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CONTINUOUS INGESTION OF DIFFERENT CHLORDECONE (KEPONE) CONCENTRATIONS AND CHANGES IN QUAIL REPRODUCTION

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To determine the effects of continuous ingestion of different chlordecone concentrations on quail reproduction and mortality, 5-wk-old female Japanese quail were fed a diet containing 10, 40, 80, and 160 ppm chlordecone for exactly 250 d. Continuous ingestion of 10–80 ppm chlordecone did not induce excessive mortality or significantly alter reproductive functions. Similar ingestion of 160 ppm chlordecone increased quail mortality, produced a lag in egg production, and affected the normal sequence of egg laying. Increased mortality occurred in quail that either did not initiate egg laying or ceased egg laying completely. Reproductive tracts of dead quail were noticeably reduced and the ovaries atrophied. Quail that continued to ingest the highest chlordecone dose produced significantly more defective eggs, which were either cracked, soft-shelled, or shell-less.

INTRODUCTION

In the past, chlordecone (Kepone)[1] was a minor pesticide that was not extensively used in the United States. Most of the insecticide was exported, and the small amounts retained in the United States were used to formulate poison traps for ants and roaches. However, within the last 5 yr, chlordecone pollution became a problem that affected both animals and humans. Initially, the source of chlordecone contamination was a manufacturing site in Hopewell, Va., that polluted the James River–Chesapeake Bay system in 1975 (Sterrett and Boss, 1977). The present-day source of chlordecone pollution is photodegradation of Mirex, a pesticide that was extensively used in the southeastern United States to control fire ants (Carlson et al., 1976; National Research Council, 1978).

Since humans have been exposed to chlordecone contamination, numerous reports are available concerning the epidemiology of chlor-

[1] Chlordecone is the common name for the chlorinated pesticide decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one, manufactured under the trade name of Kepone.

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decone poisoning, its treatment, its elimination from the body, and its adverse action in human organs (Cannon et al., 1978; Boylan et al., 1978; Guzelian et al., 1980). In addition, numerous biochemical studies with chlordecone indicate altered mitochondrial enzymatic activity and energy metabolism in different cells (Desaiah et al., 1977a, 1977b; Carmines et al., 1979). On the other hand, there are few reports concerning the effects of continuous ingestion of different chlordecone concentrations on mammalian or avian reproduction. Since environmental contamination with chlordecone can vary, such studies are important.

Earlier reports from this laboratory showed that ingestion of high chlordecone doses not only increased mortality, but also significantly reduced reproductive functions in quail (Eroschenko, 1979a). The present report describes the effects of prolonged ingestion of various chlordecone concentrations on Japanese quail reproduction.

METHODS AND MATERIALS

Five-week-old Japanese quail (*Coturnix coturnix japonica*) were randomly separated into five groups, placed into individual wire-floor cages, and exposed to 16 h of light. The room was air-conditioned and the ambient temperature remained at 22 ± 1°C.

The control group of 17 birds was fed a standard laying diet mixed with sesame oil (10 ml/kg). The remaining four groups, consisting of 14, 19, 20, and 48 birds, were fed the same diet contaminated with 10, 40, 80, and 160 ppm chlordecone, respectively. Chlordecone was first suspended in sesame oil (10 ml/kg) and then thoroughly mixed into feed with an electric mixer. Chlordecone-contaminated and control diets were administered for exactly 250 d. All birds received feed and water *ad libitum* and were checked daily for signs of illness or mortality.

Individual records were kept for each bird. All eggs were collected daily before 5 p.m., visually inspected for damage, and recorded as either intact or broken. Broken eggs were classified as soft-shelled when a partially calcified eggshell was present or as shell-less when egg contents completely lacked a calcified eggshell.

Egg production, condition of eggs, and clutch size for each group were calculated every 50 d. Mean egg production for each category was determined by dividing the total number of eggs laid by the number of quail in the group, and clutch sizes were calculated by recording the number of eggs laid by quail in a sequence. Quail that died during these intervals were not included in any calculations.

