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The Prolonged Exposure of Japanese Quail to Carbaryl and Its Effects on Neurochemical and Blood Chemical Parameters[1,2]

S. J. Bursian[3] and F. W. Edens

Department of Poultry Science, North Carolina State University, Raleigh, N.C. 27650

Carbaryl (1-naphthyl N-methyl carbamate) is an extensively used insecticide which acts as a reversible inhibitor of acetylcholinesterase (AChE). BURSIAN and EDENS (1977) reported that the prolonged administration of carbaryl (via the feed for 14 wk) caused decreased body weight and increased relative brain, liver and kidney weights in one or both sexes of Japanese quail at levels of 900 or 1200 ppm. These effects appeared at levels higher and with an exposure regime longer than is required to produce similar effects in various mammalian species and other avian species. Reproductive parameters were also studied but no treatment effects were found.

Studies conducted with mammalian species indicate that exposure to carbaryl through various routes and for various periods of time can affect cholinesterase (ChE) activity, catecholamine and indolamine metabolism and plasma glucose levels. CARPENTER et al. (1961) reported that serial i.p. injections of carbaryl caused a transitory decrease in erythrocyte and plasma ChE activity in beagle dogs. RYBAKOVA (1966) and SHTENBERG and RYBAKOVA (1968) demonstrated progressive inhibition of serum ChE activity with acute and chronic oral administration of carbaryl in rats. RICHARDSON et al. (1975) found decreased erythrocyte ChE activity in humans occupationally exposed to a mixture of DDT, parathion and carbaryl, but WILLS et al. (1968) reported no such decrease in humans which were given low levels of carbaryl over a six wk period. HASSAN and CUETO (1970) demonstrated an increased urinary excretion of catecholamine and serotonin metabolites in rabbits after the administration of a combination of DDT, para-

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[3]On leave from the Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711.

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thion and carbaryl, and HASSAN (1971) reported similar results when carbaryl alone was given to rats. HASSAN and SANTOLUCITO (1971) have shown that a single oral dose of carbaryl in rats resulted in an increase in the brain levels of serotonin and its primary metabolite 5-hydroxy-3-indolyacetic acid. Conversely, RICHARDSON et al. (1975) stated that the plasma levels and excretion rates of epinephrine were significantly lower in their occupationally exposed group, while the plasma levels of norepinephrine and the excretion rates of norepinephrine and various catecholamine metabolites were not altered. WEISS et al. (1964,1965) and ORZEL and WEISS (1966) reported that carbaryl caused an elevation in blood sugar levels presumably as a result of increased cholingergic stimulation of the adrenal gland with a concomitant increased release of epinephrine.

The present report describes the effects of the prolonged administration of carbaryl on whole-brain AChE activity, whole-brain, heart and adrenal catecholamine levels and plasma glucose levels in Japanese quail. The Japanese quail used in this study were the same quail utilized in the previous study (BURSIAN and EDENS 1977). Since the body weight, organ weight and reproductive data indicated that the Japanese quail as affected less by carbaryl than mammalian species it was of interest to determine if a similar relationship existed using neurochemical and blood chemical parameters as indices of toxicosis.

METHODS

Random bred Japanese quail were used throughout the study and correspond to the present population described in a previous report (BURSIAN and EDENS 1977). Upon hatching, the chicks were assigned randomly to seven different carbaryl levels and housed accordingly in brooders through 5 wk 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 with a one hr increase in light per week thereafter until a 14:10 lighting schedule was reached. The diet from the day of hatching up to 6 wk of age consisted of a quail starter-grower ration. The birds were provided then with a quail layer ration for the remaining 8 wk. 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 ppm. The feed containing carbaryl was provided from the day of hatching through 14 wk of age and was prepared every 2 wk.

At the end of each week for the first 6 wk and at the end of 14 wk, ten birds of both sexes were randomly selected from each treatment group, weighed, bled via cardiac stab, killed by cervical dislocation and necropsied. The brain, heart and adrenals were removed, weighed and immediately frozen for subsequent neurochemical analysis.

