

Toxicity of DCPD to Mink

TEST 1 - ACUTE (LD₅₀)

Procedure

Twenty-four adult female mink were singly dosed intragastrically with DCPD in order to determine its acute oral toxicity to mink. The following progression of doses (and number of mink per dose) were employed: 0.0 mg/kg (2); 30 mg/kg (3); 60 mg/kg (2); 120 mg/kg (2); 240 mg/kg (4); 480 mg/kg (4); 600 mg/kg (4); 720 mg/kg (3); and 960 mg/kg (2).

The larger doses (240 mg/kg and greater) were administered by gavage as described for DIMP. The smaller doses were introduced into the stomach by gelatin capsule.

In addition, 5 adult female mink were injected intraperitoneally with DCPD according to the following regime: 960, 1200, 1440, 1680, and 1920 mg/kg (1 mink per dose).

Mortality and signs of intoxication were recorded during a 2 hour observation period following dosing, and daily thereafter for 14 days. The mink were then terminated by cervical dislocation, and examined for gross pathomorphological changes.

Results

Calculation of an acute oral LD₅₀ for DCPD in mink was not possible since 100% of the animals survived the highest dosage (960 mg/kg). However, intraperitoneal injections of DCPD at 960, 1200, 1440, 1680, and 1920 mg/kg resulted in death for those animals.

The clinical signs of intoxication following oral exposure to DCPD included hyperactivity, high-pitched vocalizations, dyspnea, diarrhea, opisthotonus, convulsions, vomiting, and paresis of the hind limbs. Recovery was generally rapid with resumption of normal appearance and behavior within an hour to an hour and a half of dosing.

The mink exposed to the high doses of DCPD by I.P. injection all died within minutes of administration of the compound.

Discussion

The acute oral LD₅₀ to mink was above the maximum dose of DCPD given in this test (>1000 mg/kg). The acute oral toxicity of DCPD to Mallard ducks, (LD₅₀ > 40,000 mg/kg) and to Bobwhite quail (1010 mg/kg) as previously reported, and the LD₅₀ to mice (1041-1363 mg/kg) and rats (866-1125 mg/kg) reported by Hart and Dacre (1977), support the evidence that DCPD is slightly toxic to practically nontoxic for most species.

Pharmacologically, DCPD seemed to act as a general excitant to mink, causing increased activity and convulsions as the most pronounced clinical signs. These observations are consistent with those for Mallard ducks.

The acute intraperitoneal dosing of DCPD to mink caused mortality in all mink in doses of 960 mg/kg and above.

These data suggest a lower LD₅₀ for intraperitoneal administration than by the oral route, of DCPD to mink. Data of intraperitoneal LD₅₀'s for other species is lacking except for the mouse which was stated to be greater than 250 mg/kg (Horton, 1948).

TEST 2 - SUBACUTE (LC₅₀)

Procedure

Testing

The subacute dietary LD₅₀ test consisted of a 7-day quarantine and acclimation period, a 21-day dosing period, and a 7-day recovery period.

Sixty juvenile pastel mink were separated into 6 groups of 10 mink each. Each group consisted of 5 males and 5 females randomly chosen from healthy stock, and were approximately 8 months of age. One group was assigned to each of the following dietary concentrations of DCPD: 0 (control), 1, 10, 100, 1000, and 10,000 ppm. Dietary constituents and preparation procedures are given in Appendix I.

All animals in the subacute trial were housed indoors in an environmentally controlled cage room, at the Poultry Science Research and Teaching Center, Michigan State University. Each mink was housed individually in a 51 x 36 x 30 cm (length x width x height) cage equipped with a water cup and feed container.

Feed was provided in removable feeders attached to the inside of the cage on a swinging door such that feed consumption could be ascertained from measurement of unconsumed feed. Water was provided ad libitum.

During the 7-day predosing acclimation period, all mink were provided with a control diet.

Body weights were recorded at the beginning of the dosing period and on days 7, 14, and 21 of dosing, and on day 7 of the recovery period (termination of test).

Feed consumption was estimated by daily recovery of the unconsumed portion of a preweighed allotment of feed, and collectively weighed for each treatment level on days 7, 14, and 21 of dosing, and on day 7 of recovery.

Mortality, signs of intoxication, and behavioral changes were noted throughout both the dosing and recovery periods.

Blood for packed cell volume (hematocrit) and differential leukocyte counts was procured by toe-clip at the termination of the test. Blood was collected in heparinized microcapillary tubes (100 μ l) and centrifuged for 7 minutes at 4500 rpm on an International Microcapillary Centrifuge¹ for hematocrit determination. Blood smears were allowed to air dry and were then fixed and stained in Wright's stain (see Appendix F). After staining, slides were first rinsed with phosphate buffer, for differentiation, and then with distilled water. They were then blotted and air dried. Differential leukocyte counts were made under oil immersion (930-x), and any abnormalities in cells were recorded.

At the end of the experiment animals were terminated by cervical dislocation, and necropsied. Gross pathomorphological observations were made, and the following organs were excised, weighed, and prepared for histopathological observation according to routine laboratory procedures: brain, heart, lungs, kidneys, spleen, and liver.

Statistical Analysis

Differences in body weight changes, feed consumption, hematocrit values, differential leukocyte counts, and organ weights were analyzed by a one-way analysis of variance and Dunnett's t-test. Zero predicted feed consumption was estimated by regression analysis. Determination of approximate LC₅₀ was made by regression analysis.

Results

The mortality associated with feeding DCPD in the diet at various levels for 21 days, followed by a 7 day post-treatment period, is given in Table 103. Mortality occurred in only the highest dietary concentration of DCPD (10,000 ppm). Mortality of mink on the 10,000 ppm DCPD diet was greater for males than for females. A mean lethal concentration (LC₅₀) of 6800 ppm was calculated from the regression line shown in Figure 36.

The mean of body weights recorded weekly throughout the test are shown in Table 104 and Figure 37. There were significantly lower body weights for mink on the 10,000 ppm DCPD treatment on days 14 and 21 of the dosing period. Although the 10,000 ppm group showed a weight gain for the 7-day post-treatment period, the mean body weight was still significantly depressed compared to the controls.

Since Table 104 shows combined body weights for both sexes, the sexual dimorphism in mean body weight of mink is not represented.

¹ International Equipment Company, Boston, MA

Table 103. Mortality associated with a subacute 21-day dietary administration of DCPD and a 7-day post-treatment recovery period.

Sex	Treatment (ppm)	No. of mink surviving during treatment				No. of mink surviving post-treatment	Mortality (%)
		1/15	1/22	1/29	2/5	2/13	
Male	DCPD 0	5	5	5	5	5	0
	1	5	5	5	5	5	0
	10	5	5	5	5	5	0
	100	5	5	5	5	5	0
	1000	5	5	5	5	5	0
	10000	5	5	3	2	1	80
Female	DCPD 0	5	5	5	5	5	0
	1	5	5	5	5	5	0
	10	5	5	5	5	5	0
	100	5	5	5	5	5	0
	1000	5	5	5	5	5	0
	10000	5	4	3	3	3	40
Combined Sexes	DCPD 0	10	10	10	10	10	0
	1	10	10	10	10	10	0
	10	10	10	10	10	10	0
	100	10	10	10	10	10	0
	1000	10	10	10	10	10	0
	10000	10	9	6	5	4	60

Figure 36. Regression line for the data presented in Table 103. In the regression equation $x = \log$ concentration of DCPD in ppm; $y =$ percent mortality.

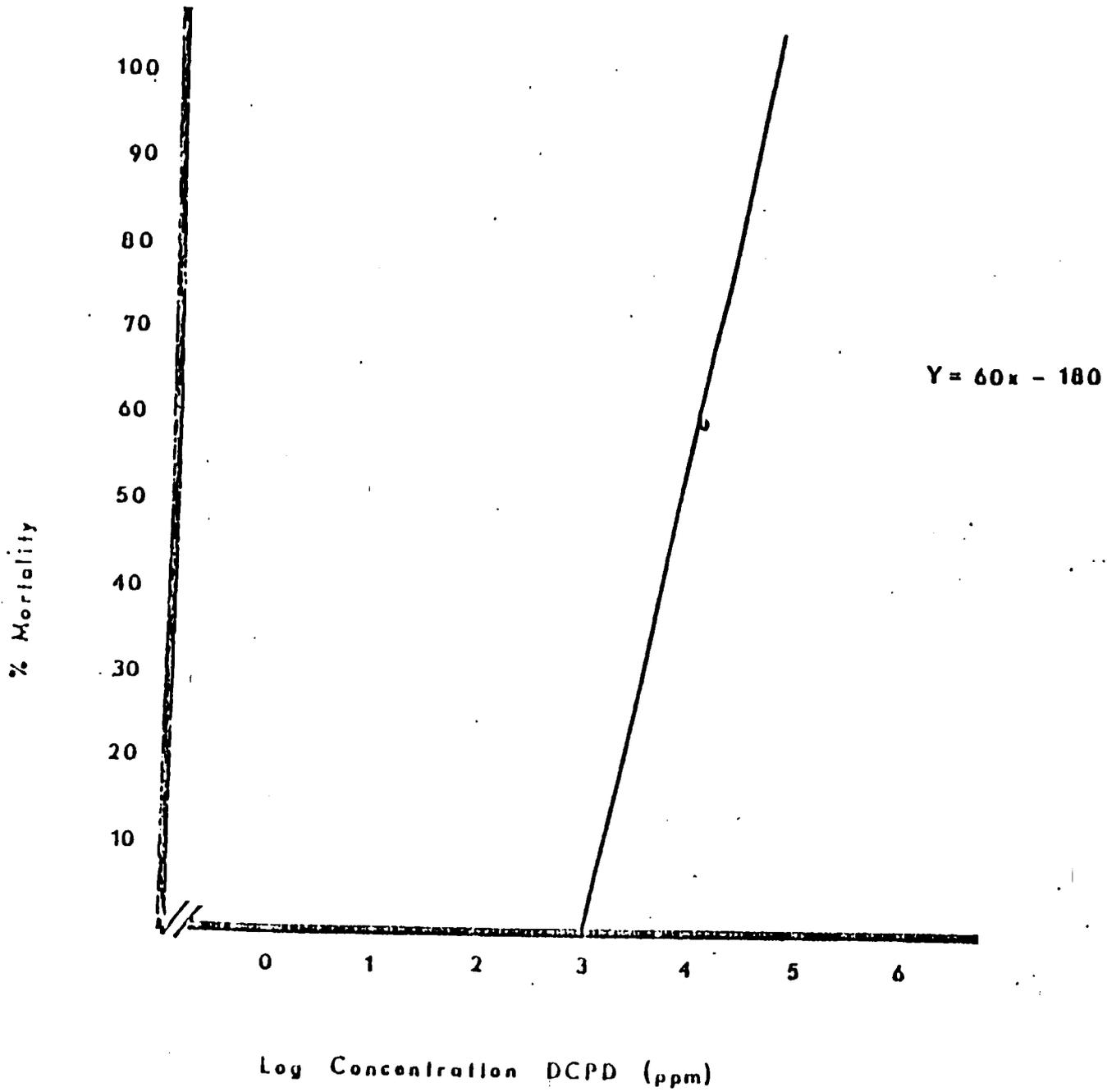


Table 104 . Change in body weight of mink on 21-day dietary LC₅₀ test and post-treatment recovery.

Treatment (ppm)	Mean body wt. (g)				
	Initial wt.	7 days	14 days	21 days	7 days post-treatment
DCPD 0	1406 ± 135 ^a	1461 ± 169 ^a	1501 ± 185 ^a	1512 ± 177 ^a	1452 ± 154 ^a
1	1440 ± 154 ^a	1581 ± 169 ^a	1483 ± 154 ^a	1577 ± 169 ^a	1511 ± 141 ^a
10	1350 ± 130 ^a	1444 ± 140 ^a	1404 ± 143 ^a	1411 ± 138 ^a	1380 ± 117 ^a
100	1392 ± 98 ^a	1529 ± 113 ^a	1475 ± 111 ^a	1518 ± 118 ^a	1496 ± 105 ^a
1000	1305 ± 92 ^a	1295 ± 98 ^a	1254 ± 94 ^a	1238 ± 94 ^a	1277 ± 89 ^a
10000	1339 ± 87 ^a	1093 ± 81 ^a	903 ± 88 ^b	697 ± 75 ^c	920 ± 200 ^b

^a Means with the same superscript are not significantly different from their controls.

^b Means significantly different from control at P<0.05 level of significance.

^c Means significantly different from control at P<0.01 level of significance.

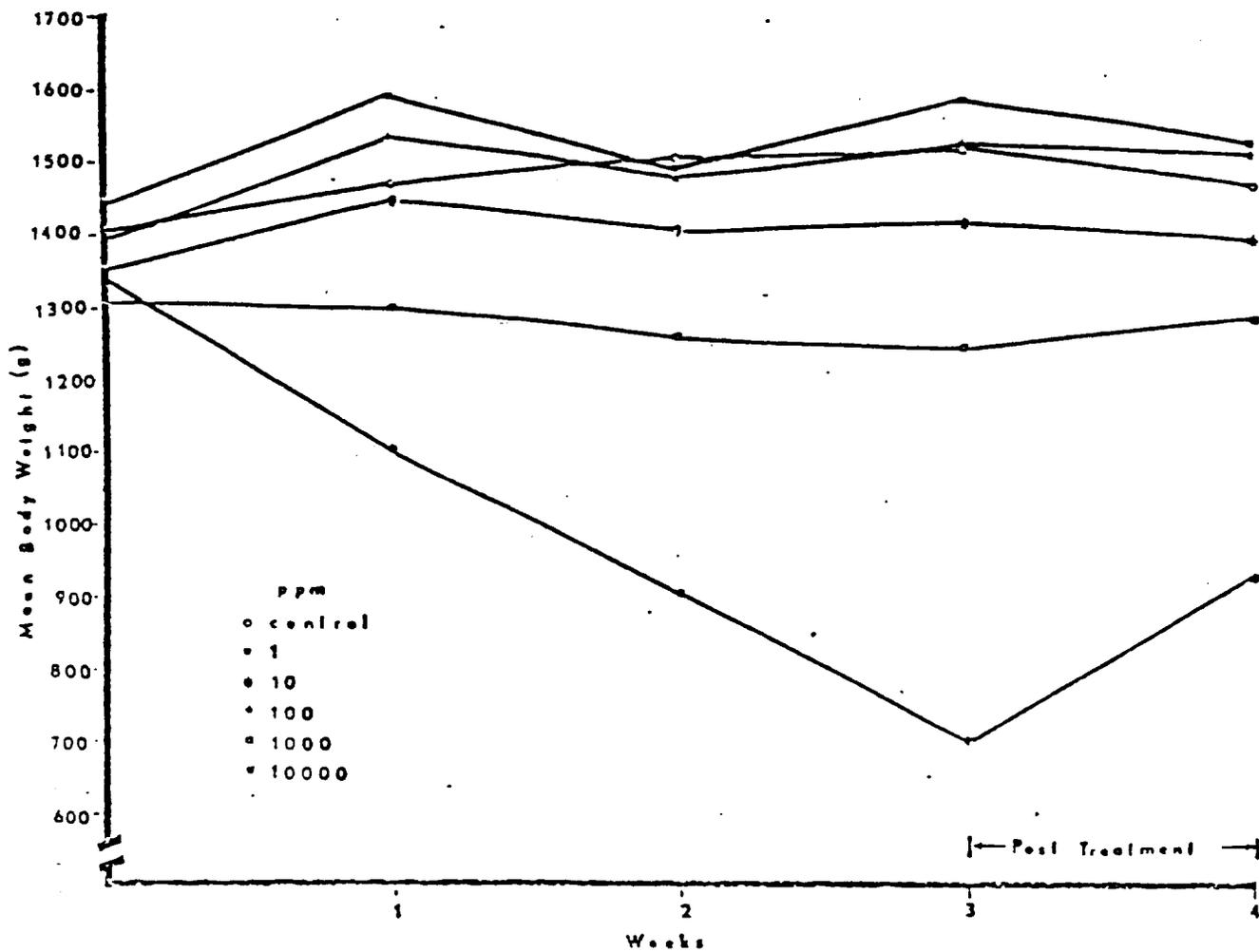


Figure 37. Mean body weights of mink on the 21-day subacute test fed DCPD at various levels.

To account for this difference, Table 105 shows mean percent change in body weight, by sex, over four weekly intervals. During the first week of DCPD administration, there was a significant loss in body weight when compared to controls, for the females on 1000 and 10,000 ppm DCPD treatment, and for males on the 10,000 ppm DCPD diet. In addition, during the first 7 days the males on the 1000 ppm DCPD treatment showed a significantly smaller body weight gain than controls. The second week of DCPD administration was characterized by a further significant loss in body weight for both males and females on the 10,000 ppm DCPD treatment, over that of control animals. Females fed 10 ppm DCPD also lost significantly more weight than controls during the second week. Both males and females on the 10,000 ppm DCPD treatment continued to lose weight during the third week of dosing. The females fed 10,000 ppm DCPD for 21 days responded to the post-treatment control diet by gaining significantly more weight than the controls during the post-treatment period. The single surviving male on the 10,000 ppm DCPD treatment also gained a considerable amount of weight during the post-treatment period.

Consumption of DCPD-supplemented feed of various concentrations is given in Table 106. The mean feed consumption for 21 days on treatment was significantly lower than controls for the animals on the 1000 ppm DCPD treatment ($P < 0.05$) and for the mink on the 10,000 ppm DCPD treatment ($P < 0.01$) than for the controls. Feed consumption resumed to somewhat above control values for the 7-day post-treatment period for both of these groups. Table 107 shows the calculated averaged amount of DCPD ingested/kg body weight, over the 21-day treatment period based on mean feed consumption and mean body weight for the period. The highest dose received daily was 754 mg/kg by animals fed the 10,000 ppm DCPD diet.

Figure 38 shows the extrapolated zero predicted feed consumption as calculated by regression analysis of the data presented in Table 106. Zero feed consumption would have occurred at 74,372 ppm DCPD according to this analysis.

The hematological parameters measured at the termination of the test are given in Table 108. Hematocrit (packed cell volume) was found to be lowered significantly ($P < 0.01$) compared to control values for mink on the 10,000 ppm DCPD treatment. The differential leukocyte counts revealed a significantly depressed percentage of band-neutrophils in the 1, 10, 100, and 1000 ppm DCPD treatments as compared with controls. All other leukocytes were not significantly different in number from the controls for any DCPD treatment.

All animals on the 10,000 ppm DCPD treatment appeared to be anorectic for the first 2-3 days on treatment. They all became progressively emaciated, lethargic, and disoriented. Their stools became loose and had a tar-black color. Before death they appeared to be immobilized with periodic, slight tonic convulsions.

Necropsy revealed no consistent macroscopic pathologies associated with any treatment group, with the exception of a severe depletion of body fat in 10,000 ppm DCPD-fed animals that died while

Table 105. Effect of subacute dietary DCPD administration upon percent change in mink body weights taken at weekly intervals.

Sex	Treatment (ppm)	Time interval and number animals included in analysis							
		1/15-1/22		1/23-1/29		1/30-2/5		2/6-2/13	
		N	% Gain (loss) in body wt.	N	% Gain (loss) in body wt.	N	% Gain (loss) in body wt.	N	% Gain (loss) in body wt. (post-treatment)
Male	DCPD 0	5	9.8 ± 2.17 ⁽¹⁾ _A	5	4.8 ± 3.32 _A	5	(0.3 ± 1.79) _A	4	(2.8 ± 2.79) _A
	1	5	11.5 ± 2.72 _A	4	(0.8 ± 1.63) _A	5	4.8 ± 2.11 _A	5	(6.6 ± 1.86) _A
	10	4	4.1 ± 3.47 _A	5	(1.1 ± 1.38) _A	5	0.6 ± 1.40 _A	5	(5.5 ± 1.12) _A
	100	5	11.4 ± 2.22 _A	5	(4.2 ± 2.25) _A	5	4.1 ± 2.00 _A	5	(4.5 ± 1.90) _A
	1000	5	1.7 ± 2.11 _B	5	(3.0 ± 1.46) _A	5	(0.6 ± 1.46) _A	5	1.3 ± 1.80 _A
	10000	5	(18.2 ± 4.05) _C	3	(20.6 ± 1.47) _C	2	(23.3 ± 5.35) _C	1	75.8 ± 0.00
Female	DCPD 0	4	4.8 ± 2.42 _A	5	(1.4 ± 0.80) _A	5	3.7 ± 1.91 _A	5	3.9 ± 1.73 _A
	1	5	8.3 ± 2.78 _A	5	(4.7 ± 1.25) _A	5	1.7 ± 1.76 _A	5	1.2 ± 2.53 _A
	10	5	6.4 ± 2.99 _A	5	(5.4 ± 0.72) _C	5	1.1 ± 0.80 _A	5	3.9 ± 1.83 _A
	100	5	7.8 ± 1.55 _A	5	(2.7 ± 0.37) _A	5	1.2 ± 0.64 _A	5	3.3 ± 2.20 _A
	1000	5	(3.9 ± 1.22) _C	5	(3.1 ± 1.22) _A	5	(2.0 ± 1.85) _A	5	6.3 ± 3.83 _A
	10000	4	(19.5 ± 0.79) _C	3	(17.1 ± 0.49) _C	3	(22.3 ± 3.13) _C	3	22.8 ± 2.98 _C
Combined Sexes	DCPD 0	9	7.6 ± 1.02 _A	10	1.7 ± 1.98 _A	10	1.7 ± 1.45 _A	9	0.9 ± 1.92 _A
	1	10	9.9 ± 2.01 _A	9	(2.9 ± 1.19) _A	10	3.1 ± 1.45 _A	10	(2.7 ± 2.00) _A
	10	9	5.4 ± 2.30 _A	10	(3.3 ± 1.04) _A	10	0.9 ± 0.81 _A	10	(0.8 ± 1.83) _A
	100	10	9.6 ± 1.47 _A	10	(3.5 ± 1.17) _A	10	2.7 ± 1.14 _A	10	(0.6 ± 1.94) _A
	1000	10	(1.1 ± 1.5) _C	10	(3.1 ± 0.95) _A	10	(1.3 ± 1.20) _A	10	3.0 ± 2.26 _A
	10000	9	(18.0 ± 2.29) _C	6	(18.9 ± 1.05) _C	5	(22.7 ± 2.85) _C	4	36.0 ± 11.71 _C

⁽¹⁾ Means with same subscript are not significantly different from controls (P > 0.05).

