Oviposition and the plasma concentrations of LH, progesterone and corticosterone in bobwhite quail (*Colinus virginianus*) fed parathion

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Summary. Bobwhite quail were fed concentrations of parathion (0, 50, 100, 200 or 400 p.p.m.) for 10 days. Food intake, body weight change, brain acetylcholinesterase activity, egg production, and ovary weight were reduced in a dose-dependent manner. In a second experiment, birds were fed 0, 25 or 100 p.p.m. parathion or pair-fed control food to equate consumption in the 100 p.p.m. group. Egg production was not affected in birds fed 25 p.p.m. or in the pair-fed group, but the daily time of oviposition was more variable than in the control group. Cessation of egg production, inhibition of follicular development, and reduced plasma LH concentration were observed in birds fed 100 p.p.m. parathion. These findings indicate that exposure to parathion can impair reproduction, possibly by altering gonadotrophin secretion.

Introduction

Organophosphorus insecticides are extensively used in agriculture as substitutes for the persistent chlorinated hydrocarbons of past decades. The seasons during which these insecticides are applied often coincide with the time of peak reproductive activity in a variety of species. Despite short half-lives in the environment and in the tissues of homeothermic animals, some organophosphorus insecticides are highly toxic to wildlife for brief periods after application (Stickel, 1975; Mendelssohn & Paz, 1977; Zinkl, Rathert & Hudson, 1978). Parathion is an organophosphate which has been implicated in the mortality of birds and small mammals (Mills, 1973), and in one instance in the impairment of reproductive success in an avian breeding colony (White, King, Mitchell, Hill & Lamont, 1979).

The mechanism of action, metabolism, and neuromuscular effects of organophosphates have been thoroughly investigated (Matsumura, 1975). Dietary exposure to organophosphates has been demonstrated to reduce food consumption, body weight, egg production, fertility, and hatchability in the chicken (*Gallus domesticus*), partridge (*Alectoris graeca*), pheasant (*Phasianus colchicus*), and quail (*Colinus virginianus* and *Coturnix coturnix japonica*) in a dose-dependent manner (Ross & Sherman, 1960; Shellenberger, Newell, Adams & Barbaccia, 1966; Stromborg, 1977; Schom, Abbott & Walker, 1979). Some of the endocrinological consequences of exposure of mammals to such anticholinesterase compounds include alteration of hypothalamo-hypophysial function (Muller, Nistico & Scapagnini, 1977; Civen, Leeb, Wishnow, Wolfson & Morin, 1980; Ruiz de Galarreta, Fanjul & Meites, 1981), changes in

plasma corticosterone rhythms (Szot & Murphy, 1971; Civen, Brown & Morin, 1977), and the inhibition of steroidogenesis (Civen *et al.*, 1977) and hepatic steroid metabolism (Conney, Welch, Kuntzman & Burns, 1967). However, little is known about the hormonal responses which accompany sublethal organophosphate exposure in birds. The present investigation was conducted to examine the effects of graded dietary concentrations of parathion on body and organ weights, food intake, egg production, and the concentrations of luteinizing hormone (LH), progesterone and corticosterone in bobwhite quail.

Materials and Methods

Female bobwhite quail (*Colinus virginianus*) were obtained commercially (Oak Ridge Game Farm, Gravette, Arkansas) at 15 weeks of age. Birds were housed individually in stainless-steel cages (22.8 cm wide $\times$ 33.0 cm high $\times$ 38.1 cm deep) fitted with food dispensers and watering tubes, and situated within environmental chambers maintained at $26.0 \pm 1.0^\circ\text{C}$. Birds were provided with mash containing corn oil vehicle (2% w/w) and water *ad libitum*. Birds receiving toxicants mixed in mash frequently spill large quantities of food. Spillage was minimized by partly filling the food dispenser and placing a plastic-coated wire grate on top of the mash.