Plasma glucose levels were determined by the method of DUBOWSKI (1962a, 1962b) and whole-brain AChE activity was determined using the technique of BERG and MAICKEL (1968). Whole-brain, heart and adrenal catecholamine levels were determined in the 0, 300, 600 and 1200 ppm groups at weeks 6 and 14. The catecholamines were isolated on aluminum oxide following the method of ANTON and SAYRE (1962). Norepinephrine (NE) and epinephrine (E) were assayed fluorometrically according to von EULER and LISHAJKO (1962) and dopamine (DA) by the method of BARCHAS et al. (1972).

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 test was significant.

RESULTS AND DISCUSSION

The effects of prolonged ingestion of carbaryl on whole-brain AChE activity, whole-brain, heart and adrenal catecholamine levels and plasma glucose levels in male Japanese quail are summarized in Table 1 through 3. Carbaryl had no significant effect on whole-brain, AChE activity (Table 1), whole-brain NE levels, heart NE levels, adrenal NE and E levels (Table 2) and plasma glucose levels (Table 3).

TABLE 1

Effect of prolonged administration of carbaryl on whole-brain acetylcholinesterase activities[+] in male Japanese quail.

Level ppm	Week 1	2	3	4	5	6	14
0	(3) .618±.018[a*]	(4) .538±.047[a]	(3) .645±.070[a]	(6) .606±.051[a]	(5) .585±.015[a]	(8) .955±.053[a]	(5) .611±.044[a]
50	(3) .615±.074[a]	(4) .659±.027[a]	(5) .541±.028[a]	(4) .584±.047[a]	(5) .503±.026[a]	(3) 1.037±169[a]	(5) .649±.024[a]
150	(4) .584±.054[a]	(3) .480±.029[a]	(3) .587±.080[a]	(7) .573±.034[a]	(5) .542±.021[a]	(6) .905±.071[a]	(5) .615±.051[a]
300	(4) .599±.069[a]	(6) .491±.047[a]	(6) .610±.041[a]	(3) .627±.032[a]	(5) .580±.070[a]	(5) .896±.062[a]	(5) .684±.022[a]
600	(2) .482±.093[a]	(6) .553±.033[a]	(7) .478±.027[a]	(2) .562±.063[a]	(3) .548±.027[a]	(5) .881±.033[a]	(5) .645±.073[a]
900	(4) .586±.042[a]	(3) .653±.040[a]	(5) .535±.081[a]	(3) .585±.055[a]	(5) .517±.021[a]	(4) .863±.076[a]	(5) .598±.021[a]
1200	(2) .471±.018[a]	(3) .527±.053[a]	(4) .613±.036[a]	(5) .610±.065[a]	(5) .589±.042[a]	(7) .870±.042[a]	(5) .587±.034[a]

[+]Expressed as millimoles of acetylcholine hydrolyzed/hr/gm.

[*]Mean ± standard error. Number in parentheses refers to n. Means with same superscripts are not significantly different.

Whole-brain DA levels were significantly elevated at the 600 and 1200 ppm levels at week 14 when compared to controls. Whole-brain AChE activities (Table 4), whole-brain, heart and adrenal catecholamine levels (Table 5), and plasma glucose levels (Table 6) were not significantly altered by carbaryl admin-

istration in female Japanese quail.

TABLE 2

Effect of prolonged administration of carbaryl on whole-brain norepinephrine and dopamine levels, heart norepinephrine levels and adrenal norepinephrine and epinephrine levels in male Japanese quail.

Week	Level ppm	Whole-Brain NE ng/gm	Whole-Brain DA ng/gm	Heart NE ng/gm	Adrenal NE µg/gm	Adrenal E µg/gm
		(5)	(5)	(5)	(5)	(5)
6	0	312±36[a*]	603±51[a]	343±93[a]	3618±860[a]	2672±451[a]
		(5)	(5)	(5)	(5)	(5)
6	300	368±36[a]	486±47[a]	421±55[a]	3977±233[a]	2419±278[a]
		(5)	(5)	(5)	(5)	(5)
6	600	398±51[a]	651±22[a]	466±37[a]	3891±191[a]	2184±230[a]
		(5)	(5)	(5)	(5)	(5)
6	1200	327±12[a]	648±38[a]	395±82[a]	3726±835[a]	2046±448[a]
		(5)	(5)	(5)	(5)	(5)
14	0	286±18[a]	283±61[a]	646±22[a]	2574±317[a]	1712±223[a]
		(5)	(5)	(5)	(5)	(5)
14	300	326±43[a]	445±88[a]	684±82[a]	2813±319[a]	1532±254[a]
		(6)	(6)	(6)	(6)	(6)
14	600	360±33[a]	585±55[b]	643±59[a]	2923±306[a]	1983±237[a]
		(5)	(5)	(5)	(5)	(5)
14	1200	323±68[a]	472±34[b]	706±71[a]	2573±233[a]	1559±157[a]

*Mean ± standard error. Number in parentheses refers to n. Means with same superscripts are not significantly different.