^b Mean significantly different from control (P < 0.05).

^c Mean significantly different from control (P < 0.01).

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Table 106. Feed consumption of mink on 21-day dietary LC₅₀ trial and post-treatment recovery period.

Treatment (ppm)	Feed consumption (g/mink/day)				
	1/15-1/22	1/22-1/29	1/29-2/5	Mean for 21 days on treatment ± S.E.	Post-treatment 2/6-2/13
DCPD 0	280	271	263	271.0 ± 4.9	245
1	258	279	271	269.3 ± 6.1	262
10	259	279	264	267.3 ± 6.0	256
100	256	279	267	267.3 ± 6.6	272
1000	190	242	244	225.3 ± 17.7 ^a	280
10000	72	70	61	67.7 ± 3.4 ^b	298

^aSignificantly different from control for P<0.05.

^bSignificantly different from control for P<0.01.

Table 107. Feed consumption, body weight, and amount of chemical ingested by adult mink fed DCPD at various levels for 21 days.

DCPD in diet (ppm)	Feed consumed (g/mink/day)	DCPD consumed (mg/mink/day)	Mean body wt. (g)	DCPD consumed (mg/kg/day)
0	271.0	0	1491.3	0
1	269.3	0.269	1547.0	0.173
10	267.3	2.67	1419.7	1.881
100	267.3	26.7	1507.3	17.717
1000	225.3	225	1262.3	178.29
10000	67.7	677	897.7	754.15

21 Day Mean Feed Consumption g/m/d

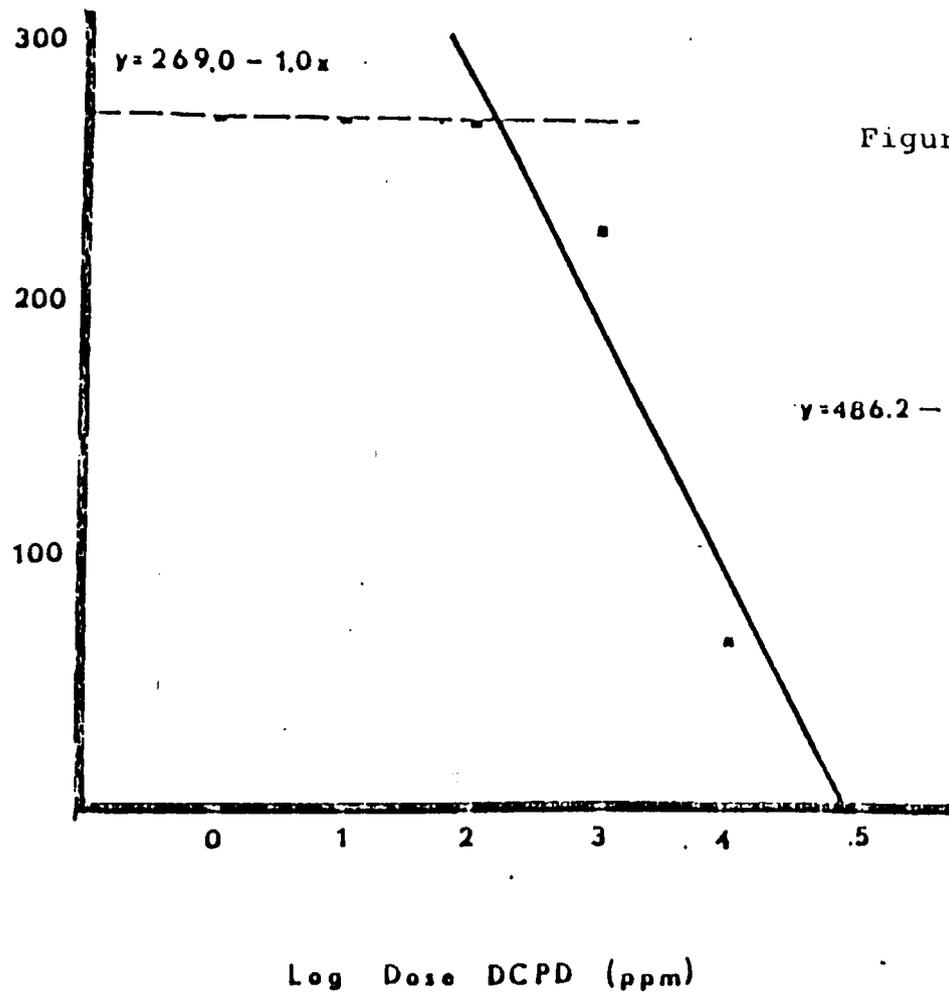


Figure 38. Regression lines for the data presented in Table 107. In the regression equation $x =$ log dose of DCPD in ppm; $y =$ mean feed consumption for 21 days in grams/mink/day.

Table 108. Effect of subacute dietary DCPD upon mink hematocrit values and differential leukocyte counts.

Treatment (ppm)	N	Hematocrit ^a (%)	Leukocyte cell type (% ± S.E.)					
			Basophils	Eosinophils	Band-Neutrophils	Segmented Neutrophils	Lymphocytes	Monocytes
DCPD 0	10	56.1 ± 1.36	0.2 ± 0.21	2.1 ± 0.08	3.0 ± 0.77	60.1 ± 4.41	24.1 ± 3.40	2.1 ± 0.53
1	10	57.6 ± 0.61	0.6 ± 0.21	1.5 ± 0.47	0.5 ± 0.29 ^a	67.7 ± 3.49	26.0 ± 3.17	3.7 ± 0.54
10	10	57.1 ± 1.27	0.3 ± 0.20	1.2 ± 0.34	0.3 ± 0.14 ^a	69.7 ± 2.47	22.0 ± 2.61	3.5 ± 0.43
100	10	56.2 ± 0.73	0.5 ± 0.16	1.9 ± 0.62	0.8 ± 0.34 ^a	66.2 ± 1.00	25.5 ± 1.96	4.1 ± 0.36
1000	10	56.1 ± 1.42	0.4 ± 0.15	1.6 ± 0.59	0.4 ± 0.21 ^a	71.7 ± 2.93	21.6 ± 2.93	4.4 ± 0.76
10000	4	39.3 ± 4.02 ^{**}	0.0 ± 0.00	1.5 ± 0.25	1.3 ± 1.00	71.0 ± 4.60	21.8 ± 5.94	4.5 ± 1.33

^aMean ± S.E.

^aTreatment mean significantly different from control mean for (P<0.05)

^{**}Treatment mean significantly different from control mean for (P<0.01).

on treatment. Males fed the 10,000 ppm DCPD diet differed significantly from controls with respect to heart, liver, and spleen weights (see Table 109). Females showed no significant differences in weights of organs among any DCPD treatments (see Table 110).

Discussion

The LC₅₀, of a 21-day dietary administration of DCPD to mink, determined in this test to be 6800 ppm, is at best a rough estimate of the true LC₅₀, since only one dietary regime (10,000 ppm DCPD) produced mortality. As previously reported a 5-day dietary concentration of up to 90,000 ppm DCPD, failed to show any mortality in Mallard ducklings. Similarly, no toxicant related mortality was noted in Bobwhite quail chicks fed DCPD in dietary concentrations of up to 18,000 ppm for 5 days. However, both of these studies did indicate marked depression in feed consumption at these high dietary concentrations.

The depression in feed consumption and the rapid reduction in body weight of mink fed 10,000 ppm DCPD, suggests that mortality was likely due to starvation in these animals. The feed consumption of 150 gm/day required for maintenance of body weight, reported by Schaible (1970) for adult female mink, was much greater than the average feed consumption recorded in this study for mink fed 10,000 ppm DCPD for 21 days. However, since a pair-fed control group was not maintained in this test, it is difficult to ascertain whether the mortality for the 10,000 ppm DCPD treatment animals was due to palatability dependent starvation or to toxic subacute effects. The fact that the animals fed the 10,000 ppm DCPD diet responded to the post-treatment recovery period by a dramatic increase in both feed consumption and body weight suggest the former reason as a more tenable explanation for the mortality seen in these animals.

The depression in hematocrit values in the animals fed the 10,000 ppm DCPD diet could have been due to either decreased erythropoiesis or increased clearance of erythrocytes, or a combination of both. The fact that these animals showed marked weight loss and decreased feed consumption during DCPD administration suggests a protein deficiency might have been responsible for a decrease in erythropoiesis. However, since neither clearance nor production of erythrocytes was measured in this study, and since a pair-fed control was not maintained, it would be premature to speculate on the exact cause for this change.

Differential leukocyte counts of animals on all the DCPD diets, except for the 10,000 ppm diet, showed a decreased number of band neutrophils. However, since the animals on the highest dietary concentration failed to show a similar effect, and since the total white cell counts were not made, the depression in band neutrophils cannot conclusively be attributed to DCPD administration. All other values for leukocyte types in the control approximate values established by other workers (Fletch and Karstad, 1972; Asher et al., 1976; and Gilbert, 1969).

The organ weight differences noted for male mink fed the 10,000 ppm DCPD diet may have been due to toxicosis, but severe depletions in body weight from starvation can cause differences in organ weights to appear at necropsy (Shärer, 1977).

Since a pair-fed control was not maintained, the cause of these organ weight changes is not conclusively due to toxicosis. Control organ weights were not different from mink organ weights reported by other workers (Wood et al., 1965).

The severe depletion in body fat in mink fed the 10,000 ppm DCPD diet was the only gross change noted in the condition of the animals at necropsy. The reduction in feed consumption by the mink fed 10,000 ppm DCPD is consistent with this loss in body fat. Since no other gross pathological changes were noted consistent with toxicosis, it cannot be concluded that the increased mortality of mink fed 10,000 ppm DCPD was associated with anything more than starvation.

TEST 3 - CHRONIC

Procedure

Testing

The chronic toxicity feeding study began with 150 immature dark variety mink (approximately 3 months of age) and continued through one reproductive season (12 months total duration). Five groups of 30 randomly selected animals (6 males and 24 females per group) were used in the test. The following dietary concentrations of DCPD were employed (1 group per concentration level): 0 control; 100; 200; 400; and 800 ppm. Diets constituents and preparation procedures are given in Appendix I. Water was provided ad libitum.

Animals were housed out-of-doors in commercial style mink ranch sheds at the experimental facilities of the Fur Animal Project, Department of Poultry Science, Michigan State University. The animals on each diet were assigned individually to single-tier cages 46 x 61 x 30 cm (length x width x height), or to double tiered cages 61 x 30 x 30 cm (length x width x height) plus a top nest box tier 38 x 30 x 30 cm (length x width x height) in 6 subgroups of 5 animals (one male, 4 females) per subgroup. The subgroups were randomly placed in one of three sheds. Each of the subgroups was specified by color coded mink identification cards placed above their cages which matched the color coding on the respective feed containers.

During the reproductive season, females were housed individually in breeder cages 76 x 61 x 46 cm (length x width x height) to which a nest box 30 x 25 x 25 cm (length x width x height) was attached to the outside of the cage.

Bedding, consisting of shredded wood, was provided for insulation in the winter and for nesting during the reproductive season.

Table 109 Effect of subacute dietary DCPD upon male mink organ weights.

Treatment (ppm)	N	Mean weight (g ± S.E.)					
		Brain	Heart	Lungs ^a	Kidneys	Spleen ^a	Liver
DCPD 0	5	10.2 ± 0.39	12.2 ± 0.45	10.2 ± 0.40	8.6 ± 0.32	3.7 ± 0.41	49.8 ± 2.12
1	5	10.4 ± 0.22	12.0 ± 0.62	10.4 ± 0.50	8.7 ± 0.25	4.3 ± 0.51	57.0 ± 4.34
10	5	10.6 ± 0.46	12.6 ± 0.89	9.5 ± 0.79	8.0 ± 0.29	3.8 ± 0.26	44.9 ± 2.52
100	5	9.9 ± 0.20	12.2 ± 0.62	10.4 ± 0.34	7.9 ± 0.37	4.2 ± 0.46	52.1 ± 1.65
1000	5	10.0 ± 0.39	12.4 ± 0.77	9.8 ± 0.52	8.0 ± 0.36	4.2 ± 0.57	48.8 ± 3.25
10000	5	9.6 ± 0.41	8.1 ± 0.37**	10.1 ± 0.63	7.5 ± 0.28	1.6 ± 0.30**	35.1 ± 3.57*

^aN=4 for 10,000 ppm DCPD treatment.

*Treatment mean significantly different from control at (P<0.05).

**Treatment mean significantly different from control at (P<0.01).

Table 110. Effect of subacute dietary DCPD upon female mink organ weights.

Treatment (ppm)	N	Organ weight (g ± S.E.)					
		Brain	Heart	Lungs	Kidneys	Spleen ^a	Liver
DCPD 0	5	7.8 ± 0.40	6.2 ± 0.25	6.6 ± 0.43	4.7 ± 0.25	3.7 ± 0.36	32.1 ± 1.94
1	5	8.8 ± 0.30	7.8 ± 0.45	6.8 ± 0.42	5.0 ± 0.20	3.2 ± 0.18	33.8 ± 2.24
10	5	8.0 ± 0.75	7.8 ± 0.54	6.6 ± 0.18	5.7 ± 0.39	3.4 ± 0.39	34.7 ± 1.59
100	5	8.4 ± 0.22	7.7 ± 0.65	7.2 ± 0.22	5.5 ± 0.33	4.3 ± 0.10	38.6 ± 2.45
1000	5	8.4 ± 0.35	7.3 ± 0.55	5.7 ± 0.38	5.1 ± 0.07	3.6 ± 0.35	35.8 ± 2.89
10000	5	8.3 ± 0.21	6.8 ± 0.68	7.6 ± 0.62	5.7 ± 0.52	4.1 ± 0.57	40.6 ± 4.10

^a N = 3 for 0 ppm DCPD; N = 4 for 10,000 ppm DCPD

Mortality and signs of intoxication were recorded throughout the experiment.

Body weight measurements were made at two week intervals, except during the gestation period.

Feed consumption was estimated once every two weeks for 5 months, by weighing unconsumed feed recovered from a preweighed allotment given the previous day, for each animal.

Blood for hematocrit (packed cell volume), hemoglobin, and blood smears was collected by toe-clip at the beginning of the experiment, at 3 month intervals (except during the gestation period), and at the termination of the test.

Hematocrit values were determined from blood drawn into heparinized microcapillary tubes (100 μ l) and centrifuged in an International Microcapillary Centrifuge¹ for 7 minutes at 4500 rpm.

Hemoglobin values were determined by the cyanmethemoglobin method, based on a quantitative spectrographic change in absorption of light relating to hemoglobin concentration (see Appendix E).

Blood smears were allowed to air dry and were then fixed and stained with Wright's stain (see Appendix F). After staining, the slides were rinsed with phosphate buffer for differentiation, followed by distilled water. They were then blotted and air dried. Differential leukocyte counts were made on the smears collected at the termination of the test. Counting was done under oil immersion and abnormalities in cell types were recorded.

Mink mating was initiated on March 1, 1978, and lasted approximately 20 days. Females were bred to males within their respective treatment group whenever possible. Breeding attempts began at 7:00 a.m. daily and were ceased at noon. Females were introduced into the males' cages every fourth day for one half of an hour to an hour, until a positive mating was secured. Positive matings were confirmed by checking post-coital vaginal aspirations for sperm. Positive matings were followed-up by a second mating attempt eight days later.

After breeding, the females were transferred to the cages described above for whelping.

During the whelping period (April 20 - May 15) the nest boxes were checked daily for evidence of whelping. New born kits were sexed and weighed on the day of whelping and at one month of age. Whelping females were also weighed on the day of whelping and one month after whelping.

Length of gestation, litter size, sex ratio, kit mortality, increase in kit "biomass" during lactation, and lactating female weight changes were recorded.

¹ International Equipment Company, Boston, MA

At the termination of the chronic test, the mink were weighed, and blood samples were taken (by cardiac puncture) and stored for future analysis.

The animals were terminated by cervical dislocation, and were then necropsied. Any gross pathomorphological changes were recorded. The following organs were then excised and weighed: brain, liver, kidneys, spleen, gonads, lungs, heart, and adrenal glands. Portions of these organs, in addition to portions of the intestine, stomach, skeletal muscle, adipose tissue, and integument were then fixed in 10% neutral, buffered formalin, and prepared for histopathological examination according to routine histological procedures.

Statistical Analysis

All parameters were analysed for significant differences by analysis of variance and Dunnett's t-test.

Results

Chronic ingestion of dietary DCPD by mink at concentrations as high as 800 ppm for 12 months resulted in no significant differential mortality. Table 111 gives mortality data determined for quarterly intervals of the chronic toxicity test.

Body weight changes associated with chronic feeding of DCPD showed no dose specific trend, although on a few scattered measurement dates, animals on the 800 ppm DCPD diet exhibited significantly lighter body weights than controls (Table 112). When analyzed on a percent change basis, body weights of treated animals failed to show a consistent difference with respect to controls (Table 113).

The measurement of feed consumption at biweekly intervals revealed an initial depression in feed consumption for animals receiving the highest dietary concentrations (Table 114). Feed consumption by treated animals resumed to a level not significantly different from controls for the majority of the remaining measurement periods. Feed consumption by the animals on the 800 ppm DCPD treatment was significantly depressed below that of controls on only one occasion (with the exception of the initial measurement), and was recorded as being greater than that shown by controls (as were several other lower level treatments) on the final measurement date. Estimated daily ingestion of DCPD, as calculated from body weight and feed consumption, for all treatments is shown in Table 115.

Hematological parameters showed no consistent changes associated with chronic DCPD administration. Hematocrit values (packed cell volume) showed no significant differences in treated animals as compared to controls, with the exception of 100 ppm DCPD-fed animals on the second blood collection date (Table 116). The depression in hematocrit values shown by these animals (combined sexes) was not apparent when separated by sex.

Table 111. Mortality of mink fed DCPD at various levels for 12 months.

Sex	Treatment (ppm)	Mortality by date (Deaths per total on treatment)			
		7/21/77	10/18/77	1/17/78	6/30/78
Males	DCPD 0	0/6	0/6	0/6	0/6
	100	0/6	0/6	1/6	1/6
	200	0/6	0/6	0/6	1/6
	400	0/6	0/6	0/6	0/6
	800	0/6	0/6	0/6	0/6
Females	DCPD 0	0/24	0/24	0/24	4/24
	100	0/24	1/24	1/24	2/24
	200	0/24	0/24	0/24	3/24
	400	0/24	0/24	0/24	1/24
	800	0/24	0/24	0/24	3/24
Combined Sexes	DCPD 0	0/30	0/30	0/30	4/30
	100	0/30	1/30	2/30	3/30
	200	0/30	0/30	0/30	4/30
	400	0/30	0/30	0/30	1/30
	800	0/30	0/30	0/30	3/30

Table 112. Effect of chronic dietary DCPD administration to male and female mink upon body weight ($g \pm S.E.$) gain by date.

Sex	Treatment (ppm)	N	7/21/77	N	8/3/77	N	8/18/77	N	9/1/77	N	9/15/77	N	9/29/77
Male	DCPD 0	6	1003 \pm 56 _a (1)	6	1297 \pm 59 _a	6	1427 \pm 69 _a	6	1569 \pm 57 _a	6	1631 \pm 60 _a	6	1649 \pm 63 _a
	100	6	1133 \pm 47 _a	6	1316 \pm 46 _a	6	1374 \pm 69 _a	6	1532 \pm 61 _a	6	1525 \pm 61 _a	6	1692 \pm 60 _a
	200	6	1029 \pm 55 _a	6	1250 \pm 50 _a	6	1351 \pm 52 _a	6	1469 \pm 57 _a	6	1473 \pm 53 _a	6	1624 \pm 57 _a
	400	6	1150 \pm 40 _a	6	1404 \pm 50 _a	6	1507 \pm 40 _a	6	1631 \pm 61 _a	6	1666 \pm 59 _a	6	1740 \pm 80 _a
	800	6	1054 \pm 42 _a	6	1229 \pm 40 _a	6	1310 \pm 32 _a	6	1418 \pm 39 _a	6	1447 \pm 47 _a	6	1534 \pm 52 _a
Female	DCPD 0	24	760 \pm 16 _a	24	861 \pm 18 _a	24	919 \pm 22 _a	24	920 \pm 23 _a	24	971 \pm 21 _a	24	999 \pm 21 _a
	100	24	739 \pm 14 _a	24	806 \pm 34 _a	24	900 \pm 19 _a	24	880 \pm 21 _a	23	905 \pm 22 _a	23	954 \pm 25 _a
	200	24	739 \pm 16 _a	24	845 \pm 17 _a	24	903 \pm 20 _a	24	914 \pm 24 _a	24	951 \pm 25 _a	24	1006 \pm 27 _a
	400	24	731 \pm 18 _a	24	829 \pm 20 _a	24	875 \pm 22 _a	24	878 \pm 24 _a	24	896 \pm 23 _a	24	967 \pm 25 _a
	800	24	731 \pm 18 _a	24	806 \pm 20 _a	24	848 \pm 21 _a	24	844 \pm 21 _a	24	874 \pm 21 _c	24	933 \pm 23 _a
Combined Sexes	DCPD 0	30	825 \pm 29 _a	30	949 \pm 37 _a	30	1021 \pm 43 _a	30	1050 \pm 52 _a	30	1103 \pm 52 _a	30	1129 \pm 52 _a
	100	30	818 \pm 32 _a	30	908 \pm 47 _a	30	995 \pm 40 _a	30	1010 \pm 52 _a	29	1033 \pm 51 _a	29	1107 \pm 61 _a
	200	30	796 \pm 27 _a	30	926 \pm 34 _a	30	993 \pm 40 _a	30	1025 \pm 46 _a	30	1056 \pm 44 _a	30	1130 \pm 51 _a
	400	30	815 \pm 35 _a	30	944 \pm 46 _a	30	1002 \pm 50 _a	30	1028 \pm 60 _a	30	1050 \pm 60 _a	30	1122 \pm 62 _a
	800	30	796 \pm 29 _a	30	891 \pm 36 _a	30	942 \pm 39 _a	30	959 \pm 46 _a	30	988 \pm 46 _a	30	1053 \pm 49 _a

(1) Means in the same row with the same subscript are not significantly different from their respective control values ($P > 0.05$).