Initially a 12 h light:12 h dark photoperiod was utilized. After 3 weeks, the light phase was advanced 2 h/week to a 16 h light:8 h dark cycle (lights on at 06:00 h). Onset of laying and the number of eggs laid were monitored daily at 11:00 h. Eggs were also removed from the cages at this time.

Experiment 1: effects of graded concentrations of parathion on mash intake, body and organ weight, egg production, and acetylcholinesterase activity

Female birds (27 weeks old), weighing 200–250 g, were randomly assigned to treatments (5 birds/group). Mash mixed with vehicle containing 0 (control), 50, 100, 200 or 400 p.p.m. technical grade parathion (ethyl parathion or *O,O*-diethyl *O,p*-nitrophenyl phosphorothioate; Monsanto Company, St Louis, Missouri) was provided *ad libitum* for 10 days (Groups P_0 , P_{50} , P_{100} , P_{200} and P_{400}). Food intake (defined as removal of mash from the dispenser) was measured daily at 10:00 h. Body weight was determined at 09:00 h on the day the study was initiated (Day 0) and after 9 days. The cages were inspected at 11:00 h, and any eggs laid during the preceding 24-h period were removed. Birds were killed by decapitation at 12:00 h on Day 10. The intact heads were sealed in plastic bags and frozen at -20°C for subsequent determinations of acetylcholinesterase activity, and the ovary and the adrenal and thyroid glands were weighed.

Experiment 2: effects of parathion on reproductive activity and plasma hormones

Female birds (30 weeks old) were randomly assigned to treatments (36 birds/group) receiving mash mixed with vehicle containing 0, 25 or 100 p.p.m. parathion for 10 days (Groups P_0 , P_{25} , P_{100}). A group of 36 birds to be pair-fed with birds in Group P_{100} were included in the experiment 72 h later and each bird was randomly assigned a partner bird in Group P_{100} . At 09:00 h daily, each pair-fed bird was given mash (0 p.p.m.) equivalent in weight to that consumed by its partner bird during the corresponding 24-h period. An additional 5 g mash was provided to the pair-fed birds to compensate for a small residual quantity of food which could not readily be removed from the feeder and spillage. Equal numbers of birds from each of the 4 groups were housed in two identical environmental chambers.

Body weight changes between Days 0 and 9 were determined for 6 birds in each group (1 bird/sampling time, see below). Cages were inspected for eggs as in Exp. 1. At 12:00 h on Day 9 and until the birds were killed, cages were examined at 1-h intervals to determine the time of

oviposition. Six birds from each treatment were killed at 00:00, 04:00, 08:00, 12:00, 16:00 and 20:00 h on Day 10. Within a 1 min period, the cage containing the bird to be killed was removed from the chamber and the bird was decapitated. Plasma obtained following centrifugation and whole brains were frozen at -20°C , and the diameters of the three largest follicles were measured.

Analytical procedures

Plasma immunoreactive LH was measured by radioimmunoassay employing a 300 μl tube volume (Follett, Scanes & Cunningham, 1972). Quail plasma assayed at two dilutions and corrected to constant volume gave comparable estimates (coefficient of variation of 10.9%; $n = 10$). Saline or 10 μg LH-RH/kg body weight i.v. (Sigma Chemical Company, St Louis, Missouri) was given to male birds which were then bled after 10 min. Plasma LH concentration was significantly elevated ($P < 0.01$) in LH-RH-treated birds (mean $\pm$ s.e.m.; 36.6 ± 2.45 ng/ml; $N = 7$) compared to saline-treated controls (14.2 ± 2.62 ng/ml; $N = 7$). Intra- and inter-assay precision (coefficient of variation) were 10.1 and 21.0% respectively, and sensitivity was 95 pg/ml.