TABLE 3

Effect of prolonged administration of carbaryl on plasma glucose[+] in male Japanese quail.

Level ppm	Week 1	2	3	4	5	6	14
	(7)	(7)	(9)	(11)	(10)	(13)	(10)
0	251±29[a*]	294±19[a]	262±7[a]	329±11[a]	316±15[a]	357±12[a]	292±17[a]
	(7)	(9)	(7)	(9)	(9)	(8)	(10)
50	275±23[a]	281±18[a]	244±9[a]	310±13[a]	282±24[a]	367±14[a]	296±19[a]
	(9)	(6)	(7)	(13)	(11)	(11)	(10)
150	245±13[a]	285±17[a]	254±18[a]	317±10[a]	351±11[a]	342±9[a]	281±15[a]
	(6)	(11)	(11)	(10)	(9)	(10)	(10)
300	219±24[a]	266±12[a]	233±16[a]	294±11[a]	315±13[a]	342±16[a]	291±18[a]
	(7)	(10)	(10)	(8)	(11)	(12)	(11)
600	229±19[a]	273±15[a]	234±15[a]	296±10[a]	320±15[a]	333±10[a]	284±38[a]
	(7)	(5)	(7)	(7)	(8)	(10)	(10)
900	229±32[a]	269±23[a]	212±13[a]	259±8[a]	331±15[a]	334±16[a]	297±15[a]
	(7)	(5)	(11)	(9)	(9)	(12)	(10)
1200	283±23[a]	290±17[a]	230±14[a]	299±17[a]	332±11[a]	335±11[a]	282±17[a]

[+]Expressed as mg %.

*Mean ± standard error. Number in parentheses refers to n. Means with the same superscripts are not significantly different..

TABLE 4

Effect of prolonged administration of carbaryl on whole-brain acetylcholinesterase activities[+] in female Japanese quail.

Level ppm	Week 1	2	3	4	5	6	14
0	(7) .597±.026[a*]	(6) .604±.041[a]	(7) .558±.045[a]	(4) .625±.041[a]	(5) .593±.055[a]	(2) .800±.033[a]	(5) .570±.056[a]
50	(7) .596±.036[a]	(5) .535±.073[a]	(5) .539±.030[a]	(4) .554±.048[a]	(5) .550±.062[a]	(7) .916±.045[a]	(5) .579±.049[a]
150	(6) .600±.056[a]	(7) .543±.025[a]	(7) .593±.028[a]	(3) .613±.040[a]	(5) .567±.039[a]	(4) 1.049±.108[a]	(5) .688±.047[a]
300	(6) .561±.035[a]	(4) .537±.043[a]	(4) .455±.049[a]	(7) .533±.044[a]	(5) .597±.037[a]	(5) .912±.065[a]	(5) .694±.027[a]
600	(8) .595±.024[a]	(4) .539±.034[a]	(3) .581±.053[a]	(8) .585±.046[a]	(5) .490±.030[a]	(3) .914±.054[a]	(5) .742±.023[a]
900	(6) .565±.053[a]	(3) .562±.010[a]	(5) .594±.027[a]	(3) .552±.035[a]	(5) .569±.018[a]	(2) 1.003±.013[a]	(5) .614±.023[a]
1200	(8) .570±.033[a]	(7) .541±.036[a]	(6) .571±.048[a]	(5) .604±.026[a]	(5) .522±.073[a]	(3) .798±.023[a]	(5) .647±.039[a]

[+]Expressed as millimoles of acetylcholine hydrolyzed/hr/gm.

[*]Mean ± standard error. Number in parentheses refers to n. Means with same superscripts are not significantly different.

TABLE 5

Effect of prolonged administration of carbaryl on whole-brain norepinephrine and dopamine levels, heart norepinephrine levels and adrenal norepinephrine and epinephrine levels in female Japanese quail.