Continued

Table 112. Continued.

Sex	Treatment (ppm)	N	10/13/77	N	10/27/77	N	11/10/77	N	11/22/77	N	12/0/77	N	12/23/77
Males	DCPD 0	6	1654 ± 30 _A	6	1600 ± 37 _A	6	1766 ± 32 _A	6	1731 ± 33 _A	6	1613 ± 42 _A	6	1649 ± 52 _A
	100	6	1674 ± 76 _A	6	1602 ± 76 _A	6	1750 ± 77 _A	6	1750 ± 77 _A	6	1601 ± 70 _A	6	1739 ± 80 _A
	200	6	1620 ± 60 _A	6	1639 ± 57 _A	6	1703 ± 63 _A	6	1703 ± 72 _A	6	1500 ± 80 _A	6	1616 ± 75 _A
	400	6	1777 ± 80 _A	6	1767 ± 84 _A	6	1613 ± 74 _A	6	1035 ± 66 _A	6	1735 ± 71 _A	6	1809 ± 90 _A
	800	6	1519 ± 40 _A	6	1500 ± 35 _A	6	1569 ± 38 _A	6	1560 ± 39 _A	6	1432 ± 36 _B	6	1397 ± 68 _B
Females	DCPD 0	24	1041 ± 24 _A	24	1031 ± 22 _A	24	1020 ± 22 _A	24	1014 ± 26 _A	24	937 ± 22 _A	24	989 ± 21 _A
	100	23	997 ± 25 _A	23	974 ± 25 _A	23	1019 ± 25 _A	23	1014 ± 30 _A	23	920 ± 26 _A	23	967 ± 28 _A
	200	24	1036 ± 30 _A	24	1019 ± 31 _A	24	1006 ± 30 _A	24	1015 ± 30 _A	24	952 ± 28 _A	24	905 ± 30 _A
	400	24	997 ± 25 _A	24	983 ± 23 _A	24	1006 ± 23 _A	24	1025 ± 23 _A	24	945 ± 25 _A	24	1008 ± 27 _A
	800	24	948 ± 23 _A	24	936 ± 24 _A	24	940 ± 27 _A	24	958 ± 26 _A	23	806 ± 25 _A	23	919 ± 29 _A
Combined Sexes	DCPD 0	30	1163 ± 49 _A	30	1162 ± 51 _A	30	1169 ± 50 _A	30	1157 ± 57 _A	30	1072 ± 53 _A	30	1121 ± 52 _A
	100	29	1137 ± 57 _A	29	1156 ± 56 _A	29	1167 ± 62 _A	29	1167 ± 62 _A	29	1077 ± 63 _A	29	1126 ± 64 _A
	200	30	1153 ± 50 _A	30	1143 ± 53 _A	30	1145 ± 50 _A	30	1152 ± 50 _A	30	1079 ± 54 _A	30	1112 ± 54 _A
	400	30	1153 ± 62 _A	30	1140 ± 62 _A	30	1168 ± 63 _A	30	1107 ± 63 _A	30	1103 ± 63 _A	30	1160 ± 65 _A
	800	30	1062 ± 46 _A	30	1049 ± 46 _A	30	1066 ± 51 _A	30	1080 ± 50 _A	29	968 ± 57 _A	29	1018 ± 45 _A

Continued

Table 112. Continued.

Sex	Treatment (ppm)	N	1/0/78	N	1/10/70	N	2/4/70	N	2/19/78	N	3/4/78	N	6/30/78
Males	DIMP 0	6	1535 ± 58 _A	6	1553 ± 50 _A	6	1591 ± 50 _A	6	1669 ± 67 _A	6	1672 ± 67 _A	6	1640 ± 62 _A
	100	6	1502 ± 84 _A	5	1534 ± 77 _A	5	1577 ± 75 _A	5	1649 ± 73 _A	5	1645 ± 71 _A	5	1573 ± 69 _A
	200	5	1524 ± 93 _A	5	1550 ± 77 _A	5	1460 ± 76 _A	5	1504 ± 60 _A	5	1527 ± 68 _A	5	1500 ± 92 _A
	400	6	1721 ± 84 _A	6	1744 ± 89 _A	6	1750 ± 105 _A	6	1709 ± 107 _A	6	1753 ± 103 _A	6	1722 ± 70 _A
	800	6	1304 ± 55 _A	6	1357 ± 52 _A	6	1398 ± 43 _B	6	1458 ± 40 _A	6	1533 ± 40 _A	6	1538 ± 43 _A
Females	DIMP 0	24	927 ± 23 _A	24	933 ± 22 _A	23	909 ± 26 _A	23	936 ± 24 _A	23	947 ± 22 _A	19	811 ± 26 _A
	100	23	895 ± 29 _A	23	880 ± 26 _A	23	870 ± 28 _A	23	897 ± 26 _A	23	891 ± 25 _A	21	746 ± 25 _A
	200	24	955 ± 32 _A	24	919 ± 29 _A	23	910 ± 30 _A	22	923 ± 32 _A	22	943 ± 29 _A	21	837 ± 27 _A
	400	24	954 ± 26 _A	24	930 ± 24 _A	24	916 ± 25 _A	24	933 ± 23 _A	24	943 ± 21 _A	23	837 ± 25 _A
	800	23	890 ± 30 _A	23	864 ± 27 _A	22	873 ± 29 _A	22	879 ± 32 _A	22	906 ± 32 _A	21	771 ± 20 _A
Combined Sexes	DIMP 0	30	1049 ± 49 _A	30	1057 ± 50 _A	29	1050 ± 56 _A	29	1087 ± 60 _A	29	1097 ± 59 _A	25	1010 ± 75 _A
	100	29	1037 ± 59 _A	28	1003 ± 53 _A	28	997 ± 50 _A	28	1032 ± 60 _A	28	1026 ± 60 _A	26	905 ± 68 _A
	200	29	1053 ± 50 _A	29	1028 ± 52 _A	28	1008 ± 40 _A	27	1031 ± 52 _A	27	1051 ± 51 _A	26	966 ± 59 _A
	400	30	1100 ± 62 _A	30	1093 ± 65 _A	30	1084 ± 60 _A	30	1104 ± 69 _A	30	1105 ± 65 _A	29	1020 ± 71 _A
	800	29	992 ± 46 _A	29	966 ± 44 _A	28	985 ± 48 _A	28	1003 ± 52 _A	28	1040 ± 56 _A	27	941 ± 64 _A

Table 113. Effect of chronic dietary administration of DCPD to male and female mink upon percent change in body weight (\pm S.E.) by date.

Sex	Treatment (ppm)	N	7/21/77-8/3/77	N	8/4/77-8/18/77	N	8/19/77-9/1/77	N	9/2/77-9/1/77
Males	DCPD 0	6	20.0 \pm 2.19 ⁽¹⁾ _a	6	10.0 \pm 2.31 _a	6	10.5 \pm 1.78 _a	6	3.9 \pm 0.8
	100	6	16.4 \pm 1.58 _a	6	4.3 \pm 2.94 _a	6	11.8 \pm 1.49 _a	6	(0.4 \pm 1.2
	200	6	22.2 \pm 3.22 _a	6	8.1 \pm 0.28 _a	6	8.8 \pm 1.39 _a	6	0.4 \pm 1.5
	400	6	22.1 \pm 0.83 _a	6	7.4 \pm 1.46 _a	6	8.1 \pm 0.97 _a	6	2.3 \pm 1.6
	800	6	17.0 \pm 2.86 _a	6	7.5 \pm 1.79 _a	6	7.6 \pm 1.27 _a	6	1.9 \pm 1.1
Females	DCPD 0	24	13.4 \pm 0.58 _a	24	6.6 \pm 0.94 _a	24	0.1 \pm 0.74 _a	24	6.0 \pm 1.0
	100	24	13.7 \pm 1.17 _a	24	7.3 \pm 0.87 _a	24	(2.2 \pm 1.24) _a	23	2.8 \pm 1.3
	200	24	16.2 \pm 1.67 _a	24	7.0 \pm 0.89 _a	24	1.1 \pm 0.91 _a	24	3.8 \pm 0.6
	400	24	14.2 \pm 1.65 _a	24	5.6 \pm 1.01 _a	24	0.2 \pm 0.91 _a	24	3.7 \pm 1.2
	800	24	10.3 \pm 1.16 _a	24	5.3 \pm 0.66 _a	24	(0.3 \pm 0.72) _a	24	3.6 \pm 0.6
Combined Sexes	DCPD 0	30	14.8 \pm 0.80 _a	30	7.3 \pm 0.92 _a	30	2.2 \pm 1.02 _a	30	5.5 \pm 0.8
	100	30	14.3 \pm 1.01 _a	30	6.7 \pm 0.94 _a	30	0.7 \pm 1.45 _a	29	2.1 \pm 1.1
	200	30	17.4 \pm 1.54 _a	30	7.2 \pm 0.72 _a	30	2.7 \pm 0.96 _a	30	3.1 \pm 0.7
	400	30	15.8 \pm 1.45 _a	30	6.0 \pm 0.87 _a	30	1.8 \pm 0.95 _a	30	3.4 \pm 1.1
	800	30	11.7 \pm 1.19 _a	30	5.7 \pm 0.66 _a	30	1.3 \pm 0.85 _a	30	3.3 \pm 0.7

(1) Means in the same column with same subscript are not significantly different from their respective control values ($P > 0.05$).

Continued

Table 113. Continued.

Sex	Treatment (ppm)	N	9/16/77-9/29/77	N	9/30/77-10/13/77	N	10/14/77-10/27/77	N	10/28/77-11/10/
Males	DCPD 0	6	1.4 ± 3.09 _a	6	0.7 ± 2.02 _a	6	1.6 ± 0.22 _a	6	5.2 ± 1.04 _a
	100	6	11.0 ± 1.07 _b	6	(1.1 ± 1.09) _a	6	(0.5 ± 1.33) _a	6	3.8 ± 0.59 _a
	200	6	10.3 ± 0.95 _b	6	(0.3 ± 1.16) _a	6	1.2 ± 0.54 _a	6	3.9 ± 1.14 _a
	400	6	4.2 ± 1.89 _a	6	2.2 ± 0.81 _a	6	(0.6 ± 0.83) _a	6	2.8 ± 1.21 _a
	800	6	6.1 ± 1.01 _a	6	(0.9 ± 1.58) _a	6	(1.1 ± 1.45) _a	6	4.6 ± 0.76 _a
Females	DCPD 0	24	3.0 ± 0.97 _a	24	4.1 ± 0.90 _a	24	(0.8 ± 0.67) _a	24	(1.0 ± 0.97) _a
	100	23	5.4 ± 0.74 _a	23	4.6 ± 0.88 _a	23	(2.0 ± 0.60) _a	23	4.7 ± 0.76 _c
	200	24	4.9 ± 0.65 _a	24	2.9 ± 0.65 _a	24	(1.7 ± 0.68) _a	24	(1.2 ± 0.74) _a
	400	24	7.0 ± 0.90 _b	24	3.3 ± 1.03 _a	24	(1.3 ± 0.52) _a	24	2.4 ± 0.53 _b
	800	24	6.8 ± 0.50 _c	24	1.8 ± 1.03 _a	24	(1.2 ± 0.77) _a	24	0.3 ± 1.06 _a
Combined Sexes	DCPD 0	30	2.7 ± 1.00 _a	30	3.4 ± 0.86 _a	30	(0.3 ± 0.56) _a	30	0.3 ± 0.92 _a
	100	29	6.5 ± 0.75 _c	29	3.4 ± 0.85 _a	29	(1.7 ± 0.56) _a	29	4.5 ± 0.62 _c
	200	30	7.0 ± 0.59 _c	30	2.3 ± 0.61 _a	30	(1.1 ± 0.59) _a	30	(0.2 ± 0.74) _a
	400	30	6.4 ± 0.84 _b	30	3.1 ± 0.84 _a	30	(1.1 ± 0.45) _a	30	2.5 ± 5.48 _a
	800	30	6.6 ± 0.45 _c	30	1.3 ± 0.90 _a	30	(1.2 ± 0.68) _a	30	1.1 ± 0.91 _a

Continued

Table 113. Continued.

Sex	Treatment (ppm)	N	11/11/77-11/22/77	N	11/23/77-12/8/77	N	12/9/77-12/23/77
Males	DCPD 0	6	(2.0 ± 0.94) _a	6	(6.8 ± 2.21) _a	6	2.2 ± 1.17 _a
	100	6	0.4 ± 1.31 _a	6	(3.9 ± 1.54) _a	6	3.5 ± 0.85 _a
	200	6	0.0 ± 1.71 _a	6	(6.9 ± 1.60) _a	6	1.9 ± 1.05 _a
	400	6	1.4 ± 0.74 _a	6	(5.5 ± 1.28) _a	6	4.1 ± 1.45 _a
	800	6	(0.0 ± 0.97) _a	6	(8.7 ± 0.89) _a	5	1.0 ± 2.64 _a
Females	DCPD 0	24	(0.7 ± 1.04) _a	24	(7.4 ± 0.74) _a	24	5.8 ± 0.96 _a
	100	23	(0.7 ± 0.80) _a	23	(9.2 ± 0.77) _a	23	5.2 ± 1.16 _a
	200	24	0.9 ± 0.93 _a	24	(5.6 ± 0.85) _a	24	3.6 ± 1.01 _a
	400	24	2.0 ± 0.94 _a	24	(7.8 ± 0.97) _a	24	6.6 ± 1.16 _a
	800	24	2.2 ± 1.15 _a	22	(7.7 ± 0.86) _a	22	4.1 ± 1.20 _a
Combined Sexes	DCPD 0	30	(1.0 ± 0.86) _a	30	(7.2 ± 0.74) _a	30	5.1 ± 0.85 _a
	100	29	(0.5 ± 0.70) _a	29	(8.1 ± 0.79) _a	29	4.8 ± 0.94 _a
	200	30	0.7 ± 0.82 _a	30	(5.9 ± 2.40) _a	30	3.3 ± 0.85 _a
	400	30	1.8 ± 0.77 _a	30	(7.4 ± 0.84) _a	30	6.1 ± 0.99 _a
	800	30	1.7 ± 0.95 _a	28	(7.9 ± 0.71) _a	27	3.5 ± 1.12 _a

Table 114. Effect of chronic administration of DCPD to mink upon feed consumption.

Date	DCPD treatment (ppm)				
	0	100	200	400	800
8/3	249 ± 8.4 ⁽¹⁾ _a	230 ± 10.1 _a	226 ± 12.9 _a	208 ± 11.0 _b	197 ± 10.6 _c
8/18	249 ± 20.0 _a	283 ± 20.2 _a	255 ± 20.3 _a	263 ± 18.6 _a	234 ± 14.2 _a
9/1	212 ± 17.1 _a	191 ± 18.0 _a	177 ± 17.9 _a	204 ± 17.0 _a	190 ± 13.4 _a
9/17	284 ± 17.0 _a	266 ± 22.6 _a	249 ± 17.6 _a	235 ± 14.4 _a	221 ± 9.4 _c
9/30	269 ± 17.9 _a	283 ± 19.5 _a	252 ± 18.5 _a	260 ± 10.7 _a	221 ± 12.5 _a
10/13	248 ± 11.0 _a	250 ± 18.3 _a	234 ± 13.9 _a	200 ± 17.0 _a	230 ± 13.8 _a
11/3	213 ± 17.8 _a	261 ± 17.1 _a	206 ± 18.0 _a	200 ± 17.1 _a	192 ± 15.5 _a
11/15	119 ± 18.5 _a	194 ± 15.1 _c	179 ± 14.9 _b	168 ± 12.5 _a	193 ± 15.6 _c

(1) Means in the same row with the same subscript are not significantly different from the control (P>0.05).

Table 115. Calculation of estimated daily intake of DCPD by mink fed DCPD at various levels for 12 months.

DCPD level in diet (ppm)	Mean daily feed consumption (g) ¹	DIMP ingested/ day (mg)	Mean body wt. (g) ²	Daily ingested dose (mg/kg/day)
0	230	0	1071	0
100	245	24.5	1038	23.6
200	222	44.4	1047	42.4
400	217	86.8	1021	85.0
800	210	168.0	989	169.9

¹ Represents mean feed consumption for 8 measurements taken over 4 months.

² Represents mean body weight for 18 measurements taken over 12 months.

Table 116. Effect of chronic dietary administration of DCPD to male and female mink upon peripheral blood mean packed cell volume (hematocrit %).

Sex	Treatment (ppm)	Date measured and number included in analysis							
		7/21/77		10/18/77		1/17/78		6/30/78	
		N	Hct. % ± S.E.	N	Hct. % ± S.E.	N	Hct. % ± S.E.	N	Hct. % ± S.E.
Males	DCPD 0	6	45.7 ± 0.88 ⁽¹⁾ _a	6	55.3 ± 0.61 _a	6	56.8 ± 0.82 _a	6	56.5 ± 0.99 _a
	100	6	45.8 ± 0.83 _a	6	52.3 ± 1.11 _a	5	52.8 ± 1.31 _a	5	53.2 ± 0.91 _a
	200	6	45.2 ± 0.91 _a	6	53.3 ± 0.70 _a	5	55.1 ± 0.61 _a	5	55.8 ± 1.68 _a
	400	6	46.8 ± 1.02 _a	6	55.5 ± 0.82 _a	6	55.4 ± 1.04 _a	6	56.3 ± 0.48 _a
	800	6	44.8 ± 0.56 _a	6	55.2 ± 0.28 _a	6	55.5 ± 0.98 _a	6	54.8 ± 0.83 _a
Females	DCPD 0	24	45.2 ± 0.38 _a	24	54.1 ± 0.49 _a	23	53.9 ± 0.64 _a	19	53.6 ± 0.78 _a
	100	24	46.4 ± 0.43 _a	23	52.6 ± 0.39 _b	23	52.9 ± 0.50 _a	21	53.5 ± 0.62 _a
	200	24	45.9 ± 0.46 _a	24	53.9 ± 0.53 _a	24	53.9 ± 0.58 _a	21	53.9 ± 0.80 _a
	400	23	46.6 ± 0.67 _a	24	52.7 ± 0.35 _a	24	54.6 ± 0.62 _a	23	54.8 ± 0.66 _a
	800	24	46.7 ± 0.47 _a	24	53.6 ± 0.47 _a	22	53.9 ± 0.53 _a	21	52.9 ± 0.95 _a
Combined Sexes	0	30	45.3 ± 0.39 _a	30	54.4 ± 0.42 _a	29	54.5 ± 0.58 _a	25	54.3 ± 0.68 _a
	100	30	46.3 ± 0.38 _a	29	52.6 ± 0.39 _a	28	52.9 ± 0.47 _a	26	53.5 ± 0.53 _a
	200	30	45.8 ± 0.42 _a	30	53.8 ± 0.45 _a	29	54.1 ± 0.50 _a	26	54.3 ± 0.68 _a
	400	29	46.6 ± 0.57 _a	30	53.3 ± 0.38 _a	30	54.8 ± 0.54 _a	29	55.1 ± 0.55 _a
	800	30	46.3 ± 0.42 _a	30	53.9 ± 0.40 _a	28	54.2 ± 0.49 _a	27	53.3 ± 0.78 _a

(1) Means in the same column with the same subscript are not significantly different from their respective control values (P>0.05).

At no time during the chronic study was hemoglobin concentration of the treated animals shown to be significantly different from that of the control (Table 117). Mean corpuscular hemoglobin concentration (derived by the division of hemoglobin concentration by the hematocrit value x 100) was not significantly different for treatment animals with respect to controls, except for an initial deviation for animals fed the 100 ppm DCPD diet. When analyzed on a sex-dependent basis, this difference failed to appear (Table 118).

Differential leukocyte counts failed to establish a dose related difference in treatment groups with respect to control (Table 119).

The effect of chronic dietary exposure to DCPD upon reproductive performance is shown in Table 120. Whelping rates, gestation length, fecundity, kit weight at birth, and secondary sex ratios were not adversely affected by DCPD administration. No differences in male fertility, as determined by presence of sperm in post-coital vaginal aspirations was noted among any of the treatment groups.

Performance of kits and whelping dams during lactation is shown in Table 121. A significant depression in kit weight at four weeks was noted for animals on the three highest DCPD-treatment levels. Kit mortality, however, was not significantly affected by DCPD treatments. Although all females exhibited marked weight loss during lactation, no significant differences in body weight were noted among treatments.

At the termination of the chronic test, no gross pathological or histopathological changes consistent with toxicosis were noted for any DCPD-treatment group. No significant differences were noted in organ weights, with the exception of spleen weight for animals on the 400 ppm DCPD treatment and testes weight for males on the 800 ppm DCPD treatment (Table 122).

Discussion

DCPD chronically fed to mink caused no differential mortality for any treatment group. Mortality was not appreciably greater than the natural mortality for the first year mink that occurs in commercial fur ranch operations (Kennedy, 1952).

