$ $n = 6$; $R^2 = 0.01$	$y = 0.3x + 5.27$ $n = 6$; $R^2 = 0.83$	$y = -0.089x + 6.29$ $n = 6$; $R^2 = 0.16$
24	—	$y = 0.3x + 2.81$ $n = 6$; $R^2 = 0.28$	$y = 0.11x + 5.71$ $n = 6$; $R^2 = 0.14$	—	—
60	$y = 0.21x + 5.75$ $n = 6$; $R^2 = 0.80$	$y = 0.06x + 3.51$ $n = 6$; $R^2 = 0.28$	$y = -0.13x + 5.33$ $n = 6$; $R^2 = 0.29$ *(0,24)	$y = 0.18x + 5.34$ $n = 6$; $R^2 = 0.70$	$y = 0.12x + 5.53$ $n = 6$; $R^2 = 5.53$
150	$y = 0.05x + 5.85$ $n = 6$; $R^2 = 0.07$ *(0,60)	$y = 0.15x + 3.35$ $n = 6$; $R^2 = 0.17$	$y = -0.11x + 5.94$ $n = 6$; $R^2 = 0.33$	$y = 0.18x + 5.13$ $n = 6$; $R^2 = 0.35$	$y = -0.28x + 4.91$ $n = 6$; $R^2 = 0.61$
375	$y = -0.03x + 5.57$ $n = 6$; $R^2 = 0.02$ *(0,60)	$y = 0.05x + 2.83$ $n = 6$; $R^2 = 0.25$ *(0)	—	$y = 0.0003x + 4.61$ $n = 6$; $R^2 = 0.0003$ *(0,60,150)	$y = 0.04x + 2.86$ $n = 6$; $R^2 = 0.019$ *(0,60,150)

† Data on eggs laid per week in each dose group were analyzed by fitting a simple linear regression model to various weeks (y = number of eggs per week; x = respective week)
* Statistically different using the multiple comparison procedure (Tukey test) on an alpha level of 0.05. Numbers within the brackets indicate where differences between the respective dose groups and the given dose level exists

Table 2. Response of the Japanese quail to tributyltinoxide in the diet during weeks 1–6. Mean egg weight (g) per week†

TBTO (mg/kg) in the diet	Laboratory A	B	C	D	E
0	$y = -0.01x + 12.35$ $n = 6$; $R^2 = 0.05$	$y = 0.15x + 11.73$ $n = 6$; $R^2 = 0.73$	$y = 0.1x + 11.93$ $n = 6$; $R^2 = 0.77$	$y = 0.08x + 11.50$ $n = 6$; $R^2 = 0.58$	$y = -0.01x + 12.20$ $n = 6$; $R^2 = 0.01$
24	—	$y = 0.08x + 11.56$ $n = 6$; $R^2 = 0.51$	$y = 0.11x + 11.85$ $n = 6$; $R^2 = 0.73$	—	—
60	$y = 0.07x + 12.47$ $n = 6$; $R^2 = 0.05$	$y = 0.02x + 12.55$ $n = 6$; $R^2 = 0.06$	$y = -0.03x + 12.13$ $n = 6$; $R^2 = 0.43$	$y = 0.17x + 10.62$ $n = 6$; $R^2 = 0.91$	$y = -0.04x + 11.70$ $n = 6$; $R^2 = 0.02$
150	$y = 0.02x + 11.51$ $n = 6$; $R^2 = 0.08$ *(0,60)	$y = 0.13x + 11.41$ $n = 6$; $R^2 = 0.86$	$y = 0.02x + 11.82$ $n = 6$; $R^2 = 0.21$ *(0,24,60)	$y = 0.13x + 10.72$ $n = 6$; $R^2 = 0.99$ *(0)	$y = 0.08x + 10.10$ $n = 6$; $R^2 = 0.06$ *(0,60)
375	$y = 0.07x + 10.89$ $n = 6$; $R^2 = 0.13$ *(0,60,150)	$y = -0.01x + 10.85$ $n = 6$; $R^2 = 0.0003$ *(0,24,60,150)	—	$y = 0.26x + 9.44$ $n = 6$; $R^2 = 0.95$ *(0,60,150)	$y = -0.15x + 9.50$ $n = 6$; $R^2 = 0.30$ *(0,60,150)

