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Changes in the Reproductive Performance of the Japanese Quail fed Kepone in Different Calcium Diets

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Kepone (decachlorotetracyclodecanone) is a persistent insecticide which, during its manufacture, caused severe contamination of certain aquatic and terrestrial environments in Virginia. In addition, occupational exposure to Kepone produced human ailment not only in the workers, but in their family members as well while its wide dispersion in the environment affected various species of animals (U.S. Senate 1976, STERETT and BOSS 1977). Kepone is a chemical that is strikingly similar in structure to Mirex, also an insecticide which has been widely used in the southern United States for fire ant control. It is presently believed that at least 10 percent of Mirex present in the environment decomposes to Kepone (CARLSON et al. 1976, HOLDEN 1976). Previous experiments with Kepone indicated that a prolonged ingestion of the chemical in different calcium diets by the Japanese quail produced eggshell thinning, eggshell weakening, increased egg breakage and an interference in ovarian function. In addition, while eggshell weight was also reduced, certain egg dimensions from Kepone treated quail exceeded those of the control (EROSCHENKO and PLACE 1977, 1978). The present paper examines the detailed effects of prolonged Kepone ingestion on additional reproductive parameters of the Japanese quail fed different calcium diets.

METHODS AND MATERIALS

Five week old Japanese quail (Coturnix coturnix Japonica) were randomly separated into two groups, kept in individual wire-floor cages and exposed to 16 hours of light and 8 hours of darkness. The ambient room temperature was kept at about 23°C. A control group of 25 quail was first fed ad libitum a low calcium diet (0.5%) for the first 16 weeks followed by 16 weeks of high calcium diet (3.5%). A second group of 42 quail received similar sequences of diets contaminated with 200 ppm Kepone.

All birds were observed daily and egg production, egg weight and mortality rate of the Kepone treated quail were recorded. All eggs were collected daily before 5 p.m., labeled and presence of broken eggs recorded. The fresh weights of

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intact, broken and soft-shelled eggs were recorded separately on a Mettler balance to the nearest mg. After weight determinations, all eggs were cut open at the equator, their contents washed out and the eggshells with membranes dried at 55°C for two days. The weight of each type of shell was then recorded to the nearest mg. Eggs that were laid without a calcified shell, membrenous, were recorded separately but not weighed due to dehydration.

Weekly and monthly egg productions were determined for each group by dividing the total number of eggs produced during the given treatment period by the number of days each quail spent in the treatment period, thus accounting for any deaths of the quail as a result of Kepone toxicity. Clutch size was determined by recording the number of eggs laid by the quail in a sequence while the interval between a clutch was the number of lag days during which no oviposition took place.

All data are reported as means, unless otherwise noted. Statistical comparisons between means was made by using Student's t test, with corrections for unequal group size.

RESULTS AND DISCUSSION

Birds ingesting Kepone in low calcium diet (0.5%) during the first four weeks did not lag behind the control quail in the production of total and intact eggs. In fact, during certain weeks, egg production by Kepone fed quail exceeded that of the control. However, at five weeks, there was an irreversible decline in the production of intact eggs by the treated quail which reached a significant level by the end of the second month, whereas the total egg production was not significantly reduced until the end of the fourth month (Table 1, Fig. 1). Following the diet change to high calcium (3.5%) at the end of the fourth month, the production of broken eggs by the control quail was greatly reduced, whereas the egg production by the treated quail declined as the egg breakage, in comparison, increased (Table 1, Fig. 1).

In the past, it was shown that Kepone and certain isomers of DDT produced estrogenic alterations in the oviducts of Japanese quail (BITMAN et al. 1969, BITMAN 1969, EROSCHENKO and WILSON 1975). When estrogenic o,p'-DDT and p,p'-DDT were fed to Japanese quail in both low (0.56%) and high (2.7%) calcium diets, a lag in egg production was recorded during the first three weeks, suggesting a delay in ovulation (BITMAN et al. 1969, BITMAN 1969, CECIL et al. 1971). In the present experiment, Kepone also interferred with egg laying, however, the lag in egg production was only noted after a prolonged ingestion of the chemical and not at start of the experiment. Continued Kepone ingestion caused a significant change in the mean clutch size and in the intervals between egg laying (Table 1). In fact, interference with egg laying was apparently so frequent that the treated quail laid twice the number of clutches containing only one egg and half the number containing four or more eggs in comparison to the control birds (Fig. 4). Interference with ovulation and ovarian function has been previously suggested after post-mortem examination of quail that died of Kepone intoxication. In all birds the oviducts were enlarged and the ovaries either contained many large, yolk-filled follicles or the ovaries were totally regressed (EROSCHENKO and PLACE 1977). It is believed that the estrogenic action of Kepone, reported previously in the Japanese quail (EROSCHENKO and WILSON 1975) was probably responsible for the decreased clutch size, increased interval between egg laying, delayed ovulation and the ovarian regression. The eventual interference with the ovarian function by Kepone may have involved the secretion and release of gonadotropic hormones by the pituitary gland which are essential for proper follicular formation, growth and ovulation.