Body weights were not consistently affected by any DCPD treatment, although the animals fed 800 ppm DCPD occasionally had lower body weights than controls. Since mink body weights are highly varied among individuals, a more accurate measure of their collective performance is to determine a mean for the individuals' percent change in body weight. When comparisons were made between DIMP-treatment groups and the control group, a difference in percent change on body weight was not consistent over the measurement periods. The growth of mink on the DCPD diets and the control was similar to growth patterns reported by other workers (Aulerich and Schaible, 1965; Kumeno *et al.*, 1970; Oldfield *et al.*, 1968; Seier *et al.*, 1970; Travis and Schaible, 1961).

Table 117. Effect of chronic dietary administration of DCPD to male and female mink upon peripheral blood mean hemoglobin concentration.

Sex	Treatment (ppm)	N	7/21/77	N	10/18/77	N	1/17/78	N	6/30/78
Males	DCPD 0	6	17.7 ± 0.32 _a ⁽¹⁾	6	21.6 ± 0.33 _a	6	20.8 ± 0.67 _a	6	21.2 ± 0.24 _a
	100	6	17.2 ± 0.23 _a	6	21.6 ± 0.41 _a	5	19.5 ± 0.53 _a	5	19.8 ± 0.50 _a
	200	6	17.2 ± 0.45 _a	6	22.2 ± 0.82 _a	5	20.6 ± 0.40 _a	5	20.9 ± 0.57 _a
	400	6	18.1 ± 0.21 _a	6	22.2 ± 0.69 _a	6	21.3 ± 0.47 _a	6	21.0 ± 0.56 _a
	800	6	17.7 ± 0.24 _a	6	22.2 ± 0.63 _a	6	20.7 ± 0.41 _a	6	20.4 ± 0.26 _a
Females	DCPD 0	24	17.6 ± 0.22 _a	24	21.7 ± 0.55 _a	24	19.5 ± 0.29 _a	20	19.1 ± 0.27 _a
	100	24	17.2 ± 0.23 _a	23	21.5 ± 0.14 _a	23	19.5 ± 0.20 _a	20	19.5 ± 0.23 _a
	200	24	17.7 ± 0.19 _a	23	22.0 ± 0.41 _a	24	19.8 ± 0.57 _a	21	20.2 ± 0.40 _a
	400	24	18.1 ± 0.29 _a	23	20.8 ± 0.34 _a	24	20.3 ± 0.24 _a	23	19.9 ± 0.23 _a
	800	24	18.2 ± 0.21 _a	24	21.3 ± 0.32 _a	23	20.3 ± 0.24 _a	21	19.4 ± 0.33 _a
Combined Sexes	DCPD 0	30	17.6 ± 0.19 _a	30	21.7 ± 0.43 _a	30	19.8 ± 0.28 _a	26	19.6 ± 0.27 _a
	100	30	17.2 ± 0.19 _a	29	21.5 ± 0.14 _a	28	19.5 ± 0.19 _a	25	19.5 ± 0.21 _a
	200	30	17.6 ± 0.18 _a	29	21.3 ± 0.81 _a	29	19.9 ± 0.48 _a	26	20.3 ± 0.35 _a
	400	30	18.1 ± 0.24 _a	29	21.1 ± 0.32 _a	30	20.5 ± 0.22 _a	29	20.1 ± 0.23 _a
	800	30	18.1 ± 0.18 _a	30	21.4 ± 0.29 _a	29	20.3 ± 0.21 _a	27	19.6 ± 0.27 _a

(1) Means in the same column with the same subscript are not significantly different from their respective control values (P>0.05).

Table 118. Effect of chronic dietary administration of DCPD to male and female mink upon mean corpuscular hemoglobin concentration (MCHC).

Sex	Treatment (ppm)	Mean corpuscular hemoglobin concentrations (\pm S.E.) by date and number of mink							
		N	7/21/77	N	10/18/77	N	1/17/78	N	6/30/78
Males	DCPD 0	6	38.8 \pm 0.70 ^a (1)	6	39.1 \pm 0.80 _a	6	36.8 \pm 0.46 _a	6	37.5 \pm 0.68 _a
	100	6	37.6 \pm 0.88 _a	6	41.3 \pm 0.35 _a	5	37.1 \pm 0.42 _a	5	37.2 \pm 0.40 _a
	200	6	38.1 \pm 0.61 _a	6	41.6 \pm 1.23 _a	5	37.1 \pm 0.72 _a	5	37.4 \pm 0.89 _a
	400	6	38.8 \pm 0.93 _a	6	39.9 \pm 1.34 _a	6	38.5 \pm 0.41 _a	6	37.3 \pm 0.86 _a
	800	6	39.4 \pm 0.67 _a	6	40.3 \pm 1.22 _a	6	37.3 \pm 0.76 _a	6	37.2 \pm 0.49 _a
Females	DCPD 0	24	38.8 \pm 0.37 _a	24	40.1 \pm 1.02 _a	24	36.9 \pm 0.43 _a	20	37.1 \pm 1.28 _a
	100	24	37.2 \pm 0.41 _a	23	40.9 \pm 0.26 _a	23	37.0 \pm 0.17 _a	20	36.3 \pm 0.32 _a
	200	24	38.6 \pm 0.33 _a	23	41.0 \pm 0.71 _a	24	37.3 \pm 0.28 _a	21	37.5 \pm 0.33 _a
	400	24	38.6 \pm 0.50 _a	23	39.4 \pm 0.53 _a	24	37.2 \pm 0.47 _a	23	36.4 \pm 0.41 _a
	800	24	38.9 \pm 0.52 _a	24	39.7 \pm 0.60 _a	22	37.7 \pm 0.30 _a	21	36.8 \pm 0.63 _a
Combined Sexes	DCPD 0	30	38.8 \pm 0.33 _a	30	39.9 \pm 0.84 _a	30	36.9 \pm 0.35 _a	26	37.2 \pm 1.00 _a
	100	30	37.2 \pm 0.37 _b	29	41.0 \pm 0.21 _a	28	37.0 \pm 0.16 _a	25	36.5 \pm 0.28 _a
	200	30	38.5 \pm 0.29 _a	29	41.2 \pm 0.62 _a	29	37.4 \pm 0.26 _a	26	37.4 \pm 0.32 _a
	400	30	38.6 \pm 0.44 _a	29	39.5 \pm 0.51 _a	30	37.4 \pm 0.40 _a	29	36.6 \pm 0.38 _a
	800	30	39.0 \pm 0.44 _a	30	39.8 \pm 0.52 _a	28	37.6 \pm 0.29 _a	27	36.9 \pm 0.50 _a

(1) Means in the same column with the same subscript are not significantly different from their respective control values ($P > 0.05$).

Table 119. Effect of chronic administration of DCPD to adult mink upon differential leukocyte count.

Treatment (ppm)	N	Leukocyte cell type (% ± S.E.)					
		Basophils	Eosinophils	Band- neutrophils	Segmented neutrophils	Lymphocytes	Monocytes
DCPD 0	22	0.4 ± 0.15 _a ⁽¹⁾	3.5 ± 0.70 _a	1.6 ± 0.57 _a	63.2 ± 2.87 _a	29.3 ± 2.40 _a	2.0 ± 0.24 _a
100	25	0.2 ± 0.10 _a	4.7 ± 1.19 _a	1.4 ± 0.35 _a	63.6 ± 3.32 _a	29.2 ± 2.85 _a	2.5 ± 0.36 _a
200	24	0.2 ± 0.08 _a	3.4 ± 0.49 _a	1.3 ± 0.37 _a	57.3 ± 2.90 _a	33.9 ± 2.30 _a	3.1 ± 0.85 _a
400	28	0.3 ± 0.10 _a	4.6 ± 0.91 _a	0.4 ± 0.14 _a	58.1 ± 3.23 _a	34.5 ± 2.70 _a	2.1 ± 0.24 _a
800	26	0.3 ± 0.10 _a	3.5 ± 0.61 _a	0.9 ± 0.20 _a	62.9 ± 2.60 _a	30.7 ± 2.56 _a	2.8 ± 0.67 _a

⁽¹⁾ Means with the same subscript are not significantly different from the control (P>0.05).

Table 120. Effect of DCPD on reproductive performance of mink.

	DCPD treatment (ppm)				
	0	100	200	400	800
No. females mated	22	22	21	24	22
Avg. no. times mated	1.9	2.0	1.8	2.0	2.0
% whelped	63.6	81.8	66.7	70.8	81.8
Avg. length of gestation (days \pm S.E.)	51.4 \pm 1.31 ⁽¹⁾ _a	49.3 \pm 1.28 _a	50.1 \pm 1.86 _a	51.7 \pm 1.19 _a	51.1 \pm 1.55 _a
No. of kits at birth:					
Alive	69	96	77	95	96
Dead	8	12	7	3	5
No. live kits/female whelped \pm S.E.)	5.21 \pm 0.62 _a	5.33 \pm 0.50 _a	5.50 \pm 0.60 _a	5.59 \pm 0.55 _a	5.33 \pm 0.40 _a
Avg. wt. of kits at birth (g \pm S.E.)	9.62 \pm 0.45 _a	9.97 \pm 0.43 _a	9.38 \pm 0.34 _a	9.32 \pm 0.37 _a	8.74 \pm 0.41 _a
Secondary sex ratio, no. male kits/no. female kits	1.24	1.02	1.11	0.98	1.02

(1) Means in the same row with the same subscript are not significantly different from the control (P>0.05).

Table 121. Performance of nursing offspring and dams fed DCPD.

Treatment	Whelping ♀ lactating at 4 wks (X)	Kit mortality to 4 wks (λ)	No. kits/ lactating ♀ ± S.E.	Avg. wt. of kits at 4 wks. (g ± S.E.)	Kit biomass ¹	Avg. wt. of whelping dam (g ± S.E.)	Avg. wt. of lactating ♀ 4 wks. post- partum (g ± S.E.)
DCPD 0	79	21.7	4.91 ± 0.51 _a	165 ± 2.6 _a	810.2	978 ± 27.4 _a	878 ± 38.2 _a
100	89	22.9	4.63 ± 0.51 _a	158 ± 2.7 _a	732.5	1000 ± 27.6 _a	867 ± 23.1 _a
200	93	33.8	3.92 ± 0.54 _a	146 ± 5.2 _b	571.9	981 ± 42.4 _a	900 ± 44.4 _a
400	100	14.7	4.76 ± 0.38 _a	147 ± 2.6 _b	701.1	995 ± 25.1 _a	913 ± 29.1 _a
800	100	15.6	4.50 ± 0.41 _a	128 ± 3.1 _b	576.0	939 ± 20.2 _a	843 ± 20.5 _a

¹ Biomass - average kit body weight gain between birth and 4 weeks of age x the average number of kits raised per lactating female.

Table 122 Effect of chronic administration of DCPD to mink on organ weights (g \pm S.E.) at necropsy.

Organs	DCPC treatment (ppm)				
	0	100	200	400	800
Liver	27 \pm 1.5 ¹ _a	24 \pm 1.4 _a	26 \pm 1.0 _a	28 \pm 1.6 _a	32 \pm 2.0 _a
Spleen	3.3 \pm 0.29 _a	2.5 \pm 0.20 _a	2.6 \pm 0.21 _a	2.4 \pm 0.16 _b	2.5 \pm 0.24 _a
Kidney	4.8 \pm 0.22 _a	4.5 \pm 0.22 _a	4.4 \pm 0.18 _a	4.7 \pm 0.21 _a	4.7 \pm 0.23 _a
Lungs	7.8 \pm 0.42 _a	7.0 \pm 0.35 _a	7.6 \pm 0.41 _a	8.1 \pm 0.43 _a	7.3 \pm 0.31 _a
Adrenals	0.10 \pm 0.015 _a	0.11 \pm 0.007 _a	0.10 \pm 0.011 _a	0.12 \pm 0.011 _a	0.13 \pm 0.012 _a
Heart	6.0 \pm 0.30 _a	5.8 \pm 0.28 _a	5.5 \pm 0.27 _a	5.9 \pm 0.26 _a	5.6 \pm 0.24 _a
<u>Genitals:</u>					
Testes	1.8 \pm 0.1 _a	1.6 \pm 0.3 _a	1.8 \pm 0.2 _a	1.8 \pm 0.2 _a	1.1 \pm 0.1 _b
Ovaries	0.10 \pm 0.01 _a	0.11 \pm 0.01 _a	0.11 \pm 0.01 _a	0.11 \pm 0.01 _a	0.11 \pm 0.01 _a
Brain	8.1 \pm 0.18 _a	7.8 \pm 0.15 _a	7.9 \pm 0.20 _a	7.9 \pm 0.13 _a	7.9 \pm 0.13 _a

¹ Means in the same row with the same subscript are not significantly different from the control (P > 0.05).

Feed consumption was depressed in several instances for mink on the 800 ppm DCPD treatment. This may have been due to a decreased palatability associated with the odor of DCPD at higher concentrations. However, this depression in feed consumption was transitory in nature, and the significantly greater feed consumption of mink fed DCPD over the control value on one occasion tends to contradict any supposed palatability problem at high dietary concentrations. In general, feed consumption for all groups was somewhat higher than reported for adult female mink by Schiabile, 1970. Based upon the amount of DCPD ingested daily by each treatment group, it is unlikely that DCPD, when ingested chronically in moderately high concentrations, adversely affects the feed-conversion efficiency of mink.

The analysis of hematological indices revealed no indications of hemopoietic disturbances caused by chronic DCPD administration. Hematocrit (packed cell volume), hemoglobin, and mean corpuscular hemoglobin concentration values were in accordance with values reported by other workers for normal adult mink (Asher *et al.*, 1976; Fletch and Karstad, 1972; Kubin and Mason, 1948; and Rotenburg and Jorgensen, 1971).

Even though there was no difference between treatment groups and controls, differential leukocyte counts made at the termination of the study, on blood collected from all treatment groups, differed in several respects from the values reported by other researchers. Mature (segmented) neutrophils and lymphocyte numbers differed from the results of counts made by Fletch and Karstad (1972), Gilbert (1969), and Kennedy (1935), who all reported nearly equal percentages of these leukocyte types at about 45-47% each. However, Asher *et al.* (1976) have shown seasonal and age dependent variations in white cell percentages in mink; a consideration not given by previous workers. When compared to values given by Asher *et al.* for an equivalent time of the year, the neutrophil and lymphocyte percentages of mink in this study were well correlated.

Monocyte percentages were less in the counts made in the animals in this test, when compared to the 6-9% values from several studies (Fletch and Karstad, 1972; Asher *et al.*, 1976). However, counts made by Gilbert (1969) and by Kennedy (1935) place monocytes in the 1-2% range, as was found for the animals in this test.

The reproductive potential of mink chronically exposed to DCPD was not adversely affected. Indices of reproductive performance were not markedly different from those present in the literature (Aulerich *et al.*, 1975; Aulerich and Ringer, 1977; Enders, 1952; Hansson, 1947; Schaible and Travis, 1958).

Performance of kits nursed by females on the 200, 400, and 800 ppm DCPD diets was poorer than that of control kits. The decreased weight gain of these kits over a four week nursing period is suggestive of mammary excretion of the chemical, especially since it is highly lipid soluble. However, disturbances in maternal metabolism such as lactogenic capability, fat metabolism and excretion, calcium metabolism, or a myriad of other problems may be responsible for the

reduced kit growth. Weight gain of control kits during this period was well correlated with data supplied by other workers (Aulerich et al., 1975; Aulerich and Ringer, 1977; Oldfield et al., 1968).

No gross or histopathological abnormalities were found to be consistent for any DCPD treatment, at the conclusion of the test. Spleen weights were substantially heavier in the 400 ppm diet than in the control, but this difference was not seen in the 800 ppm DCPD-treated animals. Since individuals on a higher dosage treatment failed to show a similar effect, the difference in spleen weights is probably associated with chance variation or sampling error. Organ weights were not far removed from values reported by other workers (Aulerich and Ringer, 1977; Wood et al., 1965). Kidney and lung weights for mink in this test were slightly lighter than the weights reported for these organs by Wood et al. (1965). Conversely, heart weights were found to be greater than heart weights reported in the same study. According to Wood et al. (1965), the method of euthanatization can affect organ weights. Since the method of euthanatization employed by Wood and co-workers (electrocution) was different from the technique used in this study (cervical dislocation), the difference in these organ weights is more easily reconciled. The reduction in testes weight exhibited by the males fed 800 ppm DCPD may have been due to an acceleration of the normal seasonal reduction which occurs in this species (Bostrom et al., 1968). However, histological examination revealed no differences in the state of seasonal regression.

CONCLUSIONS

1. The acute oral toxicity of DCPD for mink was estimated to be greater than 1000 mg/kg BW.
2. The 21-day subacute dietary LC₅₀ of DCPD for mink was determined to be 6800 ppm.
3. The chronic ingestion of DCPD in the diet by mink had no effect on growth, survival, or reproductive performance. Neonate weight gain was significantly reduced by the ingestion of 200, 400, and 800 ppm DCPD by lactating dams. Testes weight of males fed 800 ppm DCPD was significantly less than the controls.

Tissue Residues in Bobwhite Quail
and Mallard Ducks Fed or Dosed
14C - Dicyclopentadine .

Introduction

The future restoration of military installations previously exposed to pollutants requires information on the biological hazards of these pollutants. One possible hazard to consider is that wildlife, including birds, would become vectors in passing the pollutants along the food chain to their predators. Thus, consideration should be given to the possibility that the pollutants are not only a hazard to the animals being exposed, but also those preying on the exposed animals.

Birds are particularly difficult to keep out of military reservations because peripheral fencing does not limit their boundary. Insects and/or plants, as well as water on the premises may serve as reservoirs for pollutants. Consumption of these pollutants could result in tissue residues. A chemical of concern on some reservations is dicyclopentadiene (DCPD). To assess the possible residue levels this chemical may induce in two species of birds, the Bobwhite quail (Colinus virginianus) and the Mallard duck (Anas platyrhynchos) were fed and dosed ^{14}C labeled DCPD. The rate of accumulation and depletion of the radioactivity were ascertained. Presumably, this information would reveal the body burden to short exposure of these pollutants, and the rapidity for depletion of residues upon release from such exposure.

Methods and Procedures

Feeding

The experiments were conducted in Room #1 of Building #4 on the Michigan State University's Poultry Science Research and Teaching Center (PSRTC). Two adult species of birds, Bobwhite quail and Mallard ducks, were used in the study. The quail were housed in battery brooders, 6 decks high divided into 2 compartments on each deck. Each compartment was 99.4 x 68.6 x 24.1 cm (length x width x height) with 6 quail, 3 of each sex, in each compartment. The Bobwhite quail were from a colony maintained for research and teaching at the PSRTC.

Mallard ducks originated from two sources, Max McGraw Wildlife Foundation, Dundee, Illinois 60118, and Frost Game Farm, Colona, Wisconsin 54930. They were phenotypically indistinguishable from wild Mallards. They were regularly housed in pens measuring 152.4 x 152.4 x 76.2 cm (length x width x height). However, for the experiment the ducks were moved into growing-type batteries 4 decks high, each deck measuring 121.9 x 76.2 x 33.0 cm (length x width x height). Six ducks, 3 of each sex, comprised a group in a compartment.

Supplemental heat was provided in the room to maintain a temperature of 12.8°C. There was a 9-day pretest period during which feed intake and body weight were monitored. This was followed by the experimental period during which radioactive diets were fed and

the birds killed according to the schedule in Table 123. The experiment with ^{14}C -DCPD was conducted April 6 to 21, with the period of April 6-11 being the pretest period.

Animal care was in accordance with N.I.H. policy, Public Law, and the guidelines of H.E.W.

The diet fed to the quail was a stock breeder ration (Table 124) prepared by a local feedmill to specifications issued by the Michigan State University's Department of Poultry Science. The ration fed to the ducks was a commercial ration (unknown formula) specified for breeder ducks. Feed was provided ad libitum.

The radioactive DCPD for the experiments was obtained from New England Nuclear¹, and checked by them for purity just prior to shipment. DCPD was at least 99% pure and ring-labeled. The specific activity was 2.11 mCi/mM, which calculates to 15.9 $\mu\text{Ci}/\text{mg}$.

The radioactive compounds were blended into the feed via a premix. The latter was prepared by grinding 1 kg of the breeder ration to pass through a #20 (U.S. Bureau of Standards) sieve, and then adding a weighed amount of cold chemical previously blended with a weighed amount of the ^{14}C -chemical to yield the calculated dilution and quantity of the chemical to prepare a diet with 100 mg of ^{14}C -DCPD per kg of diet. Nine mg of ^{14}C -DCPD stock solution were blended with 2248 mg of non-radioactive DCPD, and 2000 mg of this was thoroughly blended with 998 g of the sifted diet to yield a premix with 2 mg ^{14}C -DCPD/g diet. The final rations containing chemical at 100 ppm (mg/kg) were blended in closed containers by tumbling the premix with diet at 5% of dietary weight.

Dosing Experiments

The procedures for housing the Bobwhite quail and Mallard ducks for the dosing experiments were the same as those used in the feeding experiment. The ducks were dosed, per os, on September 19, 1977 and the quail on September 26, with ^{14}C -DCPD, according to the protocol in Table 125, radioactive compound was administered directly into the crop using polyethylene tubing attached to a syringe. Corn oil was the carrier. The dosing solutions of corn oil with radioactive chemical were prepared by adding stock ^{14}C -DCPD to corn oil containing 5% by weight of the respective chemical. The final solutions of corn oil for dosing contained 0.39 $\mu\text{Ci}/\text{ml}$ of ^{14}C -DCPD to dose the ducks, and 1.23 $\mu\text{Ci}/\text{ml}$ of ^{14}C -DCPD to dose the quail. The calculated dose to be administered was based on 100 mg of chemical per kg body weight, and a target of about 1 μCi of ^{14}C per bird.