† Data on mean egg weight per week in each dose group were analyzed by fitting a simple linear regression model to various weeks (y = mean egg weight per week; x = respective week)
* See legend in Table 1

Table 3. Response of the Japanese quail to tributyltinoxide in the diet. Mean eggshell thickness (μm) of eggs laid during weeks 1–6

TBTO (mg/kg) in the diet	Laboratory A	C
0	188 ± 8	206 ± 8
24	—	195 ± 8
60	190 ± 12	192 ± 10 *(0)
150	190 ± 14	192 ± 10 *(0)
375	183 ± 21	—

* See legend in Table 1

Table 4. Response of the Japanese quail to tributyltinoxide in the diet during the six weeks exposure period. Percentage of cracked eggs of eggs laid

TBTO (mg/kg) in the diet	Laboratory A	C	E
0	2.23 ± 2.85	0.25 ± 0.85	3.12 ± 2.83
24	—	0.00 ± 0.00	—
60	3.02 ± 3.88	2.51 ± 4.54	6.96 ± 6.35
150	1.97 ± 2.43	2.13 ± 3.38	10.27 ± 8.31
375	12.03 ± 16.38 *(0,150)	—	20.19 ± 14.01 *(0,60,150)

* See legend in Table 1

Table 5. Response of the Japanese quail to tributyltinoxide in the diet. Percent fertile eggs laid in exposure weeks 5 and 6†

TBTO (mg/kg) in the diet	Week	LaboratoryQ A	B	C	D	E
0	5	97.50 ± 27.26	82.29 ± 32.15	85.42 ± 25.89	83.45 ± 19.10	77.34 ± 27.10
	6	91.27 ± 21.95	95.00 ± 9.26	98.15 ± 5.56	88.29 ± 18.00	87.12 ± 14.63
24	5	—	88.31 ± 22.07	86.53 ± 28.80	—	—
	6	—	86.90 ± 20.46	96.36 ± 8.09	—	—
60	5	100.00 ± 0.00	86.76 ± 25.92	51.55 ± 39.92 *(0,24)	79.00 ± 24.32	54.90 ± 33.71
	6	91.43 ± 21.95	72.68 ± 22.48	70.00 ± 42.39	83.12 ± 22.97	65.69 ± 32.33
150	5	94.85 ± 8.87	67.92 ± 36.25	83.89 ± 21.97	82.10 ± 19.37	19.33 ± 34.55 *(0,60)
	6	85.41 ± 22.22	82.86 ± 23.61	89.72 ± 19.12	80.18 ± 14.14	27.27 ± 35.96 *(0,60)
375	5	94.70 ± 11.95	18.57 ± 26.10 *(0,24,60,150)	—	27.32 ± 29.26 *(0,60,150)	4.17 ± 11.78 *(0,60)
	6	77.68 ± 33.08	11.67 ± 17.00 *(0,24,60,150)	—	22.33 ± 22.77 *(0,60,150)	12.50 ± 35.30 *(0,60)

† In the amount of laid eggs, broken and/or cracked eggs were included which, however, were not incubated.
* See legend in Table 1

Table 6. Response of the Japanese quail to tributyltinoxide in the diet. Percent hatch of fertile eggs from exposure weeks 5 and 6

