The Prolonged Exposure of Japanese Quail to Carbaryl and its Effects on Growth and Reproductive Parameters[1,2]

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INTRODUCTION

Carbaryl (1-naphthyl N-methyl carbamate) is a broad-spectrum carbamate insecticide which acts as a reversible acetylcholinesterase inhibitor. It has gained acceptance in animal agriculture as an effective agent for the control of ectoparasites while displaying an apparent low toxicity. However, studies have been conducted with some species of birds and mammals employing various exposure regimes which indicate that carbaryl can result in adverse effects.

Carbaryl injected into the yolk sac of various avian species results in decreased hatchability and teratogenesis (GHADIRI and GREENWOOD, 1966; KHERA, 1966; PROCTOR and CASIDA, 1975). Chronic administration of carbaryl caused growth depression, increased liver, kidney and adrenal weights, and alterations in the reproductive system of rats (CARPENTER et al., 1961; RYBAKOVA, 1966). Carbaryl fed to laying hens and roosters over a three week period resulted in a dose related decrease in hatchability and in embryonic deformities (GHADIRI et al., 1967). NIR et al. (1966) observed a depression in growth and decreased egg production in chickens fed carbaryl over a five week period but these effects occurred at an exposure level equivalent to 15 times that used by GHADIRI et al. (1967). LILLIE (1973) fed carbaryl to 32-week-old pullets and observed a significant decrease in adjusted body weight gains after 36 weeks of exposure. Progeny from this population also displayed a significant growth depression after 4 weeks of exposure. No effects on fertility, hatchability, or incidence of embryonic abnormalities were observed. SHERMAN and ROSS (1969) reported that the Japanese quail is more susceptible to carbofuran and SD8530 (carbamate insecticides) than the white Leghorn.

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The present report describes the effects of prolonged exposure to carbaryl on body weights, organ weights, and reproductive parameters in two generations of Japanese quail (*Coturnix coturnix japonica*).

METHODS

Random Bred Japanese quail were used throughout the study. Upon hatching, the chicks which were to be the parent (P) population were assigned randomly to seven different carbaryl levels and housed accordingly in brooders through five weeks of age. From week 6 through week 14 the birds were maintained in breeder cages, each containing a male and a female. The birds were maintained on a Light:Dark schedule of 24:0 for the first week, 10:14 for the second and third weeks, and a one hour increase in light per week thereafter until a 14:10 schedule was reached. The diet from the day of hatching up to six weeks of age consisted of a standard quail starter-grower ration. The birds were then provided with a standard quail layer ration for the remaining eight weeks. Both feed and water were available *ad libitum*.

Carbaryl (technical grade, 99% supplied by Union Carbide, New York, NY 10017) was dry mixed in the feed at dose levels of 0, 50, 150, 300, 600, 900 and 1200 mg./kg. of feed (expressed as ppm) and provided from the day of hatching through 14 weeks of age. Diets containing carbaryl were prepared every two weeks.

At the end of each week for the first six weeks and at the end of week 14, ten birds of both sexes from each treatment group were weighed, bled via cardiac stab, killed by cervical dislocation and necropsied. The brain, heart, liver, kidney, adrenals and testes were removed and weighed.

After week 6 the remaining birds within each dose group were paired in breeding cages and reproductive parameters were measured. From weeks 7 through 10 eggs were collected daily and set at one week intervals. Fertility and hatchability of all eggs were determined 17 days after the eggs were set. The number of chicks that hatched was noted and the unhatched eggs were characterized as follows: live pip (pipped eggs containing a live chick); live in shell (unpipped eggs containing a live chick); dead pip (pipped egg containing a dead chick); early dead (embryo less than 10 days old); late dead (embryo more than 10 days old); and infertile.

Chicks obtained from the last hatch (Hatch 4) were designated as the F_1 population. They were transferred to brooders and placed on the same carbaryl treatment as their parents. At the end of three weeks and six weeks, six birds of both sexes from each treatment group were weighed and necropsied. Reproductive parameters were not examined in the F_1 birds.

The experiment was conducted in duplicate and the statistical analyses were performed on the combined replicates. Analyses of variance were calculated for all the data using the procedures of the Statistical Analysis System (SERVICE, 1972). The LSD was calculated to test for differences between treatment means when the F statistic was significant. Data from the males and females were analyzed separately. Those data expressed as percent (egg production and hatchability) were transformed to arcsin values prior to analysis (SNEDECOR and COCKRAN, 1969). Data in the tables dealing with body and organ weights are presented as the mean over 14 weeks (P population) or six weeks (F_1 population) with a single standard error for each parameter since the type of analysis employed used an average standard error over all levels.