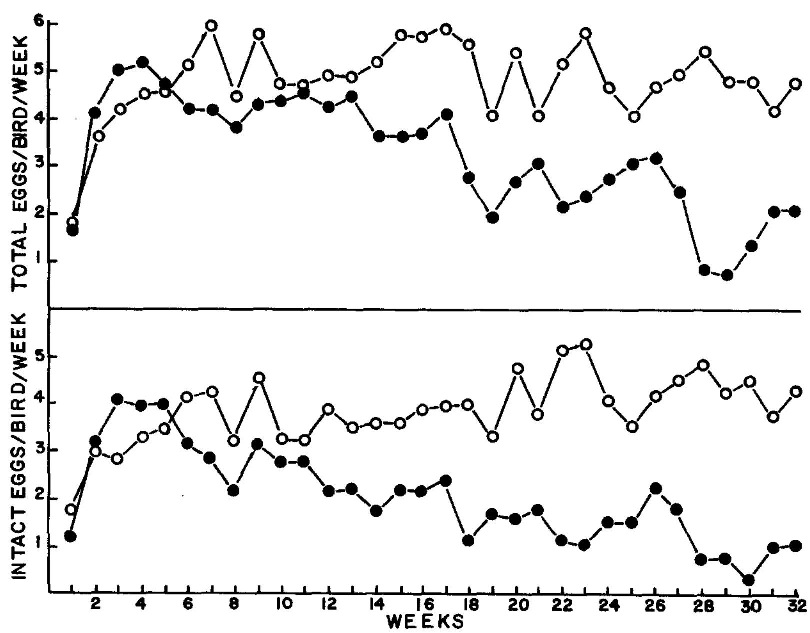

Fig. 1 Weekly egg production during low (0-16 weeks) and high (16-32 weeks) calcium diets (0-0 control; ●-● Kepone).

During the first three months of low calcium diet, the weight of the intact control eggs increased weekly and became significantly heavier; however, by the end of the fourth month, the eggs laid by the Kepone treated quail were significantly heavier. In contrast to the egg weight changes, the shells from the control intact eggs remained significantly heavier during most of the experiment (Table 1, Fig. 2). The increased weight of the eggs laid by the Kepone treated quail was comparable to similar changes reported previously for the egg dimensions. However, as the egg weight and dimensions increased, the physical eggshell strength and the actual eggshell thickness decreased significantly and irreversibly during continued Kepone ingestion (EROSCHENKO and PLACE 1977, 1978). In addition, broken eggs and shells produced by the treated quail were consistently and significantly lighter during both calcium diets (Table 1, Fig. 3), which correlated closely with the decreased eggshell thickness of similar eggs reported earlier (EROSCHENKO and PLACE 1977). In its passage through the avian oviduct, the egg spends most of its time in the shell gland where the total mass of albumin is increased by the addition of water. The increased alteration in egg size and egg weight recorded in the eggs laid by the Kepone fed quail could have been due to the delayed oviposition and increased retention of the egg in the shell gland where additional uptake of water and ions can take place (GILBERT 1971). The increased production of eggs with inferior shells by the treated birds can also indicate that Kepone interfered with the different processes of calcium trans-