TBTO (mg/kg) in the diet	Week	Laboratory A	B	C	D	E
0	5	89.01 ± 14.81	86.11 ± 18.63	86.39 ± 26.83	89.99 ± 17.44	75.69 ± 24.42
	6	87.10 ± 16.23	89.16 ± 17.89	73.03 ± 43.22	86.11 ± 30.01	72.91 ± 30.29
24	5	—	73.04 ± 31.18	83.33 ± 31.72	—	—
	6	—	78.28 ± 25.24	98.48 ± 5.03	—	—
60	5	66.68 ± 18.36	71.73 ± 34.84	34.05 ± 40.77	90.61 ± 17.44	22.64 ± 22.75
	6	60.99 ± 22.50	62.85 ± 17.25	38.21 ± 40.98	79.98 ± 20.98	34.72 ± 27.14
150	5	55.32 ± 29.41 *(0)	56.19 ± 35.61	55.00 ± 41.67	69.66 ± 21.74	0 (no hatch) *(0,60)
	6	37.14 ± 27.81 *(0,60)	45.83 ± 34.21 *(0)	45.00 ± 32.77	69.33 ± 23.07	0 (no hatch) *(0,60)
375	5	22.90 ± 23.72 *(0,60)	0 (no hatch) *(0,24,60,150)	—	13.89 ± 22.15 *(0,60,150)	0 (no hatch) *(0,60)
	6	13.49 ± 28.90 *(0,60,150)	0 (no hatch) *(0,24,60,150)	—	50.83 ± 53.89	0 (no hatch) *(0,60)

* See legend in Table 1

determined in the investigations of laboratories B and C (Table 7). The reduced hatchability, however, as found at the two highest TBTO concentrations, appeared to be correlated with the higher rate of chicks "dead-in-shell".

There was a significant dose-related decrease of the number of hatched chicks per pair in laboratories A, B, C and D (Table 8). In these laboratories the no observed effect concentration (NOEC) was 60 mg/kg, and 24 mg/kg in laboratory C. Since laboratory E reported an adverse effect even at the lowest dose tested (60 mg/kg), a NOEC could not be established in this investigation.

Regarding body weights of hatchlings, neither a dose-related decrease up to the dose of 150 mg/kg nor a definite adverse effect at 375 mg/kg was given in those laboratories (A and D) where hatching occurred at this dose (Table 9).

Regarding the number of 14 day-old survivors per dose group a significant decrease was first noted at 375 mg/kg (laboratory B) or 150 mg/kg (laboratory D) (Table 10). Data from laboratories A and C could not be related to single pens and therefore did not allow a statistical calculation. In laboratory D the decrease in the number of 14 day-old survivors appeared to be dose-related, with a NOEC of 60 mg/kg.

The weight of 14 day-old survivors was impaired by TBTO in laboratory A and D if adults were exposed at 375 mg/kg (Table 11). If, however, a TBTO amended diet up to 150 mg/kg (laboratories A, B, C, and D) or up to 60 mg/kg (laboratory E) was administered to adults the weight of survivors was not affected.

The survival of hatched chicks was not reduced up to a 150 mg/kg TBTO amended diet as shown by the results of laboratories B and D (Table 12). A respective significant adverse effect has been observed at the highest dose of 375 mg/kg in laboratory D. From the results of this and from laboratory B a NOEC of 150 mg/kg can be derived. Due to a mixed grouping of chicks during the rearing period the observed trends of dose-dependency in laboratories A and C could not be confirmed statistically. The NOEC in laboratory E appears to be 60 mg/kg.

Table 7. Response of the Japanese quail to tributyltinoxide in the diet during the six weeks exposure. Percent chicks "dead in shell of fertile eggs"

TBTO (mg/kg) in the diet	Week	Laboratory B	Laboratory C
0	5	10.19 ± 17.57	10.28 ± 25.71
	6	8.34 ± 17.82	6.28 ± 22.61
24	5	16.85 ± 24.99	8.33 ± 18.01
	6	13.08 ± 13.49	1.52 ± 5.04
60	5	12.75 ± 15.47	38.81 ± 43.26
	6	18.33 ± 13.96	41.48 ± 39.09 *(0,24)
150	5	31.90 ± 36.71	44.58 ± 41.31 *(0,24)
	6	35.44 ± 32.65	30.29 ± 30.57
375	5	61.00 ± 34.83 *(0)	—
	6	16.67 ± 28.87	—

*See legend in Table 1

Discussion

Earlier investigations with tributyltin compounds, administered for 14 days, revealed a much weaker toxic activity in Japanese quail compared to mammals (Seinen *et al.* 1977). This is remarkable, because animals of lower phylogenetic categories such as various aquatic species in both freshwater and marine ecosystems respond to these substances with acutely lethal and sublethal effects at very low concentrations (Seinen et al. 1981; Oceans 1986; BUA 1988). In addition, TBTO compounds have, if not ionized, a substantial accumulation potential as verified by measurements in invertebrates, shellfish or fish. A variety of waterfowl thus can be exposed to these substances. Under environmental conditions, however, animals may be exposed to a number of toxic substances so that impairments in reproductive success generally can not be attributed to a particular compound such as TBTO. Therefore an experimental investigation of their toxic properties appeared to be necessary.