RESULTS

Effects on Growth. The effects of prolonged ingestion of carbaryl on body weights and relative weights of brain, heart, liver, kidney and adrenals in the female, as well as relative testes weights in the male for the P and F_1 generations are summarized in Tables 1 and 2. Significant differences between treatments and controls were found for body weights and relative weights of brain, liver, and kidney in the P males over the 14 week feeding period (Table 1). Feeding levels of 900 and 1200 ppm resulted in decreased body weights and increased relative brain weights. Relative liver weights were significantly higher than control at the 900 ppm level and approached significance at the 1200 ppm level. Relative kidney weights were significantly elevated when compared to controls at the 150, 600, 900 and 1200 ppm levels.

TABLE 1.

Effect of prolonged administration of carbaryl on body weights and relative brain, heart, liver, kidney, testes and adrenal weights in P and F_1 male Japanese quail.

Population and Length of Exposure	Level (ppm)	Mean Body Wt. (gms)	Relative Organ Weights (mg/100 gm body weight) Brain	Heart	Liver	Kidney	Adrenal	Testes
P (14 weeks)	0	78 ± 1[1]^{a}[2]	1076 ± 25^{b}	1036 ± 16^{a}	2555 ± 54^{b}	462 ± 13^{b}	15 ± 1^{a}	1138 ± 75^{a}
	50	81^{a}	1081^{b}	1043^{a}	2460^{b}	451^{b}	15^{a}	1024^{a}
	150	79^{a}	1118^{b}	1052^{a}	2558^{b}	505^{a}	17^{a}	1060^{a}
	300	79^{a}	1119^{b}	1008^{a}	2515^{b}	480^{b}	15^{a}	944^{a}
	600	78^{a}	1110^{b}	1032^{a}	2624^{ab}	501^{a}	15^{a}	1083^{a}
	900	73^{b}	1203^{a}	1012^{a}	2762^{a}	502^{a}	16^{a}	1001^{a}
	1200	73^{b}	1197^{a}	1023^{a}	2682^{ab}	512^{a}	17^{a}	1027^{a}
F_1 (6 weeks)	0	81 ± 25^{a}	926 ± 91^{a}	930 ± 41^{a}	2421 ± 94^{a}	472 ± 21^{a}	17 ± 1^{a}	497 ± 102^{a}
	50	76^{a}	954^{a}	932^{a}	2398^{a}	444^{a}	15^{a}	765^{a}
	150	81^{a}	912^{a}	880^{a}	2346^{a}	439^{a}	15^{a}	710^{a}
	300	77^{a}	954^{a}	936^{a}	2438^{a}	488^{a}	15^{a}	714^{a}
	600	87^{a}	857^{a}	893^{a}	2373^{a}	469^{a}	14^{a}	646^{a}
	900	74^{a}	1006^{a}	955^{a}	2318^{a}	442^{a}	16^{a}	615^{a}
	1200	73^{a}	1125^{a}	921^{a}	2480^{a}	487^{a}	14^{a}	354^{a}

[1] Mean (over 14 or 6 weeks) ± average standard error over all levels. The n for the P population is 70 birds per dose group while the n for the F_1 population is 12 birds per dose group.

[2] Means with different superscripts are significantly different at the 5% level of probability.

Significant reductions in P female body weights over 14 weeks were found only at the 900 ppm level (Table 2). The quail fed 900 and 1200 ppm carbaryl levels had significantly higher relative liver weights when compared to controls and the quail fed 600 and 1200 ppm levels had significantly elevated relative kidney weights.

TABLE 2.

Effect of prolonged administration of carbaryl on body weights and relative brain, heart, liver, kidney, and adrenal weights in P and F_1 female Japanese quail.