Week	Level ppm	Whole-Brain NE ng/gm	Whole-Brain DA ng/gm	Heart NE ng/gm	Adrenal NE µg/gm	Adrenal E µg/gm
6	0	(5) 421±30[a*]	(5) 601±72[a]	(5) 422±56[a]	(5) 3565±490[a]	(5) 2170±251[a]
6	300	(5) 378±16[a]	(5) 523±61[a]	(5) 319±56[a]	(5) 4181±322[a]	(5) 2498±136[a]
6	600	(5) 358±72[a]	(5) 523±50[a]	(5) 289±26[a]	(5) 2996±519[a]	(5) 1882±267[a]
6	1200	(5) 371±39[a]	(5) 496±60[a]	(5) 406±48[a]	(5) 4131±509[a]	(5) 2289±343[a]
14	0	(4) 476±66[a]	(4) 377±64[a]	(4) 608±59[a]	(4) 2029±386[a]	(4) 1300±162[a]
14	300	(5) 401±58[a]	(5) 404±121[a]	(5) 721±61[a]	(5) 2211±306[a]	(5) 1205±114[a]
14	600	(4) 348±40[a]	(4) 663±145[a]	(4) 603±89[a]	(4) 2420±510[a]	(4) 1307±147[a]
14	1200	(5) 333±23[a]	(5) 407±120[a]	(5) 705±115[a]	(5) 2695±216[a]	(5) 1515±177[a]

[*]Mean ± standard error. Number in parentheses refers to n. Means with the same superscripts are not significantly different.

TABLE 6

Effect of prolonged administration of carbaryl on plasma glucose[+] in female Japanese quail.

Level ppm	Week 1	2	3	4	5	6	14
0	(13) 240±22[a*]	(13) 279±9[a]	(11) 251±15[a]	(9) 329±12[a]	(10) 308±15[a]	(7) 347±9[a]	(9) 281±12[a]
50	(13) 239±20[a]	(10) 279±14[a]	(13) 237±13[a]	(9) 329±13[a]	(11) 305±14[a]	(12) 377±17[a]	(10) 303±15[a]
150	(11) 253±24[a]	(14) 269±10[a]	(13) 263±11[a]	(7) 328±13[a]	(9) 312±21[a]	(9) 386±17[a]	(10) 279±8[a]
300	(14) 252±21[a]	(9) 287±9[a]	(9) 251±17[a]	(10) 303±13[a]	(11) 303±15[a]	(10) 396±13[a]	(10) 275±16[a]
600	(13) 221±22[a]	(10) 296±19[a]	(10) 263±13[a]	(12) 306±13[a]	(9) 318±15[a]	(8) 375±11[a]	(9) 274±8[a]
900	(9) 202±35[a]	(9) 264±14[a]	(9) 233±24[a]	(9) 269±12[a]	(8) 349±22[a]	(6) 329±20[a]	(10) 307±12[a]
1200	(13) 246±20[a]	(15) 273±10[a]	(9) 231±24[a]	(11) 289±14[a]	(11) 326±14[a]	(8) 349±19[a]	(10) 282±12[a]

[+]Expressed as mg %.

[*]Mean ± standard error. Number in parentheses refers to n. Means with the same superscripts are not significantly different.

In order to make meaningful comparisons of data from various sources, an estimation of the actual quantity of toxic compound introduced into the animal in relation to body weight is necessary. While feed consumption in this study was not determined, an estimate was made based on average body weights for each week with sexes and treatments being combined since there was no significant sex differences or week by treatment interactions. Average body weights for weeks 1 through 6 and week 14 were 18, 39, 65, 94, 103, 113 and 132 g, respectively. It has been determined (LASKEY personal communication) that the average weekly feed consumption of Japanese quail at these body weights is 25, 21, 18, 15, 15, 14 and 13 g/100 g body weight/day, respectively. This results in an average feed consumption of 17 g/100 g body weight/day over 14 weeks which in turn results in an average of 20.4, 15.3, 10.2, 5.1, 2.6 and 0.9 mg carbaryl/100 g body weight/day being ingested at the 1200, 900, 600, 300, 150 and 50 ppm levels, respectively. The quail were exposed to these levels over a 98 day period.