The birds were fasted overnight prior to receiving the single oral dose of radioactive compound in corn oil.

¹ The citation of the manufacturer's name does not constitute an endorsement by the Department of the Army.

TABLE 123. THE PROTOCOL TO DETERMINE THE DISTRIBUTION OF ^{14}C FROM ^{14}C -LABELED DCPD IN TWO SPECIES OF BIRDS (BOBWHITE QUAIL AND MALLARD DUCK) GIVEN THE RADIOLABELED COMPOUNDS IN THE DIET, AND THE PATTERN FOR DEPLETION OF ^{14}C AFTER WITHDRAWAL OF THE RADIOACTIVE DIET AND SUBSTITUTION OF FEED WITHOUT THE ABOVE CHEMICALS

Species	Sex	Number of birds sacrificed at stated time ¹							Σ
		Controls killed		^{14}C Days fed C-chemical ²		Days after withdrawal			
		Day 0	Day 10	Day 3	Day 5 ²	Day 3	Day 5		
Bobwhite quail	♀	3	3	3	3	3	3	3	18
	♂	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>18</u>
		6	6	6	6	6	6	36	
Mallard duck	♀	3	3	3	3	3	3	3	18
	♂	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>18</u>
		6	6	6	6	6	6	36	

¹ Samples to be processed for radioactivity: red blood cells, plasma, liver, muscle, kidney, skin, brain, adipose.

² Day 5 on feed containing chemical is day zero of withdrawal.

TABLE 124. THE COMPOSITION OF THE DIET FED TO QUAIL IN THE FEEDING EXPERIMENTS WITH ¹⁴C-DCPD

Ingredient	Amount per 1000 parts
Corn, #2 yellow	450.2
Soybean meal, 49%	327
Meat scrap, 50%	50
Alfalfa meal, dehy.	45
Animal fat, stabl. ¹	57
Limestone	50
Dicalcium phosphate	7
Choline chloride, 50%	3
Methionine hydroxy analogue	1
Salt, iodized	3.8
Mineral mix A	3
Vitamin mix A	3

¹ Ethoxyquin (Antioxidant) 56.8 mg/kg.

Killing and Tissue Harvesting

The procedure for procuring tissue samples was common to both the feeding and dosing experiments. Prior to being killed, a blood sample of 3 to 5 ml was obtained from a duck or quail by cardiac puncture using a heparinized syringe and stainless steel needle, 3.81 cm x 20 gauge and 2.54 x 22 gauge, for duck and quail, respectively. Ducks were killed by cervical dislocation; the quail were killed either with an overdose of chloroform in a closed container, or by cervical dislocation. The blood was processed immediately to obtain hematocrit values, and the remaining greater portion was transferred to chilled test tubes set in an ice bath. The blood was brought from the PSRTC to the laboratory for centrifugation and the plasma separated from the red blood cells (rbcs). The latter were washed twice with 3 ml of 0.9% saline. Then plasma and rbcs were frozen at -21°C , and stored in this state until thawed for ^{14}C analysis. Samples of tissues from breast muscle, skin (without feathers), adipose from the abdominal area, kidneys, liver, and brain were immediately procured from the dead bird, wrapped in individual plastic bags with identification, and stored on ice until brought into the laboratory. Then they were transferred to a freezer at -21° and stored in this state until analyzed.

Preparation of Tissues for ^{14}C Counting, and Counting Methodology

Plasma samples were thawed and 200 μl pipetted into vials for liquid scintillation counting. Twelve ml of 3a708 "Complete Counting Cocktail" (Research Products Int'l. Corp., Elk Grove Village, IL 60007) were added to the vial, the vial shaken vigorously to disperse the plasma, and then counted for ^{14}C . RBCs were thawed and stirred with a stainless steel spatula to effect uniform distribution of sample. A sample of rbcs was accurately weighed to within ± 1 mg of 100 mg in a tared vial for liquid scintillation counting by drop-wise addition of rbcs from the spatula. To this was added 1 ml of UnisolTM, a tissue solubilizer. The sample was heated at 50°C for 3 hours in an oven, and/or allowed to stand overnight to solubilize the sample. Sometimes 48 hours of solubilization were required for complete preparation of the sample. Then 10 ml of UnisolTM complement were added to the vial, followed by 2-4 drops of 30% hydrogen peroxide to reduce coloration. The vial cap was put on tightly and the vial shaken. Then the cap was unscrewed and the vial permitted to stand for 20 minutes. The cap was returned onto the vial and the vial counted for ^{14}C .

Samples from the other tissues were obtained by cutting chunks into smaller and smaller pieces, and then randomly selecting tiny pieces to obtain an accurately weighed amount to within ± 1 mg of 100 mg in a tared vial. These samples were solubilized with 1 ml UnisolTM, as indicated above. The UnisolTM complement was added, and only on liver samples, which were highly colored, were 2-4 drops of 30% hydrogen peroxide used to reduce coloration.

Samples were counted in either a Nuclear-Chicago Liquid Scintillation Counter Model 724 System; or a Nuclear-Chicago Isocap 300 Series Counter.

TABLE 125. THE PROTOCOL TO DETERMINE THE DISTRIBUTION AND DEPLETION PATTERN OF ¹⁴C-DCPD IN ADULT BOBWHITE QUAIL AND MALLARD DUCKS AFTER A SINGLE ORAL DOSE

Species	Sex	Number of birds killed at stated time to obtain samples ¹ for ¹⁴ C determination				
		Stated time birds were killed--hours				
		0	2	24	48	Σ
Bobwhite quail	♀	3	3	3	3	12
	♂	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>12</u>
		6	6	6	6	24
Mallard ducks	♀	3	3	3	3	12
	♂	<u>3</u>	<u>3</u>	<u>3</u>	<u>3</u>	<u>12</u>
		6	6	6	6	24

¹ Samples to be processed for radioactivity: red blood cells, plasma, liver, muscle, kidney, skin, brain, adipose and excreta.

A duplicate set of samples of each tissue, representative of all birds on a particular experiment, were processed before they were counted as a set. Each sample was counted for 10 minutes in a complete cycle, and all samples went through 3 cycles. Thus, total count was for 30 minutes. For example, all of the muscle samples from the quail fed ^{14}C -DCPD were removed from the freezer and prepared as one set. These were counted along with ^{14}C -DCPD standard and blank, and a ^{14}C -benzoic acid standard in toluene and a toluene blank. The latter two samples were obtained from Nuclear-Chicago Corp. to be used to establish counting efficiency to standards. A set included, in the case of the feeding experiment, samples obtained from the two groups of controls, one group of 6 quail killed at the start of the experiment, the other group of 6 at the completion of the experiment; and the muscle samples obtained from quail killed on 3 and 5 days of feeding diets with ^{14}C -DCPD, and 3 and 5 days after withdrawal of the radioactive diets.

Calculations for Radioactivity in Tissues

The data as counts per minute (cpm) were analyzed statistically in a minicomputer's program for analysis of variance (ANOV). If significant differences among group means were detected, then the samples from the control groups were compared in the ANOV program. A non-significant F-value indicated that ^{14}C dust from the radioactive diets was not a contributing factor to ^{14}C counts in tissue samples. Therefore, the data from the two control groups were pooled and considered as one group of 12 control samples. The mean value of the control group was subtracted from each cpm of the other individual values to derive a net count for that experimental sample indicative of ^{14}C chemical from the feed. Only one set of control values of the 8 tissues undergoing analyses showed a significant difference indicative of possible ^{14}C dust contamination. However, since none of the other tissues from these control birds showed a comparable effect, we considered the difference of 1.3 cpm to be an aberrant trend. The controls in this case were also pooled to arrive at a mean value for the 12 samples.

Samples were corrected for quenching using internal standards, and for machine efficiency using the ^{14}C -benzoic acid standard supplied by the manufacturer of the scintillation counter. Internal standard corrections varied with each tissue with the greatest quenching occurring in samples prepared from rbc's. Machine efficiency for ^{14}C counting ranged between 72 and 82%, depending upon which scintillation counter was used. The Isocap 300 had the best efficiency.

Detection limits were based on the specified specific activity established by the manufacturer of the ^{14}C -DCPD, and the eventual dilution factors in admixing "cold" and radioactive compound for the feeding and dosing experiments. ^{14}C -DCPD was supplied at 3.34 mCi/ μM or 2.11 mCi/ μM . The latter ^{14}C -DCPD was used in the dosing experiments, while the former was mixed in the feed. These values

are transformable to 25.3 and 15.9 $\mu\text{Ci}/\text{mg}$ for the two lots of ^{14}C -DCPD, respectively. To determine the detection limit for a particular tissue undergoing radio-metric measurements the statistical concept employed was ANOV and Dunnett's t-test for a oneway comparison at a probability value of $P = .05$ for a significant difference. The standard deviation was that associated with the 12 control values, unless the ANOV showed no significant difference in a comparison of control vs. values from birds receiving radio-active feed or solutions; in those cases, the standard deviation was derived from the Error term of the ANOV. The formula used for calculating the Dunnett's allowance value, A, (Dunnett, 1955) was as follows:

$A = \text{Dunnett's } t_{.05} \times \text{std. dev.} \times \sqrt{1/n_1 + 1/n_2}$ where:

- (a) Dunnett's t value is obtained from the table at d.f. = 30, and for 4 treatments
- (b) std. dev. is the standard deviation for the 12 control values
- (c) n_1 = number of values in the control group
- (d) n_2 = number of experimental values in the comparison to the control values.

For example: plasma samples of 200 μl counted from Bobwhite quail, each counted for 3 x 10 min. averaged 30.0 ± 1.37 (mean + S.D.) as the background.

Where $A = 2.25 \times 1.37 \times \sqrt{1/12 + 1/1}$ in a comparison of the 12 control values to any 1 experimental value.

$A = 3.2$ cpm above background would be a significantly ($P = 0.05$) higher number. Thus, a count of 33.2 ($30 + 3.2$) would indicate detectable radioactivity.

The detection limits are then calculated by transposing the allowance value from cpm to dpm and dividing by the specific activity of the radioactive compound.

In the case of plasma samples reviewed above, the calculations showed the following:

$$\begin{aligned} \text{Detection limit} &= \text{Allowance value} \times \frac{1}{\text{quench factor}} \times \frac{1}{\text{sample size}} \times \\ &\quad \frac{1}{\text{machine efficiency}} \times \frac{1}{\text{specific activity in dpm}/\mu\text{gm}} = \frac{\mu\text{gm}}{\text{g or ml}} \\ &= 3.2 \text{ cpm} \times \frac{1}{0.925} \times \frac{1}{0.2 \text{ ml}} \times \frac{1}{0.74} = 23.3 \text{ dpm/ml plasma} \end{aligned}$$

The specific activity of ^{14}C -DCPD was 3.34 mCi/mM , which is equal to 25.3 $\mu\text{Ci}/\text{mg}$. A quantity of 9.0 mg of "radioactive" ^{14}C -DCPD at 25.3 $\mu\text{Ci}/\text{mg}$ was diluted to a final weight of 2257 mg DCPD, using

non-radioactive DCPD. Therefore, a total of 27.7 μCi was diluted to 2257 mg or to a concentration of 0.1009 $\mu\text{Ci}/\text{mg}$. At 2.2×10^6 dpm per μCi , this yielded a radioactive compound with 2.2×10^6 dpm \times $0.1009 \mu\text{Ci}/\text{mg} = 0.2219 \times 10^6$ dpm/mg = $\frac{21.0 \times 10^3 \text{ cpm}}{\text{mg}} = \frac{21.9 \text{ dpm}}{\mu\text{g } ^{14}\text{C-DCPD}}$

The detection limit of 23.3 dpm of $^{14}\text{C-DCPD}$ is equivalent to 23.3 dpm \times $\frac{1}{21.0/\text{dpm}/\mu\text{g}} = 0.105 \mu\text{g } ^{14}\text{C-DCPD}/\text{ml}$.

The calculations for the detection limits of other tissues followed the above procedure, but with the proper values substituted in each case. These detection limits are listed in each table giving the values of radioactive compound(s) found in the tissues.

Extraction of Feed for Radioactivity

At the conclusion of the feeding experiments involving $^{14}\text{C-DCPD}$ to ducks and quail, samples of the feed were removed, stored in plastic bags and frozen at -21°C . About 6 months afterward they were moved into a refrigerator at 8°C and stored there for 3 months. At that time 2 g samples of the feed were weighed into 50 ml glass centrifuge tubes, and extracted 3 times with 10 ml of either dioxane or chloroform:petroleum ether (1:1) or ethyl acetate, to remove DCPD from feeds with $^{14}\text{C-DCPD}$. Total volume of extracts was determined, and aliquots of 0.5 ml counted in 12 ml of cocktail. Recoveries of ^{14}C from the feed were calculated based on original ^{14}C specific activity introduced into the feed. One-half gram residue samples of feed remaining in the test tubes after extractions were also counted, and the residue portion weighed to determine the proportion of sample that was counted. Total dpm recovered from extracts and residues after extractions represented recovery of ^{14}C in feed. The proportion of ^{14}C in extractions presumably represented initial compound. No chromatograms were developed on the extractions and residue samples to determine percentage of parent compound remaining.

Results

Body Weight, Feed Intake, and Hematocrit

Feeding Experiments

Bobwhite quail used in the feeding experiments for $^{14}\text{C-DCPD}$ lost weight during the holding period of 9 days. This can be determined from the data in Table 126, by comparison between initial weight and weight on day 0, the day the experiment started. The quail moved into the batteries to be used in the $^{14}\text{C-DCPD}$ experiment, weighed 212 g, and lost about 14 g per bird. During the time the radioactive diets were fed, the body weights improved to some extent in most groups. The controls (Group 2) fared as well as the treated quail. Generally, feed intake was higher during the time the radioactive diets were fed (Table 127), and this appeared to account for the quail regaining some of their body weight.

TABLE 126. BODY WEIGHTS OF BODWHITE QUAIL FED ¹⁴C-DCPD @ 100

JR FEED WITHOUT DCPD, AND HEMATOCRIT AT TIME OF SACRIFICE

Group	Treatment	Bird No.	Sex	Initial wt. on 4/6/77	Change in body weight from initial weight					Hematocrit %	
					day 0 on	day 3 on	day 5 on	day 3 off	day 5 off		
1	None	1857	♂	192	-9					41.8	
		1853	♂	227	-17					28.8	
		1855	♂	201	-15					42.5	
		1854	♀	226	-32					39.5	
		1856	♀	212	-6					30.0	
		1991	♀	212	-4					27.5	
		Mean (±S.D.)		211(±14)		-13.8(±9.3)					36.4(±6.6)
2	None	1999	♂	193	-11	-10	-9	-9	-10	40.0	
		2000	♂	207	-20	-17	-20	-21	-21	30.5	
		1990	♂	211	-16	-10	-11	-13	-14	49.0	
		1996	♀	217	-23	-25	-26	-32	-33	36.5	
		1995	♀	206	-21	-14	-8	-14	-10	34.0	
		1997	♀	226	-3	+2	-7	-5	-1	35.0	
		Mean (±S.D.)		210(±11)		-15.6(±7.5)	-12.3(±8.9)	-13.5(±7.7)	-15.7(±9.6)	-14.8(±11)	37.5(±6.4)
3	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11	1992	♂	183	-1	+2				39.0	
		1993	♂	193	-8	-8				46.0	
		1994	♂	209	-10	-10				43.5	
		1528	♀	264	-30	-42				39.5	
		1527	♀	202	-11	-4				36.0	
		1526	♀	226	-12	-14				30.8	
		Mean (±S.D.)		213(±29)		-13.3(±12.7)	-12.7(±15.4)				40.6(±3.6)
4	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11	1551	♂	201	-10	-15	-17			37.0	
		1529	♂	206	-13	-11	-9			42.5	
		1530	♂	198	-14	-16	-17			36.8	
		1552	♀	205	-8	-4	-5			33.3	
		1553	♀	235	-21	-24	-29			26.5	
		1554	♀	234	-14	-21	-32			37.3	
		Mean (±S.D.)		213(±17)		-13.3(±4.4)	-15.2(±7.1)	-18.2(±10.7)			35.6(±5.3)
5	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11	1657	♂	228	-12	-9	-12	-13		41.8	
		1555	♂	212	-13	-10	-12	-14		46.0	
		1557	♂	186	-8	-4	-7	9		40.3	
		1560	♀	170	-9	-6	-4	-5		42.8	
		1524	♀	273	-18	-9	-8	-19		36.5	
		1550	♀	235	-24	-30	-33	-37		35.0	
		Mean (±S.D.)		209(±26)		-15.8(±10.0)	-11.3(±9.4)	-12.7(±10.4)	-13.2(±15.2)		40.4(±4.1)
6	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11	1627	♂	205	-9	-10	-9	-9	-11	38.0	
		1626	♂	202	-16	-17	-15	-16	-19	40.0	
		1629	♂	195	-9	-11	-11	-9	-11	40.5	
		1628	♀	220	-19	-16	-18	-16	-14	33.0	
		1630	♀	219	-25	-37	-41	-39	-51	41.5	
		1654	♀	231	-9	-7	-4	-21	-21	33.0	
		Mean (±S.D.)		213(±15)		-14.5(±6.7)	-16.3(±10.8)	-16.3(±13.0)	-18.3(±11.1)	-21.2(±15.2)	37.7(±3.8)
		Avg.	♂	203(±12)							40.2(±5.0)
		Avg.	♀	221(±19)							35.8(±4.3)

300

TABLE 127. THE AMOUNT OF FEED AND ¹⁴C-DCPD CONSUMED BY BOBWHITE QUAIL FED THE RADIOACTIVE COMPOUND AT 100 PPM IN THE DIET

Group	Feed Intake - g/b/d						¹⁴ C intake mg/b	Body wt. g	¹⁴ C-mg per kg body wt.
	Pre-Exptl. Period	Experimental period		Mean	Withdrawal period				
		Days 0-3 on	Days 3-5 on		Days 0-3 off	Days 3-5 off			
1 ^a	(6) 12.3	-	-	-	-	-	0	211	0
2 ^a	(6) 12.0	(6) 17.6	(6) 17.7	17.6	(6) 17.6	(6) 16.5	0	210	0
3	(6) 11.5	(6) 15.1	-	15.1	-	-	4.5	213	21.3
4	(6) 11.5	(6) 13.6	(6) 14.6	14.0	-	-	7.0	213	32.9
5	(6) 10.6	(6) 16.1	(6) 15.0	15.6	(6) 13.5	-	7.8	209	37.5
6	(6) 12.4	(6) 16.4	(6) 17.8	17.0	(6) 15.9	(6) 14.8	8.5	213	39.8

^a Controls

^b Number of birds

Calculations to account for the amounts of ^{14}C -DCPD consumed revealed that 4.5 mg was consumed in 3 days or 21 mg per kg body weight, and 7.0-8.5 mg in 5 days, or 32.9-39.8 mg per kg weight (Table 127). On a daily basis the body burden averaged 7.3 mg ^{14}C -DCPD per kg of body weight.

Hematocrit values for the quail averaged 40 and 36 ml% for males and females in the experiment involving DCPD (Table 126). The chemicals had no effect on the hematocrit values; controls, and treated birds had comparable hematocrits.

The ducks used for the ^{14}C -DCPD experiment accepted a change in habitat quite readily, as evidenced by a maintenance of body weight in most groups used in the experiment (Table 128). The ducks to be fed ^{14}C -DCPD weighed 1107 and 1156 g for males and females, respectively (Table 128). The larger value for the females reflected either the seasonal trend for these birds to deposit migratory fat, or to be actively in egg production.

Table 129 contains the data on feed intake of the ducks during the experiment with ^{14}C -DCPD. No consistent trends were observed for feed intake to be influenced by the 100 ppm level of the chemical in the diet. Birds that consumed greater quantities of diet with the chemical consumed amounts of diet comparable to this during the withdrawal period when no chemicals were in the diet. Table 129 reveals that the ducks fed diets with ^{14}C -DCPD consumed about 35 mg of radioactive chemical in 3 days (Group 3), and 51-77 mg in 5 days. These values indicated that daily body burden of ^{14}C -DCPD averaged 12.6 mg per kg body weight.

The lower hematocrit values, averaging 33.4 ml% for the females used in the ^{14}C -DCPD experiment, as compared to the average 42.4 ml% for male ducks (Table 128) reflected the active reproductive state of these females, and was associated with the trend for females to weigh slightly more than the males.

Feeding ^{14}C -DCPD at 100 ppm in the diet for 3 or 5 days had no effect on the hematocrit values of the ducks (Table 128).

Dosing Experiments

Quail used in the dosing experiments weighed 204 and 191 g for female and male, respectively (Table 130). The dose of ^{14}C -DCPD was targeted at 100 mg per kg body weight, but the actual quantity given amounted to 102.5 mg per kg body weight (Table 130). When these values were compared to the daily body burden of ^{14}C -DCPD received via the consumption of feed, the oral dose was 14 fold greater. Hematocrit values averaged 34.2 and 36.7 ml% for female and male quail, respectively (Table 130). There was a significant ($P < .01$) difference between these values. Quail dosed with ^{14}C -DCPD had hematocrit values of 35.7, 36.0, and 36.0 ml% at 2, 24, and 48 hours after dose, as compared to an average control value of 34.7 (Table 130), no significant ($P > .05$) treatment effect was detected.