Population and Length of Exposure	Level (ppm)	Mean Body Wt. (gms)	Relative Organ Weights (mg/100 gm body weight)				
			Brain	Heart	Liver	Kidney	Adrenal
	0	85 ± 1^{1a2}	1042 ± 25^{a}	994 ± 14^{a}	2813 ± 61^{b}	497 ± 10^{b}	15 ± 1^{a}
	50	87^{a}	1037^{a}	961^{a}	2822^{b}	478^{b}	14^{a}
	150	87^{a}	1051^{a}	963^{a}	2833^{b}	492^{b}	14^{a}
P	300	82^{ab}	1091^{a}	1008^{a}	2770^{b}	506^{ab}	14^{a}
(14 weeks)	600	87^{a}	1053^{a}	982^{a}	2950^{ab}	527^{a}	15^{a}
	900	78^{b}	1119^{a}	959^{a}	3001^{a}	521^{ab}	16^{a}
	1200	81^{ab}	1080^{a}	971^{a}	3003^{a}	534^{a}	15^{a}
	0	87 ± 5^{a}	891 ± 48^{a}	935 ± 45^{a}	2365 ± 168^{a}	472 ± 16^{a}	14 ± 1^{a}
	50	79^{a}	915^{a}	899^{a}	2500^{a}	463^{a}	12^{a}
	150	87^{a}	838^{a}	935^{a}	2351^{a}	465^{a}	13^{a}
F_1	300	87^{a}	869^{a}	863^{a}	2312^{a}	503^{a}	13^{a}
(6 weeks)	600	96^{a}	789^{a}	874^{a}	2855^{a}	476^{a}	12^{a}
	900	83^{a}	904^{a}	949^{a}	2755^{a}	495^{a}	13^{a}
	1200	80^{a}	976^{a}	995^{a}	2790^{a}	510^{a}	14^{a}

[1]Mean (over 14 or 6 weeks) ± average standard error over all levels. The n for the P population is 70 birds per dose group while the n for the F_1 population is 12 birds per dose group.

[2]Means with different superscripts are significantly different at the 5% level of probability.

There were no significant differences in any of the growth parameters measured in the F_1 males or females which were fed carbaryl over a six week period (Tables 1 and 2). However, in the F_1 males and females there was a general trend toward decreased body weights and increased relative brain weights at the higher dose levels, and in the F_1 females given the higher dose levels relative weights of liver and kidney tended to be increased. The feeding of up to 1200 ppm carbaryl for 14 weeks in the P birds and for 6 weeks in the F_1 birds did not produce significant differences in mortality.

Effects on Reproduction. Egg production and hatchability data are presented in Table 3. There were no significant differences in egg production among treatment groups although there appeared to be a slight decrease in production at the 600, 900 and 1200 ppm levels. Similarly, there were no significant differences among treatments in the hatchability parameters measured. However, the 600, 900, and 1200 ppm levels resulted in a slightly lower percentage of total eggs hatched with a concomitant increase in the percentage of infertile eggs laid. Gross teratogenic effects were not observed.

TABLE 3.

Effect of prolonged administration of carbaryl on egg production and hatchability of eggs of Japanese quail.

Level (ppm)	Egg Production (hen-day) %	Hatch of Eggs Set %	Live Pip %	Live in Shell %	Dead Pip %	Early Dead %	Late Dead %	Infertile %	Hatch of Fertile Eggs %
0	60.7±3.4[a][1]	69.8±3.7[a]	5.5±1.7[a]	1.4±1.0[a]	0.3±0.2[a]	5.7±1.0[a]	5.0±0.7[a]	12.3±2.7[a]	79.6±3.4[a]
50	71.4±3.0[a]	70.4±4.8[a]	5.8±1.2[a]	1.6±0.8[a]	0.2±0.1[a]	5.0±0.9[a]	4.8±1.1[a]	12.2±2.5[a]	79.6±3.8[a]
150	64.4±3.4[a]	69.3±3.6[a]	7.4±2.0[a]	2.4±0.7[a]	0.1±0.1[a]	4.9±1.6[a]	3.0±0.8[a]	12.9±1.5[a]	79.3±3.2[a]
300	63.7±3.7[a]	68.3±3.0[a]	6.6±1.8[a]	1.5±0.4[a]	0.3±0.2[a]	6.6±1.8[a]	4.1±0.9[a]	12.8±1.7[a]	78.1±2.6[a]
600	58.5±2.7[a]	63.1±2.6[a]	8.9±2.0[a]	0.5±0.2[a]	0.0±0.0[a]	5.4±1.6[a]	4.6±1.4[a]	17.5±1.7[a]	76.5±2.5[a]
900	54.7±3.4[a]	63.8±3.6[a]	5.1±0.9[a]	0.9±0.5[a]	0.5±0.3[a]	5.3±1.7[a]	4.6±1.0[a]	19.9±3.4[a]	79.7±3.0[a]
1200	54.3±3.9[a]	62.3±3.5[a]	4.7±1.0[a]	2.0±1.3[a]	0.3±0.3[a]	6.2±1.8[a]	4.9±0.5[a]	19.6±3.2[a]	77.4±2.8[a]

[1]Means ± S.E.M. with different superscripts are significantly different at the 5% level of probability.