Plasma progesterone was assayed as previously described (Rattner, Michael & Brinkley, 1978), except that the volume of plasma extracted was 100 μl . The validity of this procedure for quail plasma was tested by the addition of progesterone (250–2000 pg) to extraction tubes containing plasma from laying and non-laying quail. The quantity of progesterone estimated by the procedure yielded a regression line with a slope of 0.97 pg estimated/pg added and a correlation coefficient of 0.97. The slope of this line was not significantly different from 1.0 ($P > 0.50$). Dilutions of an extract pool with high progesterone content yielded a regression line parallel to the standard curve ($P > 0.10$; Finney, 1964). The precision of progesterone recovery by the extraction (mean recovery of 77.7%; $n = 150$) was 9.9%. The intra- and inter-assay precisions were 3.7 and 10.9% respectively, and the lower limit of sensitivity (95% confidence interval at B_0) was 21 pg/tube.

A direct radioimmunoassay employing 5 μl plasma samples was used to measure corticosterone (Rattner & Eastin, 1981). The accuracy of this procedure for quail plasma was assessed by the addition of corticosterone (50–1000 pg) to several different plasma pools. Recovery estimates yielded a regression line with a slope 1.02 pg estimated/pg added, which was not different from unity ($P > 0.50$). This corticosterone antiserum cross-reacts equally with cortisol; however, cortisol is not a principal adrenal secretory product in avian species (Sandor, Fazekas & Robinson, 1976). Dilutions of a plasma pool with high corticosterone content (maintaining a constant volume with plasma devoid of steroids) yielded a regression line parallel to the standard curve ($P > 0.50$). The intra- and inter-assay precisions were 4.6 and 11.6% respectively, and sensitivity was 8 pg/tube.

Brains were homogenized (1:10, w/v) in 0.05 M-Tris buffer (pH 8.0). The homogenate was centrifuged for 10 min at 1500 g, and cholinesterase activity of the supernatant was determined (Ellman, Courtney, Andres & Featherstone, 1961). Four pools of brain supernatant were also assayed in the presence of 10^{-5} M-ethopropazine hydrochloride (a specific inhibitor of non-specific cholinesterase, Illsley & Lamartiniere, 1981; Sigma), and revealed that 96.6% of total activity was acetylcholinesterase (EC 3.1.1.7). The intra- and inter-assay precisions were 1.0 and 1.7% respectively.

Statistical analyses

In Exp. 1, the activities of acetylcholinesterase, eggs laid/bird, and organ weights were compared by one-way analysis of variance (ANOVA). In Exp. 2, acetylcholinesterase activity, eggs laid/bird, follicular diameters, and hormone concentrations were compared by factorial

ANOVA (4 treatments $\times$ 6 sampling times). In both experiments, body weight change (Days 0–9; g/100 g weight) was compared by one-way ANOVA and serial observations on food consumption were evaluated by ANOVA for repeated measures (Winer, 1971). When significant effects of treatment, sampling time, or the interaction of these factors were detected, means were compared by Tukey's HSD method of multiple comparisons (Kirk, 1968). The frequency distributions of oviposition with time (3-h intervals) in the control, 25 p.p.m. parathion, and pair-fed groups were compared by pair-wise tests using contingency analysis (Conover, 1971).

Results

The birds used in these experiments began laying when they were 21–23 weeks old. Eggs were generally laid between 14:00 and 17:00 h. The duration between ovipositions in a sequence was 24.4 ± 0.15 h (mean $\pm$ s.e.m.; $N = 36$ birds laying on 4 consecutive days) and the length of the laying sequence was 5.1 ± 0.37 days ($N = 144$ birds for a 21-day period).

Experiment 1

During the morning of Day 10, one bird died in each of Groups P_{200} and P_{400} . Analysis of variance revealed a significant treatment effect ($P < 0.01$) for food intake. Intake averaged over the 10 sampling periods was inversely proportional to the dietary concentration of parathion (Table 1). Weight change and brain acetylcholinesterase activity were reduced ($P < 0.05$) in a dose-dependent manner.