It could be demonstrated in this study that TBTO acts as an embryotoxic substance in the Japanese quail if administered in a dose range up to 375 mg/kg in the feed. Among the observed effects that on fertility is limited to females rather than males (data to be published).

Due to the different age of the adults at the start of this study, ranging from 8 to 12 weeks, a distinct course of body weight development was given as demonstrated by the various laboratories. In all cases, however, a dose-related effect could not be observed.

The lower food consumption observed in laboratories A, B, and E compared to C and D is correlated with an impaired hatch rate observable already at TBTO concentrations of 150 mg/kg. In the cohort of laboratory E food intake is impaired furthermore in a dose-related manner. Additionally, the mass of eggs laid in both the highest dose groups of laboratory E is far smaller than in the cohorts of the remaining laboratories. This is in accordance with the well known fact that diminished food intake leads to reduced egg weights in Japanese quails (O'Connor 1984). In comparison to the results of laboratories A to D, this is also correlated with a greater impairment of fertility rate and a cease of hatching starting at a concentration of 150 mg/kg TBTO. The cause for this quite different series of reactions is unclear. A latent but undiagnosed infection in laboratory E may have promoted the reduced food intake which itself may have led to an earlier decrease of fertility and hatching success in comparison to cohorts of the other laboratories.

There were significant effects on parameters characterizing egg production, e.g. the number of eggs laid and the mean egg mass. The mean egg mass was impaired at a lower dosage than the frequency of egg laying. The respective NOECs were 60 mg/kg and 150 mg/kg.

Among the tested eggshell quality parameters, an increased rate of cracked or broken eggs was obvious at the highest TBTO concentration (375 mg/kg) whereas the two investigations of eggshell thickness do not allow any clear interpretation in so far as the decreased thickness in eggs of laboratory C observed at 60 mg/kg and 150 mg/kg cannot be correlated with a respective increase of the rate of cracked eggs.

Except for the above mentioned peculiarity observed in labo-

Table 8. Response of the Japanese quail to tributyltinoxide in the diet. Number of hatched chicks per pen from eggs set

TBTO (mg/kg) in the diet	Week	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
0	5	5.00 ± 1.91	3.08 ± 2.31	3.58 ± 1.68	4.50 ± 1.83	2.67 ± 1.30
	6	5.00 ± 1.60	3.25 ± 2.60	3.67 ± 2.57	5.00 ± 2.00	3.25 ± 2.09
24	5	—	2.75 ± 1.91	4.00 ± 1.71	—	—
	6	—	3.42 ± 1.78	4.83 ± 1.70	—	—
60	5	4.50 ± 1.24	2.00 ± 2.34	1.42 ± 1.68 *(0,24)	4.55 ± 1.63	0.75 ± 0.87 *(0)
	6	3.58 ± 1.68	1.75 ± 1.86	1.83 ± 1.99 *(24)	4.00 ± 1.67	1.67 ± 1.61 *(0)
150	5	2.83 ± 1.70 *(0)	1.08 ± 1.31 *(0)	2.08 ± 1.68 *(24)	3.50 ± 1.57	0 (no hatch) *(0,60)
	6	1.75 ± 1.48 *(0,60)	1.58 ± 2.11	1.50 ± 1.09 *(0,24)	3.17 ± 1.85 *(0)	0 (no hatch) *(0,60)
375	5	1.17 ± 1.40 *(0,60)	0 (no hatch) *(0,24,60,150)	—	0.25 ± 0.62 *(0,60,150)	0 (no hatch) *(0,60)
	6	0.42 ± 0.67 *(0,60)	0 (no hatch) *(0,24,60,150)	—	0.33 ± 0.65 *(0,60,150)	0 (no hatch) *(0,60)

*See legend in Table 1