DISCUSSION

Growth depression and increases in relative liver and kidney weights appear to be among the usual gross changes found in animals exposed to carbaryl over extended periods of time. In this respect the present study is in general agreement with other feeding studies involving carbaryl. However, certain differences in the levels at which effects appear are apparent. Perhaps this reflects differences in the methods of administration of the compound, feed consumption/kg. body weight/day and/or the length of the exposure regime. In order to take meaningful comparisons of data from various sources, an estimation of the actual quantity of toxic compound ingested in relation to body weight is necessary.

While feed consumption in this study was not determined it has been estimated (MARKS, personal communication) that the average feed consumption of Japanese quail is 12 to 13 gms./day. Assuming an average body weight of 80 gms., the average food consumption would be approximately 15 gms./100 gms. body weight/ day. This feed intake would result in an average of 18.0, 13.5, 9.0, 4.5, 2.3 and 0.8 mg. carbaryl/100 gm. body weight/day being ingested at the 1200, 900, 600, 300, 150 and 50 ppm levels, respectively. The P population was exposed to these levels over a 98 day period while the F_1 population exposure was 42 days.

Table 4 presents a comparison among the present study and other carbaryl feeding studies involving both mammalian and avian species. The effective doses of carbaryl are expressed as mg. carbaryl/100 gm. body weight/day as estimated by the present authors based on average body weight and feed consumption data presented in the other studies.

TABLE 4.

A comparison of studies dealing with the effects of carbaryl administered over prolonged periods of time.

Species	Estimated Effective Dose (mg/100gm b.w./day)	Length of Exposure (days)	Effects[1] Growth Depression	Increased Relative Liver Wt.	Increased Relative Kidney Wt.	Increased Relative Adrenal Wt.	Reference
Rat	6	96	-	-	+ (F)		Carpenter *et al.* (1961)
	9	96	+ (F)[2]	-	+ (M F)		
	2	730	+ (M)				
Rat	8	50	-	+		+	Rybakova (1966)
	11	365	+			+	
Chicken	18	60	-				Nir *et al.* (1966)
	54	35 (100% mortality)	+				
Chicken	3	252	+				Lillie (1973)
	6	252	+				
	6	28 (progeny of above)	+				
Japanese Quail (present study)	18 (1200 ppm)	98	+ (M)	+ (M F)	+ (M F)	-	Present Study
	13.5 (900 ppm)	98	+ (M F)	+ (M F)	+ (M)	-	
	9 (600 ppm)	98	-	-	+ (F)	-	
up to	18	42 (progeny of above)	-	-	-	-	

[1]Indicated as + for change; - for no change; blanks indicate that no definitive statement was made concerning the parameter.

[2]M designates male; F designates female.

It appears that the rat is more sensitive to carbaryl than the Japanese quail in that growth depression was evident at 6 mg./100 gm. body weight/day after 96 days (CARPENTER et al., 1961) but the effect was seen in the Japanese quail only at levels of 13.5 and 18 mg. after 98 days. Kidney weights were elevated in both sexes of the rat by 9 mg. but Japanese quail displayed increased kidney weights in both sexes only at 18 mg. Neither the rat nor Japanese quail had increased liver weights at 9 mg. carbaryl/100 gm. body weight/day. The differences in effects could be accounted for by the inherently higher metabolic rate of avian species which could result in a faster detoxification and elimination of carbaryl.

Comparison of the present study with other avian feeding studies is made more difficult by the wide variation in exposure periods. LILLIE (1973) observed growth depression in chickens by levels as low as 3 mg. for 252 days in the parent population and 6 mg. for only 28 days in the progeny population. In the present study only levels of 13.5 mg. and above for 98 days in the P population resulted in growth depression while levels up to 18 mg. for 42 days in the F_1 population had no effect. However, NIR et al. (1966) observed no growth depression at 18 mg. over 60 days in chickens. It is possible that the differences in the length of the exposure regimes account for the variation in levels at which effects are seen.