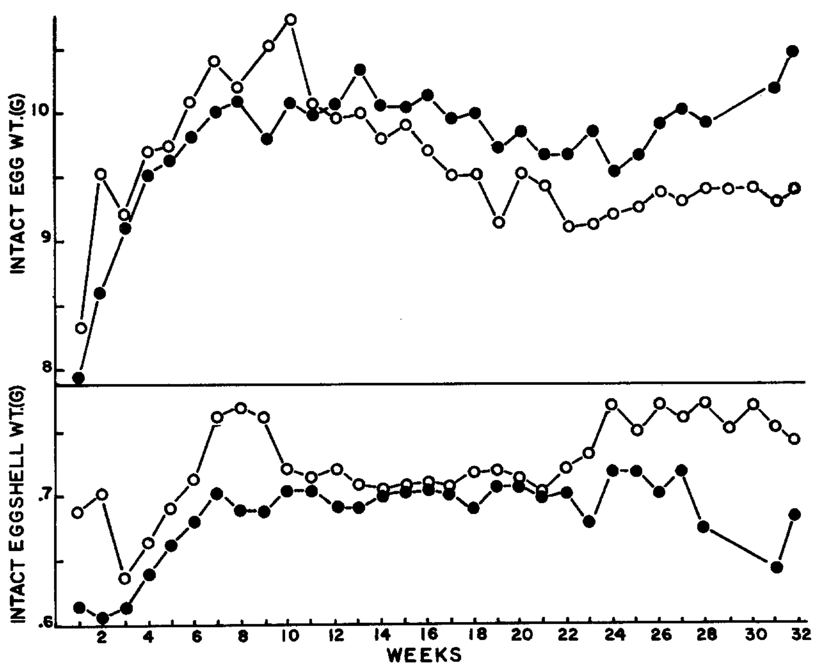

Fig. 2 Intact egg and eggshell weight produced during low (0-16 weeks) and high (16-32 weeks) calcium diets (O-O control; ●-● Kepone).

fer and its incorporation into eggshell formation since the ingestion of high dietary calcium did not alter the deliterious effects of Kepone on the eggshell formation that became evident during the low calcium diet. In fact, as ingestion of Kepone continued, more soft-shelled and membrenous eggs were laid during the high calcium diet (Tables 1 and 2). On the other hand, increased dietary levels of calcium did effect the control Japanese quail, since more intact eggs were laid with improved shell quaility. Since almost all soft-shelled and broken eggs were pigmented when collected, it is not believed that Kepone induced premature extrusion of the eggs, as has been suggested earlier (BITMAN et al. 1969). However, the increased production of membrenous eggs (Table 2) can be considered as additional evidence for interference of Kepone with proper calcification process in eggshell formation.

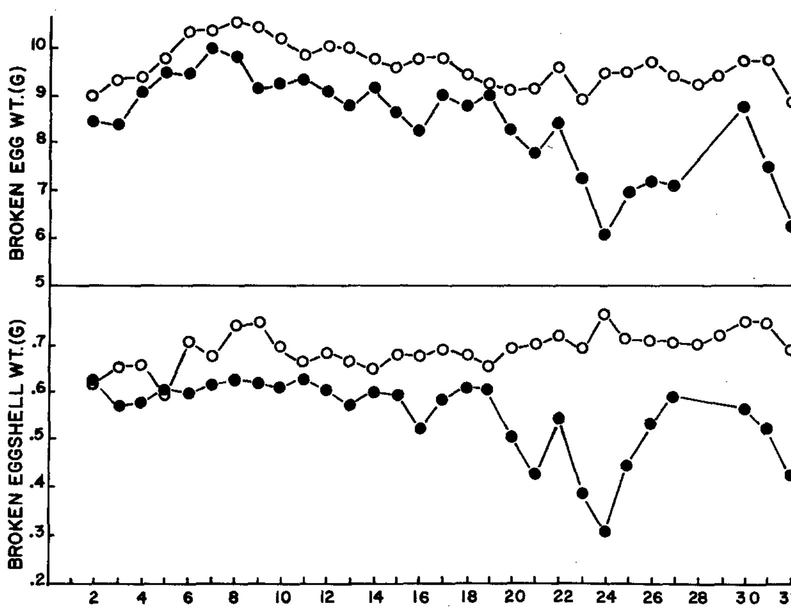

Fig. 3 Broken egg and eggshell weight produced during low (0-16 weeks) and high (16-32 weeks) calcium diets (0-0 control; ●-● Kepone).

In the past, it was shown that pesticide contents in the eggs reflected pesticide consumption, provided an important route for pesticide elimination and prolonged bird longevity (AZAVEDO et al. 1965, SMITH et al. 1969, CECIL et al. 1972, 1973). In this experiment, Kepone ingestion, egg laying and longevity appeared related (Table 1). However, once egg production decreased or ceased, the quail were probably deprived of an important route for Kepone elimination and greater body burdens of Kepone could have accumulated. Quail that either failed to lay eggs, exhibited increased intervals between egg-laying or ceased egg-laying completely, exhibited intoxication symptoms and died within an average of 17 days after laying the last egg.

Prolonged ingestion of Kepone by the Japanese quail produced various changes in their reproductive performance. Some of these changes became obvious early in the experiment, whereas others were recorded months later. One of the earlier and more important effects of Kepone on reproduction was the rapid decline in production of intact eggs. Such an effect could be of great ecological significance to various avian populations should a heavy and continous exposure to Kepone occur in their environment during the breeding season.