All birds that died during the experiment were necropsied and the condition of their reproductive organs recorded. Duncan's multiple range test was used to determine the significant differences between the means of different reproductive parameters for all groups.

RESULTS

Mortality rate in the controls and the groups ingesting 10, 40, or 80 ppm chlordecone remained variable. However, continuous ingestion of 160 ppm chlordecone decreased quail longevity, and by the end of the experiment 31 quail died. Fourteen quail died without initiating egg laying within an average period of 34 d, while another 13 quail died within an average period of 48.2 d after egg laying ceased. Death of the remaining four quail was not associated with either inhibition or interruption of egg laying (Table 1). Postmortem examination showed that in 24 of 27 quail, the oviducts were reduced and the atrophied ovaries lacked follicular development. In the remaining three quail, the oviducts were enlarged with impacted egg yolks, while the ovaries exhibited numerous preovulatory follicles.

Ingestion of low to intermediate (10–80 ppm) chlordecone doses for 250 d did not significantly alter total egg production during any of the measured intervals. On the other hand, except for a significant decrease in total egg production during the first 50 d of the experiment, the observed effects of 160 ppm chlordecone ingestion were primarily on egg quality. The effects of chlordecone ingestion on the type of eggs laid by quail in all groups during every measured interval are summarized and statistically compared in Table 1. Although there were some variations in the means, differences in average clutch size and numbers of intact or cracked eggs between quail in the control and the first three groups ingesting chlordecone remained insignificant. The only significant change in every measured parameter was seen in quail ingesting 160 ppm chlordecone. From the start to the end of the 250-d experiment, the quality of eggs produced in this group remained consistently and significantly different from that of all other groups (Table 1). In addition, quail ingesting 160 ppm chlordecone for the first 50 d laid a significantly smaller ($p < 0.05$) clutch size of 4.2 eggs, compared to the control clutch size of 7.1 eggs.

DISCUSSION

The results of this study extend our previous work and demonstrate that chronic ingestion of chlordecone affects quail reproduction and longevity in a dose-dependent manner.

Continuous ingestion of low to intermediate doses of chlordecone did not significantly alter quail reproduction or induce excessive mortality. In contrast, ingestion of 160 ppm chlordecone produced high mortality and significantly altered quail reproduction through inhibition or cessation of egg laying as well as through increased production of defective eggs. Mortality was increased among quail that either did not initiate egg laying or ceased egg laying completely. Interrupting normal egg laying can deprive the birds of an effective means of eliminating chlordecone from

TABLE 1. Effects of Continuous Chlordecone Ingestion on Mean Number of Intact, Cracked, and Soft-shelled or Shell-less Eggs Laid per Quail during Various Treatment Intervals[a,b]

	Time interval (d)															
	1-50			51-100			101-150			151-200			201-250			Mortality (alive/dead)
Treatment	I	C	SS	I	C	SS	I	C	SS	I	C	SS	I	C	SS	1-250
Control	24.2^{a}	4.7^{b}	0.2^{b}	28.0^{a}	6.9^{b}	0.1^{b}	29.5^{a}	7.2^{b}	0.1^{b}	28.9^{a}	7.6^{b}	0.5^{b}	26.5^{a}	10.2^{b}	2.3^{b}	17/4
10 ppm	23.7^{a}	5.2^{b}	0.4^{b}	28.9^{a}	6.4^{b}	0.3^{b}	31.1^{a}	$8.7^{b,c}$	0.5^{b}	29.8^{a}	13.1^{b}	0.8^{b}	26.6^{a}	13.9^{b}	1.6^{b}	14/3
40 ppm	21.5^{a}	5.5^{b}	0.2^{b}	28.8^{a}	9.0^{b}	0.8^{b}	$26.5^{a,b}$	$14.6^{a,b}$	2.6^{b}	26.9^{a}	13.5^{b}	1.6^{b}	22.0^{a}	17.0^{b}	4.1^{b}	19/0
80 ppm	20.4^{a}	4.7^{b}	0.3^{b}	30.6^{a}	9.1^{b}	0.8^{b}	32.1^{a}	$12.0^{b,c}$	1.0^{b}	26.6^{a}	13.7^{b}	2.2^{b}	17.5^{a}	18.9^{b}	3.1^{b}	20/2
160 ppm	9.8^{b}	10.1^{a}	1.8^{a}	19.4^{b}	13.5^{a}	2.1^{a}	19.7^{b}	19.8^{a}	5.6^{a}	9.0^{b}	26.3^{a}	6.3^{a}	3.4^{b}	28.9^{a}	9.7^{a}	48/31