Table 1. Food intake, body weight change, brain acetylcholinesterase (AChE) activity, egg production, and ovary weight of birds fed graded concentrations of parathion for 10 days

Group	Food intake Days 1–10 (g)*	Body weight change Days 0–9 (Δ g/100 g)	Brain AChE Day 10†	Egg production Days 1–10	Ovary weight Day 10 (g)
P_0	23.7 ± 2.91^a	0.0 ± 0.52^a	5.60 ± 0.288^a	9.2 ± 0.80^a	6.38 ± 0.692^a
P_{50}	18.0 ± 2.93^b	-4.9 ± 1.90^b	3.47 ± 0.211^b	5.6 ± 0.98^b	4.17 ± 0.494^a
P_{100}	10.2 ± 3.13^c	$-18.0 \pm 1.53^{b,c}$	$2.75 \pm 0.362^{b,c}$	$3.6 \pm 0.25^{b,c}$	1.38 ± 0.848^b
P_{200}	$6.9 \pm 3.72^{c,d}$	$-30.7 \pm 4.53^{c,d}$	$2.32 \pm 0.139^{b,c}$	$3.8 \pm 0.58^{b,c}$	0.76 ± 0.339^b
P_{400}	2.9 ± 0.74^d	-36.1 ± 5.27^d	1.82 ± 0.312^c	2.8 ± 0.37^c	0.79 ± 0.398^b

Values are mean $\pm$ s.e.m. for 5 birds/group.

Means with different letter superscripts are significantly different ($P < 0.05$) by Tukey's HSD method of multiple comparisons.

* Calculated from the average intake/bird for Days 1–10.

† Expressed as μ mol acetylthiocholine iodide hydrolysed/min/g tissue.

Birds receiving parathion laid fewer eggs ($P < 0.05$) than did control birds (Table 1). No eggs were laid after Day 7 by birds in Groups P_{100} , P_{200} or P_{400} . Large follicles (15–20 mm) were observed in the ovaries of birds in Groups P_0 and P_{50} . Large follicles were also present in one bird in each of Groups P_{100} , P_{200} and P_{400} ; however, follicles in the remainder of these birds were < 7 mm in diameter. The weight of the ovary was significantly reduced ($P < 0.05$) in the birds in Groups P_{100} , P_{200} and P_{400} when compared to the other groups; but the weights of the thyroid and adrenals were not affected by treatment.

Experiment 2

All birds survived until the end of the experiment. Mash intake did not differ between birds in Group P_{100} and those in the pair-fed groups on Days 1–10, although weight loss was greater

($P < 0.05$) in the former (Table 2). Brain acetylcholinesterase activity was differentially inhibited ($P < 0.05$) in birds in Groups P_{25} and P_{100} , but activity was not altered in the pair-fed group.

Table 2. Body weight change, brain acetylcholinesterase (AChE) activity, egg production, follicular diameter, and plasma LH concentration of birds fed parathion for 10 days

Group	Body weight change (Δ g/100 g)	Brain AChE*	Egg production Days 1–10	Follicular diameter (mm)			LH (ng/ml)
				Largest	2nd largest	3rd largest	
P_0	-1.7 ± 1.16^a	7.68 ± 0.237^a	8.4 ± 0.32^a	20.5 ± 0.43	16.1 ± 0.50^a	10.4 ± 0.52^a	7.4 ± 0.41^a
P_{25}	-3.0 ± 1.00^a	5.51 ± 0.208^b	8.0 ± 0.36^a	20.6 ± 0.42	16.0 ± 0.43^a	10.3 ± 0.44^a	6.5 ± 0.36^a
P_{100}	-19.2 ± 1.68^c	3.14 ± 0.110^c	4.3 ± 0.31^b	13.1 ± 1.01	8.4 ± 0.65^b	5.2 ± 0.52^b	4.3 ± 0.16^b
Pair-fed	-8.5 ± 2.31^b	8.10 ± 0.231^a	8.1 ± 0.27^a	19.4 ± 0.60	14.9 ± 0.64^a	9.7 ± 0.53^a	6.2 ± 0.29^a

Values are mean $\pm$ s.e.m.