Whole-brain AChE activity was not significantly depressed by the consumption of up to 20.4 mg carbaryl/100 g/day for 98 days (Tables 1 and 4). It should be noted that carbaryl inhibition of ChE activity is reversible and slight recovery of such activity in frozen serum samples has been reported (SMITH 1974). However, in the present study, brain tissue was stored for a relatively short period of time before analysis and therefore recovery of AChE activity was felt to be minimal. Comparison with other studies is made difficult by the differences in tissues assayed for AChE activity. The usual tissue employed is blood but we felt that in the present study brain AChE activity might serve as a more functional indicator of the anti-ChE effect of carbaryl since the physiological significance of blood

AChE is questionable (SILVER 1974). CARPENTER et al. (1961) have indicated that carbaryl levels which cause a depression of blood ChE activity also cause a similar depression of brain ChE activity and thus comparisons are possible. CARPENTER et al. (1961) reported that a single oral dose of 56 mg carbaryl/100 g body weight in rats cause a 42% decrease in erythrocyte ChE activity and a 30% decrease in brain ChE activity after 30 min. ChE activity in body tissues returned to normal after 24 hr. RYBAKOVA (1966) administered orally 5 mg carbaryl/100 g/day to rats for 50 days and reported a 41% decrease in blood ChE activity. SHTENBERG and RYBAKOVA (1968) administered orally up to 7 mg carbaryl/100 g daily to rats for a period of 365 days. By 84 days levels of 1.4 and 7 mg/100 g resulted in moderate inhibition of blood ChE activity and by 365 days the 1.4 and 7.0 mg/100 g levels had depressed blood ChE activity by 33% and 94%, respectively.

Plasma glucose levels were not altered by carbaryl administration in the present study (Tables 3 and 6). ORZEL and WEISS (1966) reported that intraperitoneal injections of 0.5 and 2.5 mg/100 g body weight significantly elevated blood glucose levels in fasted and non-fasted rats within 30 min. Using brain AChE activity as an index of ChE inhibition by carbaryl they postulated that the hyperglycemia was induced by inhibition of ChE activity in the adrenal medulla thereby causing secretion of epinephrine. If elevation of blood glucose is dependent upon ChE inhibition by carbaryl, then the absence of such an effect reported here is not unexpected since whole-brain AChE activity was not altered by carbaryl administration.

Whole-brain NE, heart NE and adrenal NE and E levels were not significantly altered by carbaryl administration while brain DA levels were significantly elevated at the 20.4 and 10.2 mg/100 g/day levels in the males at week 14 (Tables 2 and 5). HASSAN (1971) reported that while carbaryl did not induce changes in endogenous levels of cardiac NE, it did result in increased NE turnover and increased urinary excretion of 3-methoxy-4-hydroxymandelic acid (VMA), a primary catecholamine metabolite. HASSAN (1971) postulated that carbaryl caused increased release and synthesis of the catecholamine as a result of increased sympathoadrenergic activity. Since catecholamine turnover and excretion rates were not determined in the present study, it cannot be ascertained if carbaryl caused similar changes in brain and heart NE and adrenal NE and E. The plasma glucose responses reported in this study might indicate that there was not an increased release of adrenal catecholamines. The fact that carbaryl administration results in an increase in cardiac NE turnover (HASSAN 1971) and an increase in brain levels of serotonin and its primary metabolite (HASSAN and SANTOLUCITO 1971) might suggest that the elevated levels of whole-brain DA reported in the present study were due to an increase in DA synthesis. Whether this effect is a result of central cholinergic involvement or is due to a direct effect of carbaryl on catecholamine metabolism remains a matter of speculation.

This report extends the previous conclusion (BURSIAN and EDENS 1977) that the Japanese quail is less affected by carbaryl

than are mammalian species. This difference may be accounted for by a faster rate of detoxification and elimination of carbaryl or by less absorption of carbaryl through the digestive tract in this avian species. These possibilities remain matters of speculation. It is of interest that changes in brain DA levels occurred while brain AChE activity was not altered. Perhaps changes in catecholamine levels or metabolism might serve as more sensitive indicators of carbaryl intoxication than ChE activities.

SUMMARY

Whole-brain dopamine levels were significantly elevated in male Japanese quail fed 600 and 1200 ppm carbaryl in the diet for 14 weeks. Whole-brain AChE activity, whole-brain and heart norepinephrine levels, adrenal norepinephrine and epinephrine levels and plasma glucose levels were not altered by carbaryl. No effects were noted in the females.

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