[a]Means followed by different superscripts are significantly different at $p < 0.05$.
[b]Condition of eggs: I, intact; C, cracked; SS, soft-shelled, shell-less. Soft-shelled and shell-less eggs were recorded as a separate category of cracked eggs.

their systems, since chlordecone can be eliminated from the body in egg yolk. Eggs laid by hens ingesting 75–150 ppm chlordecone daily and eggs collected from ospreys and bald eagles that consumed fish from the chlordecone-contaminated James River–Chesapeake Bay Tidewater area contained increased chlordecone residues in their yolk (Naber and Ware, 1965; Stafford et al., 1978). It was also shown that continuous ingestion of a higher chlordecone dose (200 ppm) produced the same inhibitory effect on quail reproductive functions; however, the time of death was reduced to 17 d after egg laying ceased (Eroschenko and Place, 1977; Eroschenko, 1979a).

The mechanism of action of higher chlordecone doses on quail reproduction is not known; however, it may involve the potent estrogenicity of chlordecone in birds and mammals (Eroschenko and Palmiter, 1980). Chlordecone doses exceeding 80 ppm inhibited follicular development in some quail and prevented egg transport as well as egg laying in others. Production of smaller clutch sizes and a significant lag in egg production suggest a delay in ovulation and interference with ovarian function. In earlier experiments, feeding of DDT analogs to Japanese quail (Bitman et al., 1969; Cecil et al., 1971) or to Bengalese finches (Jefferies, 1967) produced a similar delay in ovulation and a lag in egg production. Since these DDT analogs also showed estrogenic activity in the reproductive tracts of different species (Bitman et al., 1968; Welch et al., 1969), it was postulated that DDT affected the function of the pituitary gland or the hypothalamus (Jefferies, 1967). It is possible that estrogenic action of the highest chlordecone dose influenced quail reproduction in a fashion similar to that reported for DDT.

Quail that reached sexual maturation and remained active egg layers after ingesting 160 ppm chlordecone for a prolonged period continued to produce significantly more defective eggs. This may indicate that chlordecone also influences the function of cells in the shell gland of the quail oviducts that are responsible for eggshell formation. Experiments with DDT showed that chronic ingestion of the chemical by mallard ducks produced abnormal changes and swelling of the cells in the shell gland epithelium. Since these cells are believed to transport Ca, it was suggested that the recorded abnormalities indicated decreased Ca transport, which in turn might cause the DDT-induced eggshell thinning (Koloja and Hinton, 1978). We also showed that ingestion of different doses of chlordecone produced abnormal cell swelling in the luminal epithelium of immature quail oviducts (Eroschenko, 1979b). Since the abnormality occurred in the shell gland cells of the oviduct, it is possible that chlordecone had a similar effect on such cell functions in adult quail, which could have resulted in the production of significantly more defective eggshells.

CONCLUSION

In a controlled laboratory environment, continuous ingestion of chlordecone concentrations in excess of 80 ppm affected quail reproduction

and longevity. Delay in ovulation, a lag in egg production, inhibition or cessation of egg laying, and increased production of defective eggs indicate that the high chlordecone dosage interfered with the functioning of the quail reproductive system.

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