Body weight change (Days 0–9; $n = 6$) and other variables ($n = 36$) with different letter superscripts are significantly different ($P < 0.05$) by Tukey's HSD method of multiple comparisons.

* Expressed as μ mol acetylthiocholine iodide hydrolysed/min/g tissue.

There was a reduction ($P < 0.05$) in the number of eggs laid/bird in Group P_{100} , but not in the pair-fed group relative to controls (Table 2). In the control group (P_0), 81.5% of the eggs on Day 10 were laid from 14:00 to 17:00 h (Table 3). The time of oviposition in Group P_{25} and pair-fed birds was variable and differed ($P < 0.05$) from that in the control group.

Table 3. Time of oviposition and number of birds laying eggs between Days 9 and 10

Day	Time interval (h)	Phase of photoperiod*	Group P_0 (control)	Group P_{25}	Group P_{100}	Pair-fed Group
9	12:00–14:00	L	0	4	1	8
9	15:00–17:00	L	22	12	3	9
9	18:00–20:00	L	4	9	1	3
9	21:00–23:00	L $\rightarrow$ D	1	1	0	3
10	00:00–02:00	D	0	0	0	2
10	03:00–05:00	D	0	1	0	1
10	06:00–08:00	D $\rightarrow$ L	0	0	0	0
10	09:00–11:00	L	0	0	1	0
Total laying			27/36	27/36	6/36	26/36

The frequency distributions of oviposition in Group P_{25} and pair-fed groups birds were significantly different ($P < 0.05$) from that in the control group by contingency analysis.

* D = dark, L = light.

Follicular development appeared to be inhibited in many of the birds in Group P_{100} because only small and atretic follicles were present. A significant interaction of treatment and sampling time ($P < 0.01$) was detected for the diameter of the largest ovarian follicle. The size of the largest follicle did not differ in Group P_{25} and pair-fed birds compared to that in control birds. However, the diameter of the largest follicle in Group P_{100} was less ($P < 0.05$) than that in the control group between midnight and 12:00 h, but not thereafter. The diameter of the largest follicle (pooled across sampling times) is presented for comparison in Table 2. There was a significant effect of treatment ($P < 0.01$) for the size of the second and third largest follicles; these follicles were consistently smaller ($P < 0.05$) in birds in Group P_{100} (Table 2).

Plasma hormone concentrations at 4-h intervals on Day 10 are presented in Text-fig. 1. The

Text-fig. 1. Hormone concentrations in bobwhite quail in Group P_0 (control), Group P_{25} , Group P_{100} and the pair-fed group after 10 days of treatment (Exp. 2). Values are mean $\pm$ s.e.m. for 6 birds/sampling time. The thickened portion of the time axes corresponds to the dark phase of the photoperiod. Arrows indicate the usual time of oviposition in the control birds.

concentration changes varied considerably among groups. The ANOVA indicated that there was a significant effect of treatment ($P < 0.05$) on plasma LH concentration. Plasma LH levels in Group P_{100} were less ($P < 0.05$) than in the other groups (Table 2). A significant effect of sampling time ($P < 0.01$) was detected for plasma progesterone concentration; values (pooled across treatment) at 08:00, 16:00 and 20:00 h (8.2 ± 1.03 , 7.3 ± 1.08 and 7.8 ± 1.18 ng/ml, respectively) were less than at 12:00 h (13.4 ± 1.61 ng/ml). An interaction of treatment and sampling time ($P < 0.07$) was present for plasma corticosterone concentration. Corticosterone values in control birds were higher ($P < 0.10$) at 16:00 h (coincident with oviposition) than at 00:00 and 20:00 h. In each of the other groups the highest corticosterone concentration differed ($P < 0.10$) from the lowest level.