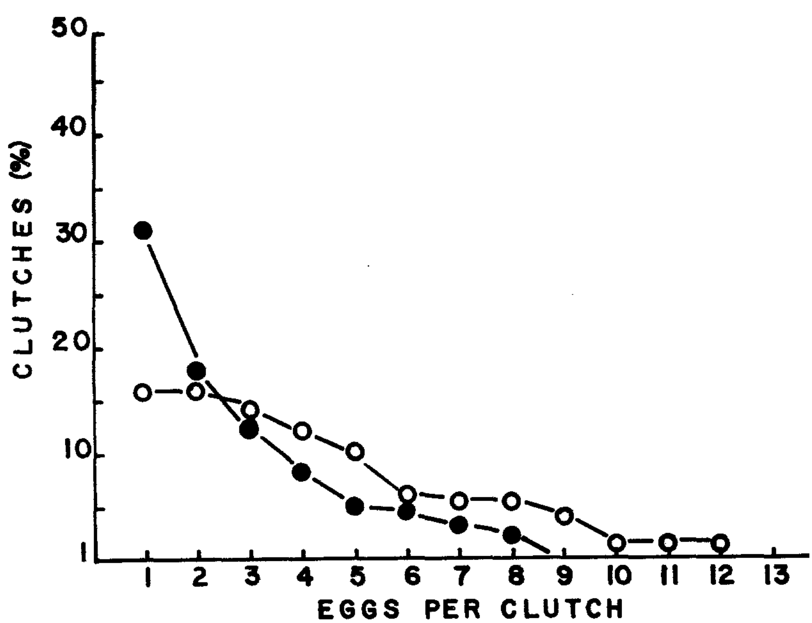

Fig. 4 Percent of clutches and number of eggs per clutch produced during the entire experiment (0-0 control; ●-● Kepone).

TABLE I

Monthly Effects of Dietary Calcium and Pesticide Kepone on Various Reproductive Parameters and Mortality Rate of the Japanese Quail

Parameters	Group	Month of Study 1	2	3	4	5	6	7	8
Mean Intact Egg Wht. (g)	C	9.40	10.13	10.53	9.85	9.42	9.20	9.34	9.35
	K	9.08[b]	9.82[c]	9.99[a]	10.19[c]	9.90[c]	9.56[c]	9.86[c]	9.98[b]
Mean Broken Egg Wht. (g)	C	9.25	10.22	10.08	9.72	9.31	9.20	9.38	9.25
	K	8.60[b]	9.62[c]	9.14[c]	8.72[c]	8.79[a]	7.31[c]	7.55[b]	7.31[b]
Mean Intact Eggshell Wht. (g)	C	.66	.73	.73	.70	.71	.74	.79	.75
	K	.62[b]	.68[c]	.70[c]	.70	.70	.70[c]	.71[c]	.68[c]
Mean Broken Eggshell Wht. (g)	C	.65	.67	.69	.66	.68	.75	.70	.72
	K	.58[b]	.61[c]	.61[c]	.57[c]	.58[c]	.41[c]	.51[c]	.50[c]
Total Eggs/Bird/Month	C	10.3	19.9	19.9	21.2	20.7	19.2	18.7	17.9
	K	13.7	16.0	16.7	14.5[c]	11.1[c]	9.2[c]	8.9[c]	5.4[c]
Mean Intact Eggs/Bird/Month	C	7.3	14.8	14.6	14.3	15.6	17.0	16.5	16.3
	K	10.5[a]	11.4[a]	10.2[b]	7.7[c]	5.9[c]	4.9[c]	5.5[c]	2.3[c]
Mean Broken Eggs/Bird/Month	C	2.9	5.6	5.2	6.9	5.1	2.2	2.2	1.7
	K	3.2	4.6	6.6	6.8	5.2	4.3	3.5	3.1
Mean No. Eggs/Clutch	C	4.2	4.8	4.6	5.7	4.4	4.9	4.8	4.1
	K	5.1	4.7	4.5	3.8[b]	2.8[c]	2.5[c]	2.7[b]	2.6[b]
Mean No. Days Between Egg Laying	C	1.9	2.0	2.1	1.5	2.2	1.8	2.1	2.6
	K	2.4	2.9	2.3	3.1[b]	3.5[a]	4.2[b]	5.4[c]	3.2
Mortality Rate (%)	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	K	0.0	28.6	40.5	54.8	66.7	76.2	85.7	90.5

a = Significantly different from control at P<.05
b = Significantly different from control at P<.01
c = Significantly different from control at P<.001
C = Control quail
K = Kepone treated quail

TABLE 2

Number of soft-shelled and non-calcified, membrenous, eggs laid during ingestion of different calcium diets and Kepone.

Group	Calcium Diet (%)	Soft-Shelled Eggs	Membrenous Eggs Laid	Total No. Eggs Laid	Membrenous Eggs (%)	Soft-Shelled Eggs (%)
Control	0.5	15	0	1151	0.00	0.01
Kepone	0.5	107	33	1977	0.02	0.05
Control	3.5	3	1	1297	0.00	0.00
Kepone	3.5	50	25	517	0.05	0.10

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