TABLE 128. BODY WEIGHTS AND HEMATOCRITS OF MALLARD DUCKS FED ¹⁴C-DCPD @ 100 PPM OR FEED WITHOUT DCPD

Group	Treatment	Bird No.	Sex	Initial wt. on 4/6	Change in body weight from initial weight					Hematocrit %
					day 0 on	day 3 on	day 5 on	day 3 off	day 5 off	
1	None	933	♂	1091	+109					43.0
		6479	♂	1066	+ 48					44.0
		6476	♂	1075	- 9					41.0
		929	♀	960	+165					36.8
		6790	♀	1174	+114					31.5
		930	♀	972	-135					30.3
		Mean (±S.D.)		1056(±80)	49(±108)					37.0(±5.9)
2	None	6485	♂	1125		- 66	- 54	- 53	- 65	46.5
		4729	♂	1150	- 9	- 10	+ 2	+ 21	+ 26	46.0
		4620	♂	1136	+ 64	+ 91	+118	+164	+185	39.0
		931	♀	1251	+169	+235	+167	Dead		37.0
		6753	♀	1360	- 32	- 54	-111	- 85	-144	33.0
		932	♀	1106	+ 79	+ 74	+ 40	- 36	- 12	34.5
		Mean (±S.D.)		1109(±98)	50(±76)	45(±113)	27(±104)	2(±98)	-2(±122)	39.8(±6.3)
3	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11/77	4647	♂	1020	+ 34	- 83				40.0
		4629	♂	1129	- 10	+ 9				34.5
		4730	♂	951	- 51	- 44				39.3
		935	♀	1107	+ 70	+119				28.0
		6752	♀	1184	- 5	+ 5				36.0
		937	♀	1135	- 6	+158				36.0
		Mean (±S.D.)		1008(±86)	6.7(±44)	27(±93)				35.6(±4.3)
4	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11/77	949	♂	1110	-100	Dead				42.0
		6478	♂	1075	- 35	- 68	-173			37.0
		934	♂	1076	- 49	+ 23	- 12			42.5
		939	♀	1139	+127	+146	+106			39.0
		942	♀	975	-115	-170	-178			22.8
		944	♀	1016	+ 72	+146	+135			34.0
		Mean (±S.D.)		1065(±60)	-16(±96)	15(±137)	-24(±148)			35.2(±7.6)
5	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11/77	936	♂	1097	+ 61	- 45	+ 75	+ 75		45.0
		940	♂	1152	+ 53	+ 12	- 2	- 5		43.0
		938	♂	1035	+ 72	+ 11	+ 52	+ 53		42.8
		6751	♀	1130	+ 59	+ 40	- 78	- 29		25.6
		6760	♀	1260	-	- 65	+ 2	- 41		41.0
		947	♀	1351	- 73	-183	- 69	- 40		34.0
		Mean (±S.D.)		1171(±115)	34(±60)	-38(±81)	-3(±62)	2(±50)		38.7(±7.5)
6	¹⁴ C-DCPD in feed @ 100 ppm starting 4/11/77	941	♂	1083	- 13	+ 32	+ 40	+ 57	+ 43	44.5
		945	♂	1018	+ 9	+ 35	+ 44	+ 67	+ 42	45.5
		943	♂	1533	-166	-102	-115	-127	-161	46.5
		6788	♀	1199	-151	- 79	-115	-120	- 75	35.0
		6784	♀	1156	- 34	+ 35	+ 35	+ 49	- 25	37.0
		948	♀	1343	- 61	+ 20	- 25	+ 29	- 42	34.0
		Mean (±S.D.)		1222(±108)	-69(±73)	-10(±63)	-23(±76)	-7(±91)	-36(±77)	40.4(±5.7)

TABLE 129. THE AMOUNT OF FEED AND ¹⁴C-DCPD CONSUMED BY MALLARD DUCKS FED THE RADIOACTIVE COMPOUND AT 100 PPM IN THE DIET

Group	Feed Intake - g/b/d						¹⁴ C intake mg/b	Body wt. g	¹⁴ C-mg per kg body wt.
	Pre-Exptl. Period	Experimental period		Mean	Withdrawal period				
		Days 0-3 on	Days 3-5 on		Days 0-3 off	Days 3-5 off			
1 ^a	(6) ^b 115.9 ^c	-	-	-	-	-	0	1056	0
2 ^a	(6) 84.7	(6) 129.5	(6) 121.5	126.3	(5) 133.1	(5) 122.3	0	1189	0
3	(6) 98.1 ^c	(6) 115.0	-	115.0	-	-	34.5	1088	31.7
4	(6) 84.9	(5) 155.2	(6) 150.0	153.1	-	-	76.6	1065	71.9
5	(6) 93.6	(6) 81.6	(6) 91.4	85.5	(6) 115.9	-	51.3	1171	43.8
6	(6) 103.0 ^c	(6) 139.9	(6) 119.0	131.6	(6) 121.4	(6) 153.3 ^c	65.8	1222	53.8

^a Controls

^b Number of birds

^c Feed wasted, not an accurate value for intake

TABLE 130. BODY WEIGHT, HEMATOCRIT AND AMOUNT OF RADIOACTIVE CHEMICAL GIVEN TO DOUBLED QUAIL DOSED ORALLY WITH ¹⁴C-DCPD AT 100 MG PER KG BODY WEIGHT

Group	Band No.	Sex	Body wt.-g	Dose	body wt. mg/kg	Compound given	Time of killing	Hematocrit %
1	2447	♀	205	None	0	None	0 hr. !	31.0
	2449	♀	180	"	0	"	"	31.0
	2440	♀	210	"	0	"	"	35.5
	2430	♀	217	"	0	"	"	34.2
	2430	♀	199	"	0	"	"	32.5
	2429	♀	210	"	0	"	"	27.5
	2451	♂	212	"	0	"	"	30.0
	2450	♂	174	"	0	"	"	30.0
	2452	♂	102	"	0	"	"	30.5
	2433	♂	107	"	0	"	"	36.5
	2431	♂	200	"	0	"	"	35.0
	2432	♂	177	"	0	"	"	35.0
				196(115)				
2	2471	♀	220	22	100	DCPD	2 hr.	30.0
	2474	♀	181	16	99	"	"	40.3
	2472	♀	188	20	106	"	"	33.3
	2475	♂	194	20	103	"	"	36.5
	2473	♂	200	21	100	"	"	33.3
	2476	♂	162	10	111	"	"	39.0
			192(120)	19.0(11.6)	103(15)			35.7(13.9)
3	2455	♀	245	25	102	DCPD	24 hr.	33.0
	2454	♀	184	20	109	"	"	36.3
	2453	♀	225	23	102	"	"	35.0
	2457	♂	170	20	112	"	"	34.0
	2456	♂	196	20	102	"	"	35.0
	2458	♂	182	19	104	"	"	41.0
			202(127)	21.2(12.3)	105(14)			36(13.0)
4	2460	♀	188	20	105	DCPD	48 hr.	41.9
	2459	♀	209	21	104	"	"	30.0
	2461	♀	215	21	98	"	"	29.0
	2464	♂	174	20	115	"	"	30.0
	2462	♂	210	21	96	"	"	39.0
	2463	♂	222	21	95	"	"	30.0
			204(119)	21(10.5)	107(10)			36(15.3)
	Avg.	♀	204(110)					34.2(14.1)
	Avg.	♂	191(110)					36.7(13.0)

The ducks used for the ^{14}C -DCPD dosing experiment had an average body weight of 1153 and 1281 g for female and male ducks, respectively (Table 131). The dose of each chemical was targeted for 100 mg per kg of body weight and the dose was on target (Table 131). The oral dose was 7.9 fold greater than the body burden of ^{14}C -DCPD, received from consuming the diets with 100 ppm of the chemical. Hematocrit values for these ducks were 42.3 and 40.6 ml% for females and males, respectively (Table 131). There was no significant ($P > .05$) treatment effect on hematocrit values over the 48-hour period following the single oral dose at 100 mg per kg of body weight.

Tissue Residues

A. In order to compare the residue values in tissue obtained from feeding or dosing ^{14}C -DCPD to quail and ducks, the comparative body burden of these chemicals must be reconsidered. In the following table are the amounts of ^{14}C -DCPD consumed on a daily basis with the values adjusted for the body weight, in kg, of these birds.

Body burden - mg ^{14}C -chemical per kg body weight

<u>Route of administration</u>	<u>^{14}C-DCPD</u>	
	<u>Quail</u>	<u>Ducks</u>
A. Fed @ 100 ppm	7.3	12.6
B. Dosed, <u>per os</u> , @ 100 mg/kg body wt.	103	99
B/A	14.1	7.9

One should recall that the above comparison is based upon a single oral dose of a chemical in a solvent which is a natural food-stuff, in this case, corn oil, as compared to the feeding approach which introduces the chemical in a dry state in much smaller quantities per unit of time, and with a mixture of feed ingredients that may interfere with or enhance absorption. Therefore, not necessarily may the ^{14}C residue values in tissues be at the same comparative relationship as the "B/A values" in the table above.

Single oral doses of ^{14}C -DCPD to quail or ducks resulted in high ^{14}C residues in all tissue samples, except rbc's, at the 2nd hour after dosing (Table 132). The comparison of ^{14}C residues at that time for quail vs. duck is given in the table below:

^{14}C -equivalents in tissues from quail and ducks dosed with ^{14}C -DCPD @ 100 mg/kg body weight. Samples obtained 2nd hour after dose

	Adipose	Kidney	Liver	Skin	Plasma	Brain	Muscle	RBCs
Quail (Q)	50.1	26.1	17.4	13.7	19.1	6.87	5.60	0.0
Duck (D)	34.3	40.9	31.7	20.8	12.1	10.8	11.8	0.0
D/Q	0.7	1.6	1.8	1.5	0.6	1.6	2.1	---

TABLE 131. BODY WEIGHT, HEMATOCRIT AND AMOUNT OF RADIOACTIVE CHEMICAL GIVEN TO MALLARD DUCKS DOSED ORALLY WITH ^{14}C -DCPD AT 100 MG PER KG BODY WEIGHT. DUCKS WERE KILLED AT 0, 2, 24, OR 48 HOURS AFTER DOSE.

Group	Band No.	Sex	Body wt.-g	Dose mg	Body wt. mg/kg	Compound given	Time of killing	Hematocrit %
1	6049	♀	1060	None	0	None	0 hr.	42.3
	6004	♀	800	"	0	"	"	41.0
	6005	♀	1220	"	0	"	"	41.8
	6006	♀	1100	"	0	"	"	43.1
	6009	♂	1322	"	0	"	"	38.8
	6097	♂	1105	"	0	"	"	41.0
	6092	♂	1405	"	0	"	"	42.3
	6001	♂	1180	"	0	"	"	40.3
			1169(±161)					41.3(±1.4)
2	6022	♀	1205	120	100	DCPD	2 hr.	47.0
	6023	♀	1185	120	101	"	"	40.3
	6024	♀	1145	115	100	"	"	43.3
	6019	♂	1610	160	99	"	"	41.8
	6020	♂	1340	130	97	"	"	40.0
	6021	♂	1310	130	99	"	"	45.5
			1300(±170)	129(±16)	99(±1)			43.0(±2.8)
3	6031	♀	1330	130	98	DCPD	24 hr.	45.0
	6032	♀	1365	135	99	"	"	41.0
	6033	♀	1200	120	100	"	"	42.0
	6034	♂	1305	130	100	"	"	42.3
	6035	♂	1245	125	100	"	"	34.5
	6036	♂	1275	130	102	"	"	40.3
			1287(±160)	128(±5)	99(±1)			40.9(±3.5)
4	6027	♀	1100	110	100	DCPD	48 hr.	43.5
	6026	♀	1010	100	99	"	"	41.0
	6025	♀	1115	110	99	"	"	38.0
	6030	♂	1120	110	98	"	"	44.3
	6028	♂	1115	110	99	"	"	42.3
	6029	♂	1320	130	98	"	"	40.3
				1130(±102)	112(±10)	99(±0.6)		
	Avg.	♀	1153(±133)					42.3(±2.2)
	Avg.	♂	1281(±138)					40.6(±2.6)

TABLE 132 . SUMMARY OF DATA, BASED ON GROUP MEANS, OF ¹⁴C ACTIVITY IN TISSUES FROM BODWHITE QUAIL AND MALLARD DUCKS GIVEN ¹⁴C-DCPD VIA THE FEED OR DOSED PER OS

Tissue ¹⁴ C-equivalents from ¹⁴ C-DCPD - µg/g (ppm)											
Feed @ 100 ppm in diet			Dose @ 100 mg/kg body wt.			Feed @ 100 ppm in diet			Dose @ 100 mg/kg body wt.		
Sample Time	Quail	Duck	Sample Time	Quail	Duck	Sample Time	Quail	Duck	Sample Time	Quail	Duck
PLASMA ¹⁴C						RBC ¹⁴C					
Day 3 on	0.0 ^a	0.25	0 hour	0.0	0.0	Day 3 on	0.0	0.20	0 hour	0.0	0.0
Day 5 on	0.0	0.0	2nd hour	19.11	12.07	Day 5 on	0.0	0.25	2nd hour	0.0	0.0
Day 3 off	0.0	0.0	24th hour	3.50	0.0	Day 3 off	0.0	0.0	24th hour	0.0	0.0
Day 5 off	0.0	0.0	40th hour	1.06	0.0	Day 5 off	0.0	0.0	40th hour	0.0	0.0
Detection Limit	0.048	0.017	Detection Limit	0.94	3.84	Detection Limit	0.06	0.09	Detection Limit	0.53	1.78
LIVER ¹⁴C						KIDNEY ¹⁴C					
Day 3 on	0.13	0.65	0 hour	0.0	0.0	Day 3 on	0.17	0.01	0 hour	0.0	0.0
Day 5 on	0.25	0.60	2nd hour	17.54	31.7	Day 5 on	0.17	0.99	2nd hour	26.1	40.9
Day 3 off	0.10	0.27	24th hour	4.44	7.15	Day 3 off	0.0	0.10	24th hour	9.15	5.5
Day 5 off	0.0	0.61	40th hour	1.90	2.96	Day 5 off	0.0	0.07	40th hour	2.06	2.15
Detection Limit	0.009	0.04	Detection Limit	0.55	1.03	Detection Limit	0.05	0.05	Detection Limit	0.32	1.30
ADIPOSE ¹⁴C						BRAIN ¹⁴C					
Day 3 on	0.0	0.39	0 hour	0.0	0.0	Day 3 on	0.0	0.0	0 hour	0.0	0.0
Day 5 on	0.0	0.16	2nd hour	50.1	34.3	Day 5 on	0.0	0.27	2nd hour	6.07	10.0
Day 3 off	0.0	0.0	24th hour	5.72	16.5	Day 3 off	0.0	0.13	24th hour	2.26	3.40
Day 5 off	0.0	0.0	40th hour	2.20	4.0	Day 5 off	0.0	0.0	40th hour	0.54	0.0
Detection Limit	0.048	0.044	Detection Limit	0.32	0.61	Detection Limit	0.028	0.033	Detection Limit	0.40	0.079
SKIN ¹⁴C						MUSCLE ¹⁴C					
Day 3 on	0.15	0.43	0 hour	0.0	0.0	Day 3 on	0.0	0.14	0 hour	0.0	0.0
Day 5 on	0.19	0.21	2nd hour	13.7	20.0	Day 5 on	0.0	0.13	2nd hour	5.60	11.0
Day 3 off	0.00	0.0	24th hour	2.93	0.04	Day 3 off	0.0	0.0	24th hour	1.21	1.06
Day 5 off	0.05	0.0	40th hour	0.94	2.49	Day 5 off	0.0	0.0	40th hour	0.60	0.0
Detection Limit	0.033	0.044	Detection Limit	0.33	1.05	Detection Limit	0.022	0.044	Detection Limit	0.26	1.60

^a0.0 = less than detection limit based upon comparison of 12 control vs. 6 exptl. values

Of the 8 tissues analyzed only adipose and plasma samples from quail exceeded ^{14}C levels found in these tissues from ducks. All others in duck were only 1.5 and 2.1 of the residue level detected in similar tissues from quail. This occurred despite the fact that the single oral dose at 100 mg per kg body weight was very much alike for both species.

^{14}C residues were detected in most tissues from quail and ducks at the 48th hour after the single oral dose of ^{14}C -DCPD (Table 132). Detection limits for duck tissues were, in most cases, at or greater than 1 ppm, except for brain and adipose tissues. These data from the quail indicate that when detection limits were in the range of 0.3 to 0.4 ppm, ^{14}C residues were detected at 48 hours after the single oral dose. The possibility exists that had the detection limits from the duck tissues been comparable to those of quail, then ^{14}C residues would have also been detected in brain and muscle samples. Based upon the data from the dosing study with quail, rbc's were not particularly permeable to ^{14}C -DCPD or its metabolites.

At 100 ppm of ^{14}C -DCPD in the diet of ducks and quail, the ^{14}C residues in tissues obtained at days 3 and 5 of feeding radioactive diets were less than 1 ppm. The table below compared the ^{14}C residues of 8 tissues obtained from the 2 species of birds at day 5 of feeding.

^{14}C -equivalents in tissues from quail and ducks fed diets with 100 ppm ^{14}C -DCPD. Samples obtained on the 5th day.

	Kidney	Liver	Skin	Adipose	Brain	Muscle	RBCs	Plasma
Quail (Q)	0.17	0.25	0.19	0.0	0.0	0.0	0.0	0.0
Duck (D)	0.99	0.63	0.21	0.16	0.27	0.13	0.25	0.0
D/Q	5.8	2.7	1.1	--	--	--	--	--

The table above shows that of the 8 tissues counted in only 3 were residues detectable in quail. Furthermore, duck tissues had higher levels of radioactivity, and the magnitude of this higher level depended upon the tissues studied. Thus, skin had ^{14}C residues of about 0.2 ppm in both ducks and quail, but kidney samples counted with a 6-fold higher radioactivity when obtained from ducks. Apparently the metabolism of the compound is different for these two species.

The other comparison to be made is that of the ^{14}C -residue level from dosing vs. that obtained from feeding. Recall, that the ratios of the body burden ranged from about 9 to 16-fold higher for dosing as feeding. The ^{14}C residues for particular tissues compared on the basis of dosing vs. feeding indicates there is no correlation of the residue levels to the body burden when routes of administration differ. For example, kidneys from quail dosed or fed ^{14}C -DCPD had residues of 26.1 vs. 0.17 $\mu\text{g/g}$, respectively; a ratio of 154/1 and far higher than the 9 to 16 ratio one would predict based upon body burden. In ducks the same comparison yields a value of 35/1 for kidney samples.

Feeding ^{14}C -DCPD at 100 ppm in the diet for 5 days followed by 5 days of withdrawal resulted in only 2 of 8 tissues, skin from quail and kidney from ducks, having detectable residue in the order of 0.05-0.07 ppm (Table 132). Most samples procured on the 3rd day after withdrawal were already at or below detection limits. Thus, DCPD consumed with food is not retained for long periods of time, and is readily depleted from the bodies of quail and duck.

The tables in Appendix K of this report contain the individual values for each tissue of each bird used to obtain the data in Table 132.

Radioactivity in Stored Feed

Nine months after the feed had been stored samples were extracted with different solvents to determine recovery values. The results are presented in the following table.

Percent recovery of ^{14}C from diets containing ^{14}C -DCPD

Chemical in feed	Solvent for extraction	% ^{14}C recovered
^{14}C -DCPD	Chloroform:petroleum ether (1:1)	24.7
	Ethyl acetate	18.6
	p-Dioxane	19.7
	DCPD	19.7
	Butanol	23.5

Butanol or chloroform:petroleum ether (1:1) extractions yielded the highest recovery values, but the values were extremely low, 24-25%. The volatile nature of DCPD presumably accounted for the very low recovery value of it from feeds.

Discussion

DCPD does not belong in the classification of those compounds which persist for long periods of time within the animal's body. Instead, it is rapidly depleted from body tissues of wild-type fowl (Bobwhite quail and Mallard ducks) as evidenced from dosing and feeding experiments. The dosing experiments revealed that despite high levels of ^{14}C residues induced within 2 hours from ^{14}C -DCPD, the residue levels were at or below detection limits of 0.3 to 1.0 ppm in 48 hours. Adipose tissue, known to be a reservoir for certain pesticides and environmental contaminants, did not show the persistence to retain ^{14}C from DCPD. Based upon these data, one can conclude that the parent compound and/or its metabolites are not particularly lipophilic upon entrance into the animal's body. The compound was soluble in corn oil, which is comprised of almost 55% linoleic acid and 30% oleic acid (85% unsaturated fatty acids). Poultry fat is 24 and 40% linoleic and oleic acids, respectively (64% unsaturated fatty acids) (Scott et al., 1976). Therefore, solubility in corn oil, a lipid of one type, does not guarantee that

the compound would be soluble and become bound to a lipid of another type; particularly when the comparison being made is one of an active metabolic tissue vs. a passive lipid solution. Thus, the fact that DCPD was soluble in a lipid stored in a test tube was in no way a measure of predictability that the compound would have a particular affinity for lipids in the bird's body. As it turned out, the highest ¹⁴C values were generally found in organs associated with metabolism and excretion, i.e., kidney and liver.

The rate of elimination of ¹⁴C residues from ducks and quail dosed with ¹⁴C-DCPD could be calculated for most tissues. These values are as follows:

Rate of elimination (t_{1/2}-hours) of ¹⁴C residues from ducks and quail given ¹⁴C-DCPD at 100 mg/kg body weight - based on data from Table 132.

	Plasma	Liver	Adipose	Skin	RBCs	Kidney	Brain	Muscle
Quail (Q)	11.1	14.7	10.4	11.9	- ^a	14.5	12.5	14.4
Duck (D)	- ^a	13.6	14.8	15.1	- ^a	10.9	13.5	8.3

^a Cannot be calculated

The average t_{1/2} for quail and duck tissues is 12.7 hours. Therefore, in 9 half-lives the tissue residue values would be 1/512 of the initial amount, and in 10 half-lives at 1/1024 of the initial amount. If the initial level were 28 ppm, which was the average residue level at 2 hours, then in 127 hours, 5.3 days, the level in tissue would be 0.027 ppm. Relating these calculations to the observation on residues in the feeding experiments, one can expect that with detection limits in the range of .02 to .09 ppm, no residues would be detected at day 5, particularly because the residue levels at steady-state values were in the range of 0.5 to 0.9 ppm rather than 28 ppm as found in the dosing experiments. Based on steady-state values being < 1.0 ppm and a t_{1/2} of 12.7 hours, about 64 hours would be required for the residue to reach detection limits of 0.04 ppm. A survey of the data on DCPD residues reveals that 5 of the 8 tissues followed this pattern.