Discussion

Exposure of reproductively active female bobwhite quail to parathion for 10 days decreased egg production (≥ 50 p.p.m. parathion) and inhibited follicular development (≥ 100 p.p.m. parathion). Parathion exposure diminished food intake, reduced body weight, and inhibited brain acetylcholinesterase activity in a dose-dependent manner. It is well known that starvation causes ovarian atrophy, reduced gonadotrophin secretion, and the cessation of egg production in domestic fowl (Morris & Nalbandov, 1961; Brake & Thaxton, 1979; Tanabe, Ogawa & Nakamura, 1981); therefore, anorexia or food avoidance during parathion ingestion could have caused the observed effects on reproductive function. However, follicular development, egg production, and brain acetylcholinesterase activity were unaffected in pair-fed birds which had consumed reduced quantities of mash and lost body weight. Therefore, it would appear that the impairment of reproductive activity in birds receiving 100 p.p.m. parathion and with 50–60% inhibition of brain acetylcholinesterase activity is primarily due to the pharmacological action of

this organophosphate, although these results do not preclude some subtle interaction of parathion and an altered nutritional plane.

It was not the intention of this study to determine effects of organophosphates on the processes which control LH secretion; however, there is evidence to indicate that the observed reduction in LH levels could reflect changes in hypothalamo-hypophyseal function or steroid metabolism. Monoamines have been intimately linked with the regulation of gonadotrophin secretion in birds and mammals (Weiner & Ganong, 1978; El Halawani, Burke & Ogren, 1980). Nevertheless, cholinergic input may also be involved in the modulation of steroid feedback and hypothalamic control of gonadotrophin release as demonstrated in the rat (Muller *et al.*, 1977; Muth, Crowley & Jacobowitz, 1980; Ruiz de Galarreta *et al.*, 1981). In several avian species acetylcholinesterase activity is high in the diencephalon, median eminence and adenohypophysis relative to that in other brain regions (Aprison, Takahashi & Folkerth, 1964; Russell, 1968; Bursian & Edens, 1978). These neurochemical findings, and the inhibition of brain acetylcholinesterase activity accompanied by reduced plasma LH concentrations observed in the present study (i.e. birds fed 100 p.p.m. parathion), suggest an as yet uninvestigated role for cholinergic neurones in the control of gonadotrophin secretion in birds. Alternatively, inhibition of steroidogenesis (Civen *et al.*, 1977) or hepatic steroid metabolism (Conney *et al.*, 1967) could impair feedback control of gonadotrophin secretion and subsequent follicular development and ovulation.

Neither the 10-day exposure to 25 p.p.m. parathion (about 30% inhibition of brain acetylcholinesterase activity) nor the restriction of food intake (pair-fed group) altered follicular development or egg production, but the time of oviposition was more variable than in the control group. Xenobiotics such as polybrominated and polychlorinated biphenyls lengthen the reproductive cycle of mammals, presumably due to an alteration in the rate of steroid metabolism (Orberg & Kihlstrom, 1973; Lambrecht, Barsotti & Allen, 1978; Johnston, Demarest, McCormack, Hook & Moore, 1980). Underfeeding has also been demonstrated to lengthen the dioestrous period in rats (Mulinos & Pomerantz, 1940) by the inhibition of LH secretion (Howland, 1971) or possibly by changes in pituitary-adrenal and temperature rhythms (Krieger, 1974). It seems likely that low level dietary exposure to parathion or underfeeding may affect the duration of the ovulatory cycle or synchrony of oviposition. This would substantiate the dissimilarity of hormone profiles in the birds fed 25 p.p.m. parathion and the pair-fed birds to controls.

In conclusion, dietary exposure of bobwhite quail to 25 p.p.m. parathion may have subtle effects on the timing of oviposition, whereas higher concentrations inhibit egg production altogether. Since parathion has a relatively long environmental half-life compared to other organophosphates, judicious use in the vicinity of breeding wildlife species seems warranted.

We thank D. Andros, A. Gilliam, J. Haaser, J. Henry, C. Hillman, R. Knopp, F. Mersereau, H. Murray, C. O'Leary, K. Phelan and S. Watt for maintaining the animal colony and for providing assistance in analytical procedures, and C. Bunck and G. Hensler for advice and assistance in the statistical analyses.

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Received 16 November 1981