While there were no significant differences in egg production or hatchability there was a slight decrease in egg production and percentage of total eggs hatching at doses equivalent to 18, 13.5 and 9 mg. carbaryl/100 gm. body weight/day with a concomitant increase in the percentage of infertile eggs laid. These results tend to agree with previous studies dealing with the reproductive effects of carbaryl on avian species. MCCAY and ARTHUR (1962) reported no differences in egg production of chickens when carbaryl was administered at an effective dose of 0.1 mg./100 gm. body weight/day for a seven day period. NIR et al. (1966) reported a complete cessation of egg production in white Leghorns at a level equivalent to 54 mg./100 gm. body weight/day over a 60 day period while a level equivalent to 18 mg. had no effect. LILLIE (1973) reported no embryonic abnormalities or changes in egg production in white Leghorns at levels up to 6 mg./100 gm. body weight/day for 252 days. With respect to reproductive parameters the Japanese quail is perhaps more sensitive to carbaryl than is the chicken.

It is apparent that the prolonged administration of carbaryl has various adverse effects on the Japanese quail but the causation of these effects is still a matter of speculation. Growth depression could be a result of decreased feed consumption or conversion. GEORGIEV (1967) reported that carbaryl administered at a level of 10 mg./100 gm. body weight/day for 45 days to rats resulted in a transient decrease in appetite and feed intake in addition to disturbances in enzyme secretion by the intestine. WILLS et al. (1968) stated that small doses of carbaryl administered to human subjects impaired the ability of the proximal convoluted tubule of the kidney to reabsorb amino acids. However, HASSAN and CUETO (1970) observed no effects on tubular reabsorption and protein and carbohydrate metabolism in rabbits administered small doses of carbaryl. Moreover, NIR et al. (1966) and LILLIE (1973) reported no differences in feed consumption in chickens fed doses of carbaryl which caused growth depression.

The increase in P male relative brain weights which occurred at the two highest levels was probably a result of normal brain development concomitant with the overall decrease in body weights. Increases in relative liver and kidney weights may reflect the major role that these organs play in the detoxification of foreign compounds. There is evidence for morphological alteration of the liver and kidney by carbaryl in other studies. RYBAKOVA (1966) reported a carbaryl induced fatty degeneration of liver cells and cloudy swelling of kidney tubular epithelium in rats. NIR et al. (1966) reported that fatty degeneration of the liver occurs in chickens fed high doses of carbaryl.

While the effects of carbaryl on reproductive parameters were not statistically significant in this study, trends were evident that would suggest that the reproductive capacity of the exposed birds was impaired to some extent by carbaryl. RYBAKOVA (1966) demonstrated a disruption of spermatogenesis and inhibition of the estrus cycle in rats exposed to carbaryl at a level equivalent to 11 mg. carbaryl/100 gm. body weight/day. Disturbances in the secretion of gonadotropic hormones from the adenohypophysis have been implicated as the reason for the reproductive alterations. It is possible that a similar situation existed in this study.

Studies dealing with the effects of carbaryl on cellular metabolic processes seemingly do not have a direct relationship to this study but cellular alterations could effect the organism as a whole and thus could conceivably account for gross changes in growth and reproductive parameters. BLEVINS and DUNN (1975) reported that carbaryl at levels of 1 and 2 ppm stimulated the division of HeLa cells (a malignant human cell line) in vitro while levels of 4 and 8 ppm inhibited cell division with a slight decrease in cellular protein composition at the 4 ppm level. The phospholipid composition of these cells was also altered by 2 and 4 ppm. WALKER et al. (1975) have reported that carbaryl resulted in a significant inhibition of the development of the Ehrlich ascites tumor in mice and reduced nucleic acid and protein synthesis in these cells in vitro. The manifestation of such effects in an organism's own cells could possibly alter its normal growth and reproductive capacity.

SUMMARY

Carbaryl fed to Japanese quail over a 14 week period resulted in decreased body weight, increased relative brain, liver and kidney weights in one or both sexes at levels of 900 ppm or higher. Significant differences were not found in the F_1 birds at the same levels after 6 weeks. It appears that the time of exposure is critical and that parental diet is not. There were no significant differences in egg production, hatchability or fertility although trends toward reduction of these parameters were developing at the 900 and 1200 ppm levels.

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