DCPD is a starting material for insecticides, and also used in the manufacture of plastics, rubber hydrocarbons, and resin coatings. Although DCPD is a fluid at 18-22°C (bird has body temperature of 41°C) and presumably insoluble in water (oil/water distribution of 60,000/1), it has been detected in surface water and wells nearby its dumping grounds. Some spillage of DCPD occurred around a manufacturing site, and this spillage was traced to a nearby stream and lake where migratory waterfowl have died (Jones, 1978). Jones (1978) also reported that ducks, treated per os with DCPD at 40,000 mg per kg body weight, 400 fold greater than the amounts used in the dosing portion of the residue study, showed in about 10% of the birds only slight intoxication and moderate tremors. Care had to be taken to prevent the ducks from drowning in order to dose such a large quantity of DCPD into them. One would suspect from such information that the compound was being absorbed to a minute extent. However,

the tissue residue studies revealed that at a dose of 100 mg per kg body weight (1/400th of the maximum dose given by Jones) residues of DCPD and its metabolites occurred at 10-40 mg per kg in 7 of the 8 tissues analyzed from ducks. On the other hand, a lower level of contamination of 12.6 mg per kg body weight, obtained in the feeding experiments produced residue levels in the range of 0.1 to 1.0 mg per kg of tissue; levels lower than predicted from dosing experiments, when considered in proportion to the dosage. One can estimate from these residue studies that the ducks dosed by Jones (1978) at 400 fold greater levels than those used in this residue study must have had high residue levels of about 1/50 to 1/10 of the dose, while resisting toxic effects.

Residues persisted in most tissues from ducks killed at 40 hours after the single oral dose. As pointed out earlier, about 5 days were estimated as a withdrawal time for the residues to reach a detection limit of 0.04 ppm. There was less of a problem with the persistence of residues in quail. Only skin and liver samples from the latter species contained detectable residues at day 3 after withdrawal. Nevertheless, the contribution of these fowl to the persistence of DCPD residues in the food chain should be very limited. The compound DCPD is not characterized as a persistent environmental contaminant within wild fowl (Bobwhite quail and Mallard duck) to be passed along the food chain.

CONCLUSIONS

Ducks and quail fed diets with radioactive DCPD had ¹⁴C residues averaging less than 1 ppm which declined to less than detection limits, averaging 0.04 ppm in most tissues by the 3rd day after withdrawal. All tissues, except quail skin and duck kidney were clear of residues by day 5 off radioactive diets.

In the dosing experiments, maximum residues at the second hour were 5.6 to 50.1 ppm, depending upon tissue and species. These values, however, declined rapidly with a biological half-life of 12.7 hours. Most tissues were at or above detection limit in 48 hours.

DCPD was not concentrated in adipose of either species. Therefore, the rapid biological half-life and lack of binding to fat cells in the carcass indicate that DCPD is not retained for passage along the food chain by predators of these fowl.

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GLOSSARY

- ad libitum: Free choice, without restriction as to amount or time.
- Dead in shell: Embryo dead at 24 (Bobwhite) or 28 (Mallards) days of incubation.
- Early dead: Embryo died within the first 14 days of incubation.
- Live in shell: Embryo failed to break shell.
- Pair - fed: Limiting an untreated dietary group to the amount of feed consumed by an ad libitum - treated dietary group.
- per os: By mouth.
- Pipped dead: Shell broken, embryo dead.
- Pipped live: Shell broken, embryo alive but failed to hatch.
- Secondary sex ratio: Sex ratio at birth.

Appendixes

APPENDIX A
ANALYSIS OF FEED

Table A-1

Feed ¹	Crude protein (Not < Percent)	Crude fat (Not < Percent)	Crude fiber (Not > Percent)
Duck Starter	19	3	6
Breeder Developer	14	2.5	10
Breeder Layer	17	2.5	7.5

¹Feed obtained from Ralston Purina Co., 5620 Millett Road, Lansing, Michigan 48917. Complete analysis not available, these feeds are in a closed book formula (privileged information).

APPENDIX B

DIET PREPARATION

Test 2

A pre-mix of DCPD or DIMP was prepared by adding the 97 percent pure chemical to corn oil and mixing this by hand to duck starter ration. The final individual diets were prepared in 0.4 to 4 kg quantities (depending on predicted amount, from range finding test, to be consumed) by combining a quantity of pre-mix with the duck starter diet (Table B-1). All final diet mixing was done on a Paul G. Abbe feed mixer² by tumbling the mixture for 15 minutes in a seven kilogram capacity feed can. The total amount of chemical-corn oil solution was not more than 2 percent of the diet containing DIMP.

For the DCPD-repeat group of ducks the diets were made by adding the chemical-corn oil solution to the duck breeder developer diet and mixing in seven kilogram capacity feed cans on a Paul G. Abbe, Inc., feed mixer². Total chemical-corn oil mixture was approximately two percent of the four kilogram diets made (Table B-2).

² Paul G. Abbe, Inc., Little Falls, NJ 07424

Table B-1

Premix

Chemical	Amount (gms)	Feed (gms)	Total (gms)	ppm
DCPD	400	3600	4000	100000
DIMP	100	4900	5000	20000

Diets

Chemical	Premix (gms)	Feed (gms)	Total (gms)	ppm
DCPD	400	3600	4000	10000
	600	2400	3000	20000
	600	1400	2000	30000
	400	600	1000	40000
	250	250	500	50000
	300	200	500	60000
	350	150	500	70000
	400	100	500	80000
	450	50	500	90000
DIMP	400	3600	4000	2000
	800	3200	4000	4000
	900	2100	3000	6000
	1200	1800	3000	3000
	200	200	400	10000
	240	160	400	12000
	280	120	400	14000
	320	80	400	16000
	360	40	400	18000

Table B-2

DCPD	Corn oil (gms)	Feed (gms)	Total (kg)	ppm
0.00	80	3920	4	0
0.04	80	3920	4	10
0.4	79.6	3920	4	100
4.0	76	3920	4	1000
20.00	60	3920	4	5000
40.00	40	3920	4	10000

Test 3

Diets for Test 3 were made by adding a chemical-corn oil solution to the duck breeder developer or breeder layer diet and mixing in a Mix-mill³ for 25 minutes. Only 80 kg of diet were prepared at a time so that the diets would be fresh at all times (Table B-3). A pre-mix was not made since the chemical-corn oil solution was found to be well distributed on the pelleted feed in the Mix-mill.

Table B-3

<u>Chemical</u>	<u>Amount added (gms)</u>	<u>Oil added (gms)</u>	<u>Feed (kg)</u>	<u>Total (kg)</u>	<u>ppm</u>
DCPD	0.0	1600	78.4	80	0
	2.56	1597	78.4	80	32
	8.0	1592	78.4	80	100
	25.6	1574	78.4	80	320
DIMP	0.0	1600	78.4	80	0
	80.0	1520	78.4	80	1000
	256.0	1344	78.4	80	3200
	800.0	800	78.4	80	10000

³ Mix-mill, Inc., Bluffton, IN 46714

APPENDIX C

HISTOPATHOLOGIC TECHNIQUE

At least 48 hours of fixation in 10 percent neutral buffered formaldehyde were allowed prior to processing tissues for paraffin embedding. Processing was accomplished with ethanol dehydration, xylene clearing, and a combination of Tissuemat^{®1} and Paraplast^{®2} infiltration and embedding.

Paraplast[®]-embedded tissues were sectioned at five micra per section for non-neural and fifteen micra per section for neural tissues. Sections were floated onto slides from the surface of a warm (47°C) water bath that contained approximately 0.03 percent gelatin. After drying and warming, the slides were stained by a regressive Harris' hematoxylin and eosin method using Harris' stain without glacial acetic acid (see Luna, 1968 p. 34). A 0.5 percent Eosin Y stain dissolved in absolute ethanol served as cytoplasmic counterstain solution. The differentiating solution was one percent concentrated hydrochloric acid in 80 percent ethanol. Xylene was used as both deparaffin and clearing agents, and graded ethanol solutions were miscible intermediaries between xylene and aqueous solutions. The mounting media, Flo-Texx^{®3} Liquid Cover Slip, was used with glass coverslips.

APPENDIX D

PREPARATION OF DRABKIN'S REAGENT

1000 mg	Sodium bicarbonate (NaHCO ₃)
50 mg	Potassium cyanide (KCN)
<u>200 mg</u>	Potassium ferricyanide (K ₃ Fe(CN) ₆)
1250 mg	

Mix to dissolve and dilute to 1 liter.
The solution was stored in a sealed amber bottle and kept refrigerated.

¹ Fisher Scientific Co., Pittsburgh, PA 15219

² Sherwood Medical Industries, St. Louis, MO 63103

³ Lerner Labs., Stamford, CN 06902

APPENDIX E

DETERMINATION OF HEMOGLOBIN CONCENTRATION

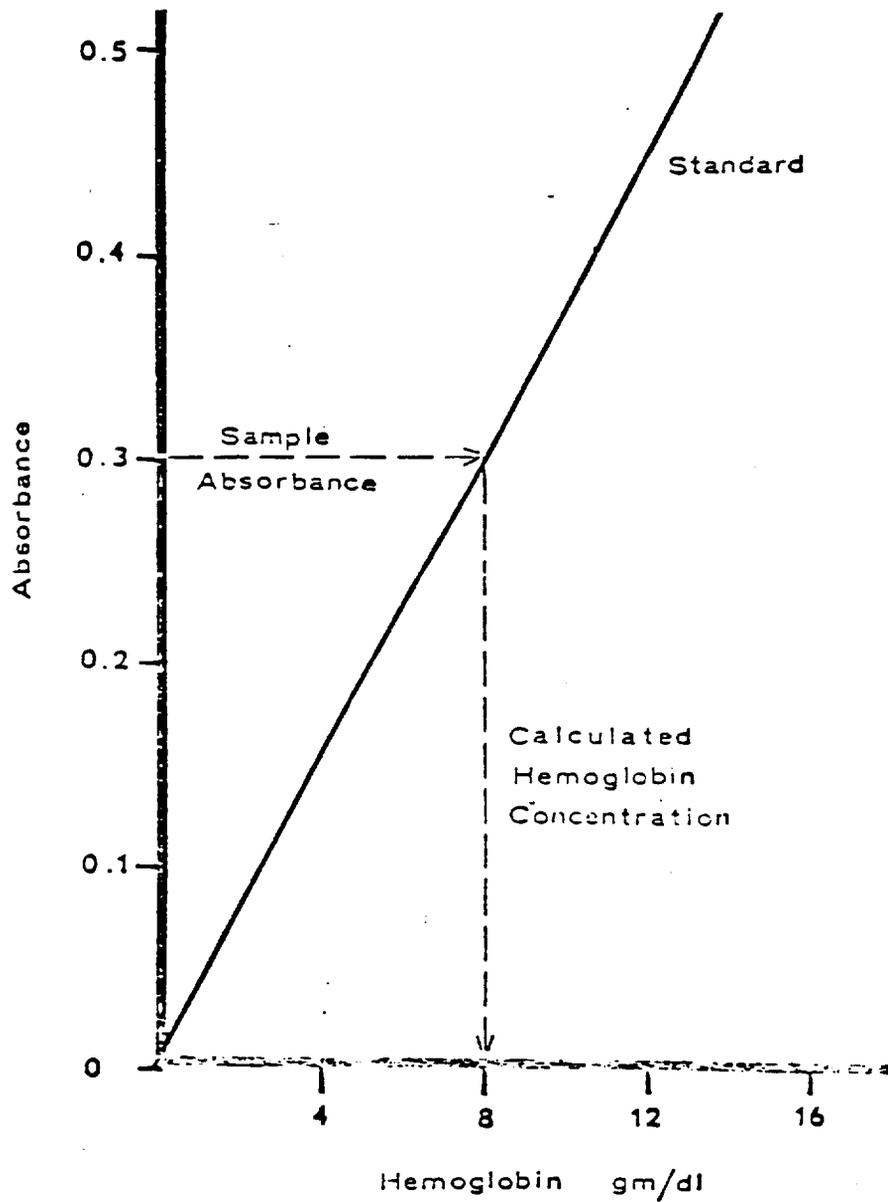
Hemoglobin concentration was determined by the cyanmethemoglobin method. Twenty microliters of blood were added to 5 ml of Drabkin's Reagent (see Appendix D), mixed, and allowed to stand for 10 minutes for maximum conversion of hemoglobin to cyanmethemoglobin. This mixture was then placed in a quartz cuvette and optical density determined at 540 nm in a Spectronic 20 calorimeter-spectrophotometer¹.

The optical density of the sample was then compared to a standard curve. The standard curve was constructed from values of optical density and hemoglobin concentration which were previously determined with human hemoglobin standards².

¹ Bausch and Lomb, Rochester, NY

² Cyanmethemoglobin certified standard, Hycel, Inc., Houston, TX

Figure E-1. Sample hemoglobin concentration calculation. The line was constructed by plotting the percent absorbance of each standard against its known hemoglobin concentration.



APPENDIX F

PROCEDURES FOR HEMATOCRIT AND DIFFERENTIAL COUNT DETERMINATIONS AND PREPARATION OF WRIGHT'S STAIN AND BUFFER

Hematocrit

Hematocrits were determined by collecting blood, from a venous puncture of the wing into a heparinized capillary tube. After sealing one end of the capillary tube, it was centrifuged at 4500 rpm for 7.5 minutes in an International Microcapillary Centrifuge¹. After centrifugation, the packed red cell volume in each tube was measured using a microcapillary reader.

Differential Counts

Blood smears for differential counts were prepared using fresh flowing blood, containing no anticoagulants, on a clean glass slide. The blood was allowed to air dry before staining. The blood film was fixed by flooding with Wright's stain and left to stand for approximately five minutes. The buffer was then added to differentiate the cells. After five more minutes, distilled water was used to wash the slides which were drained and blotted dry.

Wright's Stain

3.3 grams Wright's powder was added to 500cc fresh, pure methyl alcohol. The stain was ripened for several months to room temperature in a stoppered brown bottle.

Buffer

3.80 gm Na_2HPO_4
5.47 gm KH_2PO_4

Dissolve in 500 ml distilled water and bring total volume to 1000 ml. Set pH at 6.4.

¹ International Equipment Company, Boston, MA

APPENDIX G
COMPOSITION OF FEED

Quail Breeder (QB 72)

<u>Ingredients</u>	<u>kg</u>
Corn	408.41
Soybean meal, 49%	296.65
Meat scrap, 50%	45.36
Alfalfa meal, dehy.	40.82
Animal fat, stabl.	51.71
Limestone	45.36
Dicalcium phosphate	6.35
Choline chloride, 50%	2.72
Methionine hydroxy analogue	0.91
Salt, iolized	3.45
Mineral mix A	2.72
Vitamin mix A	2.72
Antioxidant	0.11
	907.29

Quail Starter (QS 72)

<u>Ingredients</u>	<u>kg</u>
Corn	348.64
Soybean meal, 49%	384.55
Fish meal	28.18
Meat scraps, 50%	31.82
Alfalfa meal, dehy.	40.91
Animal fat, stabl.	53.64
Dicalcium phosphate	11.82
Choline chloride, 50%	2.73
Methionine hydroxy analogue	0.91
Salt, iodized	3.18
Vitamin mix A	2.73
Mineral mix A	2.73
Antioxidant	0.11
	911.95

APPENDIX H

DIET PREPARATION

LC₅₀ Diets:

A premix of DCPD or DIMP was prepared by adding the pure chemical to corn oil and mixing by hand with quail starter diet. The final individual diets were prepared in one kilogram quantities by combining a quantity of premix with quail starter diet (Table H-1). All final diet mixing was completed by tumbling the mixture for 15 minutes in a seven kilogram capacity mixer¹. The total amount of chemical-corn oil solution was not more than two percent of the diets containing DCPD.

Table H-1

Premix

Chemical	Amount of chemical (gms)	Amount of feed (gms)	Total (gms)	ppm
DCPD	90	4410	4500	20000
DIMP	180	4320	4500	40000

Diets

Chemical	Amount of premix (gms)	Amount of feed (gms)	Total (gms)	ppm
DCPD	100	900	1000	2000
	200	800	1000	4000
	300	700	1000	6000
	400	600	1000	8000
	500	500	1000	10000
	600	400	1000	12000
	700	300	1000	14000
	800	200	1000	16000
	900	100	1000	18000
DIMP	100	900	1000	4000
	200	800	1000	8000
	300	700	1000	12000
	400	600	1000	16000
	500	500	1000	20000
	600	400	1000	24000
	700	300	1000	28000
	800	200	1000	32000
	900	100	1000	36000

¹ Paul G. Abbe, Inc., Little Falls, NJ 07424

Chronic Diets

A premix of DCPD or DIMP was prepared by the same method as employed in the premix preparation of the LC₅₀ experiment (see Table H-2). Final individual diets were prepared by the addition of an appropriate amount of premix to quail breeder diet. All mixing was completed by handmixing for ten minutes.

Table H-2

Premix

Chemical	Amount of oil (gms)	Amount of chemical (gms)	Amount of feed (gms)	Total (gms)	Total (ppm)
DCPD	60	120.0	2820.0	3000	40000
	60	37.5	2902.5	3000	12500
	60	12.0	2928.0	3000	4000
DIMP	60	360	2580	3000	120000
	60	114	2826	3000	38000
	60	36	2904	3000	12000
	60	11.4	2928.6	3000	3800

Diets

Chemical	Premix (gms)	Feed (gms)	Total (gms)	Total (ppm)
DCPD	100	900	1000	4000
	100	900	1000	1250
	100	900	1000	400
DIMP	100	900	1000	12000
	100	900	1000	3800
	100	900	1000	1200
	100	900	1000	380

APPENDIX I

MINK FEED CONSTITUENTS AND DIET PREPARATION

Mink Feed Constituents

Mink feed used in these experiments consisted of the following constituents:

Commercial cereal (XK-40 ¹)	25%
Whole Chicken	20%
Ocean fish (cod, haddock, & flounder trimmings)	20%
Beef tripe	15%
Beef lung	7.5%
Beef liver	5%
Beef trimmings	5%
Corn oil (during lactation)	1%
Powdered milk	0.1%
Vitamin E (March 1 to weaning)	55,000 units/1000 kg finished feed

The chicken, fish, and beef by-products were ground in a 6 inch commercial feed grinder², and added to the remaining constituents in a commercial three-quarter ton feed mixer². Feed was allowed to mix for 15 minutes, and was then unloaded from the mixer for further diet preparation.

Diet Preparation

For each diet, the amount of chemical (DIMP or DCPD) required for the proper final dietary concentration (dilution to 100 kg feed) was preweighed, and added to 500 ml of corn oil as a vehicle. The chemical-vehicle mixture was then combined with one kg of ground cereal, and mixed until absorbed. This premix was then added to 98.5 kg of feed (described above) in a one-quarter ton commercial feed mixer and allowed to mix thoroughly. The finished diet was then unloaded into premarked color-coded cans and frozen for future use.

¹ XK Sales and Development Co., Thiensville, WI

² Weiler and Co., Whitewater, WI

APPENDIX J

^{14}C Activity in Tissues of Bobwhite Quail and
Mallard Ducks Fed or Dosed With ^{14}C -DIMP

TABLE J1 ¹⁴C ACTIVITY IN TISSUES OF ROSSWHITE QUAIL FED 100 PPM OF ¹⁴C-DIMP AT 2/3.4 dpm/μg. SAMPLES WERE PROCURED, AS STATED, FROM QUAIL DURING AND AFTER THE FEEDING OF THE RADIOACTIVE CHEMICAL.

Bird No.	PLASMA		RBC's		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g	Day 3 on μg/g	μg/g
1685	0.31		0.0		0.37		1.22		0.53		0.0		0.46		0.13	
1686	0.54		0.0		1.01		1.11		0.53		0.11		1.16		0.15	
1688	0.52	0.51 ^a	0.0	0.0	0.96	0.764	0.97	1.103	0.63	0.046	0.12	0.005	1.14	1.109	0.17	0.143
1687	0.74	±0.159	0.0		1.03	±0.276	1.51	±0.241	1.23	±0.33	0.12	±0.047	2.44	±0.71	0.16	±0.034
1681	0.61		0.0		0.70		0.99		1.19		0.11		0.91		0.16	
1684	0.36	6/6 ^b	0.0	0/6	0.52	6/6	0.82	6/6	0.97	6/6	0.06	5/6	0.55	6/6	0.00	6/6
	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g	Day 5 on μg/g	μg/g
1689	0.17		0.0		0.20		0.49		0.25		0.10		0.43		0.06	
1694	0.11		0.0		0.27		0.33		0.25		0.08		0.43		0.21	
1692	0.14	0.135	0.0	0.0	0.39	0.322	0.30	0.432	0.46	0.35	0.0	0.056	0.43	0.432	0.05	0.116
1691	0.12	±0.008	0.0		0.39	±0.115	0.56	±0.123	0.57	±0.13	0.0	±0.044	0.33	±0.091	0.12	±0.069
1690	0.27		0.0		0.48		0.57		0.26		0.00		0.60		0.19	
1693	0.0	5/6	0.0	0/6	0.20	6/6	0.34	6/6	0.33	6/6	0.00	4/6	0.38	6/6	0.07	6/6
	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g	Day 3 off μg/g	μg/g
1625	0.0		0.0		0.10		0.0		0.0		0.0		0.10		0.0	
1623	0.0		0.0		0.0		0.0		0.0		0.0		0.11		0.0	
1683	0.0	0.0	0.0	0.0	0.22	0.067	0.0	0.0	0.0	0.0	0.0	0.0	0.12	0.122	0.0	0.0
1624	0.0		0.0		0.0	±0.105	0.0		0.0		0.0		0.14	±0.024	0.0	
1622	0.0		0.0		0.0		0.0		0.0		0.0		0.16		0.0	
1682	0.0	0/6	0.0	0/6	0.0	2/6	0.0	0/0	0.0	0/6	0.0	0/6	0.11	6/6	0.0	0/6
	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g	Day 5 off μg/g	μg/g
1593	0.0		0.0		0.0		0.0		0.0		0.0		0.09		0.0	
1596	0.0		0.0		0.0		0.0		0.0		0.0		0.10		0.0	
1600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.07	0.101	0.0	0.0
1595	0.0		0.0		0.0		0.0		0.0		0.0		0.16	±0.036	0.0	
1597	0.0		0.0		0.0		0.0		0.0		0.0		0.12		0.0	
1599	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/0	0.0	0/6	0.0	0/6	0.07	6/6	0.0	0/6
Detection Limit μg/g	0.005		0.19		0.12		0.06		0.066		0.063		0.046		0.057	

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted

TABLE J2 ¹⁴C ACTIVITY IN TISSUES OF MALLARD DUCKS FED 100 OF ¹⁴C-DIMP AT 273.4 dpm/μg. SAMPLES WERE PROCURED, AS STATED, FROM THE DUCKS DURING AND AFTER THE FEEDING OF THE RADIOACTIVE CHEMICAL.

Bird No.	PLASMA		RUC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4006	0.53		0.23		0.54		0.50		0.05		0.09		0.25		0.23	
4008	0.12		0.0		0.11		0.22		0.0		0.06		0.14		0.10	
4010	0.33	0.24 ^a	0.21	0.11	0.48	0.29	0.40	0.32	0.0	0.000	0.07	0.053	0.20	0.17	0.13	0.143
4009	0.13	±0.162	0.00	±0.12	0.30	±0.182	0.23	±0.143	0.0	±0.020	0.0	±0.044	0.15	±0.052	0.17	±0.052
4005	0.22		0.19		0.17		0.29		0.0		0.10		0.19		0.13	
4007	0.13	6/6 ^b	0.0	3/6	0.15	6/6	0.22	6/6	0.0	1/6	0.0	4/6	0.10	6/6	0.09	6/6
	Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4012	0.43		0.20		0.44		0.60		0.05		0.11		0.11		0.15	
4014	0.36		0.0		0.65		0.52		0.0		0.11		0.19		0.09	
4016	0.63	0.40	0.26	0.13	0.83	0.51	0.81	0.51	0.15	0.040	0.06	0.070	0.16	0.16	0.20	0.140
4011	0.26	±0.126	0.00	±0.11	0.22	±0.226	0.25	±0.189	0.0	±0.062	0.0	±0.041	0.12	±0.045	0.09	±0.048
4013	0.37		0.14		0.60		0.45		0.09		0.08		0.23		0.18	
4015	0.34	6/6	0.16	4/6	0.32	6/6	0.41	6/6	0.0	3/6	0.06	5/6	0.15	6/6	0.18	6/6
	Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4096	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
4089	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
4087	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.06	0.02	0.0	0.0
4088	0.0		0.0		0.0		0.0		0.0		0.0		0.07	±0.034	0.0	
4086	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
4085	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	2/6	0.0	0/6
	Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4713	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
904	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
905	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6733	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
6732	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
995	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	0/6
Detection Limit	0.02		0.11		0.06		0.06		0.05		0.05		0.04		0.04	
	μg/g															

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted.

TABLE J3. ¹⁴C ACTIVITY IN TISSUES OF DOBSONTIC GUinea PIGS ADMINISTERED ORALLY ¹⁴C-DIMP AT A DOSE OF 100 µg/kg BODY WEIGHT. THE SPECIFIC ACTIVITY OF THE DIMP WAS 2000 dpm/µg.

Bird No.	PLASMA		RBC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
2465	135.5		7.74		93.9		80.5		141.2		6.09		50.15		8.39	
2466	160.2		6.44		124.0		107.2		65.39		9.05		54.36		13.27	
2467	181.7	154.14 ^a	10.18	7.47	101.4	115.43	145.1	117.46	4.26	71.20	9.13	9.31	30.28	47.97	11.29	11.10
2468	122.7	±22.66	6.31	±1.43	70.07	±20.99	91.3	±28.17	135.1	±55.46	10.24	±3.06	30.28	±0.21	10.30	±3.25
2469	174.6		6.00		145.8		141.3		39.26		14.63		50.10		16.11	
2470	150.2	6/6 ^b	7.29	6/6	149.4	6/6	139.5		41.93	6/6	5.89	6/6	40.65	6/6	7.25	6/6
	24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
2431	2.16		0.0		0.0		2.00		2.25		0.0		1.13		1.07	
2435	c		0.0		0.66		2.53		0.0		0.73		0.78		0.79	
2436	0.0	0.43	0.0	0.0	0.0	0.11	0.80	1.26	0.0	0.38	0.56	0.50	0.95	0.79	0.59	0.87
2437	0.0	±0.97	0.0		0.0	±0.27	0.0	±0.52	0.0	±0.92	0.78	±0.40	0.54	±0.22	0.95	±0.17
2438	0.0		0.0		0.0		0.90		0.0		0.0		0.57		0.88	
2440	0.0	1/5	0.0	0/6	0.0	1/6	1.22	5/6	0.0	1/6	0.90	4/6	0.78	6/6	0.96	6/6
	48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
2441	0.0		0.0		0.0		0.0		0.0		0.0		0.85		0.0	
2442	0.0		0.0		0.0		0.0		0.0		0.0		0.93		0.0	
2443	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.12	0.0	0.0	0.0	0.0	0.61	0.90	0.0	0.19
2444	0.0		0.0		0.0		0.0	±0.29	0.0		0.0		0.0	±0.89	0.0	±0.30
2445	0.0		0.0		0.0		0.0		0.0		0.0		0.41		0.51	
2446	0.0	0/6	0.0	0/6	0.0	0/6	0.70	1/6	0.0	0/6	0.0	0/0	2.57	5/6	0.64	2/6
Detection Limit	1.15		0.66		0.67		0.39		0.39		0.49		0.41		0.32	
	µg/g		µg/g		µg/g		µg/g		µg/g		µg/g		µg/g		µg/g	

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted

^c Tube broke in centrifuge

TABLE J4. ¹⁴C ACTIVITY IN TISSUES OF MALLARD DUCKS GIVEN ORALLY ¹⁴C-DIMP AT A DOSE OF 100 µg/kg BODY WEIGHT. THE SPECIFIC ACTIVITY OF THE DIMP WAS 16.7 dpm/µg.

Bird No.	PLASMA		RBC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
6013	139.44		4.17		674.44		190.37		13.56		16.94		30.60		20.12	
6014	129.00		4.49		932.70		103.43		15.65		22.39		45.44		25.63	
6015	129.74	137.93 ^a	5.28	5.05	712.34	756.32	161.62	179.98	16.50	15.78	25.92	22.85	39.30	45.13	25.63	26.45
6016	134.90	±8.36	4.25	±1.29	662.17	±103.99	175.03	±19.64	20.13	±2.37	29.90	±4.95	52.43	±8.80	33.94	±4.46
6017	150.34		7.55		825.59		211.10		14.57		17.72		49.17		25.72	
6018	144.05	6/6 ^b	4.56	6/6	730.60	6/6	150.23	6/6	14.26	6/6	24.23	6/6	53.75	6/6	27.65	6/6
	24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
6043	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
6044	0.0		0.0		0.0		0.0		1.23		0.0		0.0		0.0	
6045	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.78	0.50	0.0	0.00	0.0	0.0	0.0	0.0
6046	0.0		0.0		0.0		0.0		0.0	±0.80	1.76	±0.88	0.0		0.0	
6047	0.0		0.0		0.0		0.0		0.0		1.53		0.0		0.0	
6048	0.0	6/6	0.0	6/6	0.0	0/6	0.0	0/6	0.0	2/6	1.53	3/6	0.0	0/6	0.0	0/6
	48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
6037	0.0		0.0		2.00		0.0		0.0		0.0		0.0		0.0	
6038	0.0		0.0		0.0		0.0		1.00		0.0		0.0		0.0	
6039	0.0	0.0	0.0	1.01	0.0	0.47	0.0	0.0	1.15	0.50	0.0	0.27	0.0	0.0	0.0	0.0
6040	0.0		0.0	±2.47	0.0	±1.14	0.0	0.0	1.31	±0.64	1.61	±0.66	0.0		0.0	
6041	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
6042	0.0	0/6	6.06	1/6	0.0	1/6	0.0	0/6	0.0	3/6	0.0	1/6	0.0	0/6	0.0	0/6
Detection Limit µg/g	7.19		3.32		1.93		2.43		1.14		1.48		1.98		3.14	

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted

APPENDIX K

¹⁴C Activity in Tissues of Bobwhite Quail and
Mallard Ducks Fed or Dosed With ¹⁴C-DCPD

TABLE XI. ¹⁴C ACTIVITY IN TISSUES OF DOMESTIC QUAIL FED 100 μg OF ¹⁴C-DCPD AT 221.7 dpm/μg. SAMPLES WERE PROCURED, AS STATED, FROM QUAIL DURING AND AFTER THE FEEDING OF THE RADIOACTIVE CHEMICAL.

Bird No.	PLASMA		RBC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
1992	0.0		0.0		0.23		0.15		0.0		0.0		0.12		0.0	
1993	0.0		0.0		0.0		0.14		0.12		0.0		0.10		0.0	
1994	0.0	0.0	0.0	0.0	0.23	0.13 ^a	0.20	0.173	0.0	0.030	0.0	0.010	0.13	0.152	0.0	0.0
1520	0.0		0.0		0.0	±0.15	0.23	±0.036	0.0	±0.060	0.0	±0.023	0.21	±0.046	0.0	0.0
1527	0.0		0.0		0.33		0.18		0.12		0.06		0.09		0.0	
1527	0.0	0/6 ^b	0.0	0/6	0.0	3/6	0.14	6/6	0.0	2/6	0.0	1/6	0.19	6/6	0.0	0/6
	Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
1551	0.0		0.0		0.25		0.11		0.0		0.0		0.17		0.0	
1529	0.0		0.0		0.29		0.11		0.0		0.0		0.17		0.0	
1530	0.0	0.037	0.0	0.0	0.34	0.247	0.14	0.173	0.14	0.024	0.0	0.0	0.19	0.100	0.0	0.0
1552	0.13	±0.050	0.0		0.36	±0.131	0.20	±0.068	0.0	±0.058	0.0		0.32	±0.071	0.0	0.0
1553	0.09		0.0		0.24		0.17		0.0		0.0		0.11		0.0	
1554	0.0	2/6	0.0	0/6	0.0	5/6	0.23	6/6	0.0	1/6	0.0	0/6	0.16	6/6	0.0	0/6
	Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
1657	0.0		0.0		0.29		0.0		0.0		0.0		0.0		0.0	
1555	0.0		0.0		0.0		0.0		0.0		0.0		0.12		0.0	
1557	0.0	0.0	0.0	0.0	0.0	0.030	0.0	0.0	0.0	0.0	0.0	0.0	0.06	0.003	0.0	0.0
1560	0.0		0.0		0.0	±0.151	0.0		0.0		0.0		0.10	±0.052	0.0	0.0
1524	0.0		0.0		0.0		0.0		0.0		0.0		0.15		0.0	
1550	0.0	0/6	0.0	0/6	0.30	2/6	0.0	0/6	0.0	0/6	0.0	0/6	0.06	6/6	0.0	0/6
	Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
1627	0.0		0.0		0.0		0.0		0.0		0.0		0.14		0.0	
1626	0.0		0.0		0.25		0.0		0.0		0.0		0.0		0.0	
1629	0.0	0.0	0.0	0.0	0.0	0.070	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.046	0.0	0.0
1620	0.0		0.0		0.0	±0.121	0.0		0.0		0.0		0.0	±0.071	0.0	0.0
1670	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0	
1654	0.0	0/6	0.0	0/6	0.22	2/6	0.0	0/6	0.0	0/6	0.0	0/6	0.13	2/6	0.0	0/6
Detection Limit μg/g	0.006		0.10		0.16		0.09		0.006		0.050		0.06		0.04	

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted

TABLE K2. ¹⁴C ACTIVITY IN TISSUES OF MALLARD DUCKS FED 160 PPM OF ¹⁴C-DCCP AT 221.7 dpm/μg. SAMPLES WERE PROCURED, AS STATED, FROM DUCKS DURING AND AFTER THE FEEDING OF THE RADIOACTIVE CHEMICAL.

Bird No.	PLASMA		RBC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on		Day 3 on	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
4647	0.13		0.18		0.30		0.94		0.19		0.15		0.23		0.09	
4629	0.17		0.29		1.07		1.00		0.29		0.19		0.16		0.0	
4730	0.33	0.25 ^a	0.44	0.20	0.77	0.65	0.54	0.61	0.36	0.393	0.40	0.267	0.38	0.43	0.24	0.143
935	0.33	±0.003	0.31	±0.093	0.57	±0.264	0.65	±0.212	0.32	±0.201	0.33	±0.095	0.46	±0.202	0.22	±0.093
6752	0.20		0.26		0.48		1.04		0.43		0.22		0.40		0.20	
937	0.25	6/6 ^b	0.20	6/6	0.68	6/6	0.67	6/6	0.77	6/6	0.31	6/6	0.93	6/6	0.11	5/6
	Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on		Day 5 on	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
949 ^c																
6470	0.16		0.40		0.49		1.01		0.68		0.23		0.26		0.10	
934	0.10	0.10	0.20	0.25	1.22	0.602	0.97	0.908	0.15	0.164	0.09	0.120	0.12	0.200	0.15	0.130
939	0.08	±0.040	0.17	±0.090	0.69	±0.364	1.03	±0.039	0.26	±0.072	0.07	±0.062	0.16	±0.064	0.12	±0.029
942	0.05		0.26		0.24		0.93		0.12		0.13		0.26		0.17	
944	0.10	5/5	0.22	5/5	0.77	5/5	1.00	5/5	0.21	5/5	0.12	5/5	0.24	5/5	0.11	5/5
	Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off		Day 3 off	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
936	0.0		0.0		0.46		0.0		0.00		0.0		0.0		0.0	
940	0.0		0.0		0.11		0.11		0.00		0.0		0.0		0.0	
938	0.0	0.0	0.0	0.0	0.35	0.27	0.0	0.10	0.00	0.0	0.0	0.0	0.0	0.022	0.0	0.013
6751	0.0		0.0		0.33	±0.129	0.14	±0.065	0.00		0.0		0.0	±0.053	0.0	±0.033
6760	0.0		0.0		0.21		0.18		0.00		0.0		0.13		0.08	
947	0.0	0/6	0.0	0/6	0.18	6/6	0.19	4/6	0.00	0/6	0.0	0/6	0.0	1/6	0.0	1/6
	Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off		Day 5 off	
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
941	0.0		0.0		0.99		0.13		0.0		0.0		0.0		0.0	
945	0.0		0.0		1.10		0.10		0.0		0.0		0.0		0.09	
943	0.0	0.0	0.0	0.0	0.72	0.61	0.0	0.07	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.015
6780	0.0		0.0		0.31	±0.377	0.09	±0.056	0.0		0.0		0.0		0.0	±0.037
6784	0.0		0.0		0.26		0.10		0.0		0.0		0.0		0.0	
948	0.0	0/6	0.0	0/6	0.29	6/6	0.0	4/6	0.0	0/6	0.0	0/6	0.0	0/6	0.0	1/6
Detection Limit μg/g	0.03		0.16		0.08		0.09		0.08		0.06		0.08		0.08	

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted

^c Died 4/13/77 (other 5 died 4/14/77)

TABLE K3. ^{14}C ACTIVITY IN TISSUES OF DOBBIITE QUAIL GIVEN ORALLY ^{14}C -DCPD AT A DOSE OF 100 mg/kg BODY WEIGHT. THE SPECIFIC ACTIVITY OF THE DCPD WAS 53.5 dpm/ μg .

Bird No.	PLASMA		RDC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	2 Hr. $\mu\text{g/g}$															
2471	12.06		0.0		9.56		26.53		91.26		4.32		17.49		2.51	
2472	26.07		0.0		21.01		29.07		56.46		10.53		16.55		0.12	
2473	24.72	19.11 ^a	1.49	0.50	21.93	17.54	27.47	26.11	25.40	50.13	7.11	6.07	14.33	13.69	7.88	5.60
2474	22.43	± 6.46	1.49	± 0.77	10.36	± 5.60	26.51	± 4.27	91.09	± 35.30	6.01	± 2.46	11.21	± 3.16	6.21	± 2.30
2475	10.80		0.0		11.61		17.75		8.77		4.06		9.14		3.38	
2476	18.49	6/6 ^b	0.0	2/6	21.96	6/6	29.31	6/6	27.69	6/6	8.30	6/6	13.39	6/6	5.49	6/6
	24 Hr. $\mu\text{g/g}$															
2453	0.0		0.0		2.41		3.69		1.46		0.00		0.09		0.0	
2454	0.0		0.0		3.13		3.62		1.05		1.10		1.99		0.0	
2455	4.76	3.58	1.01	0.17	5.94	4.44	9.99	9.15	6.55	5.72	2.32	2.26	1.37	2.93	1.79	1.21
2456	3.92	± 3.78	0.0	± 0.41	2.04	± 3.20	10.19	± 5.63	5.63	± 4.51	2.02	± 1.50	2.61	± 2.02	2.11	± 0.97
2457	10.18		0.0		10.33		18.95		13.54		5.04		6.11		1.34	
2458	2.62	4/6	0.0	1/6	2.01	6/6	8.47	6/6	6.06	6/6	2.27	6/6	4.60	6/6	2.01	4/6
	48 Hr. $\mu\text{g/g}$															
2459	0.0		0.0		2.24		2.24		1.39		0.0		0.0		0.64	
2460	3.02		0.0		2.63		3.10		1.32		0.0		1.12		0.57	
2461	0.0	1.06	0.0	0.0	0.0	1.98	2.44	2.06	3.19	2.28	0.0	0.54	0.09	0.94	0.57	0.60
2462	1.84	± 1.27	0.0		3.92	± 1.32	4.13	± 0.70	4.36	± 1.34	1.50	± 0.64	1.34	± 0.49	1.04	± 0.34
2463	1.50		0.0		1.29		2.05		2.56		0.67		1.09		0.0	
2464	0.0	3/6	0.0	0/6	1.79	6/6	2.41	6/6	0.85	6/6	1.05	3/6	1.22	5/6	0.77	5/6
Detection Limit $\mu\text{g/g}$	1.69		0.96		0.99		0.58		0.57		0.72		0.60		0.47	

^a Mean \pm S.D.

^b Number showing ^{14}C vs number of samples counted

TABLE K4. ¹⁴C ACTIVITY IN TISSUES OF MALLARD DUCKS GIVEN ORALLY ¹⁴C-DCPD AT A DOSE OF 100 µg/kg BODY WEIGHT. THE SPECIFIC ACTIVITY OF THE DCPD WAS 17.4 dpm/µg.

Bird No.	PLASMA		RBC		LIVER		KIDNEY		ADIPOSE		BRAIN		SKIN		MUSCLE	
	2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.		2 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
6019	14.97		0.0		40.00		73.56		69.62		12.61		34.60		10.83	
6020	15.04		0.0		27.35		34.85		21.21		9.00		15.32		10.90	
6021	13.54	12.07 ^a	0.0	0.0	29.59	31.71	37.74	40.94	44.64	34.26	13.20	10.79	24.96	20.81	8.96	11.79
6022	12.07	±3.30	0.0		41.62	±0.63	34.03	±16.32	20.61	±19.75	11.80	±2.87	19.51	±7.82	13.53	±4.04
6023	10.30		0.0		32.13		37.07		29.00		12.39		16.74		11.13	
6024	6.39	6/6 ^b	0.0	0/6	18.76	6/6	20.39	6/6	19.57	6/6	5.75	6/6	13.75	6/6	7.32	6/6
	24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.		24 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
6031	0.0		0.0		7.55		6.45		14.75		4.79		12.63		3.29	
6032	0.0		0.0		5.83		2.09		12.16		2.22		5.90		0.0	
6033	0.0	0.0	0.0	0.0	4.56	7.15	3.63	5.51	13.12	16.52	0.0	3.40	4.78	8.04	0.0	1.06
6034	0.0		0.0		0.67	±2.14	7.42	±2.76	33.14	±3.20	5.40	±2.03	12.10	±3.30	3.51	±2.06
6035	0.0		0.0		5.90		3.04		13.79		4.35		7.40		0.0	
6036	0.0	0/6	0.0	0/6	10.39	6/6	9.64	6/6	12.16	6/6	4.06	5/6	10.16	6/6	4.33	3/6
	48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.		48 Hr.	
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
6025	0.0		0.0		1.07		0.0		1.78		0.0		0.0		0.0	
6026	0.0		0.0		0.0		0.0		0.82		0.0		0.0		0.0	
6027	0.0	0.0	0.0	0.0	2.91	2.96	0.0	2.15	2.74	4.01	0.0	0.70	2.99	2.49	0.0	0.0
6028	0.0		0.0		3.06	±2.47	2.30	±2.70	4.90	±3.16	0.0	±1.22	2.39	±3.71	0.0	0.0
6029	0.0		0.0		7.47		6.45		9.71		2.66		9.56		0.0	
6030	0.0	0/6	0.0	0/6	2.47	5/6	4.15	3/6	4.00	6/6	1.99	2/6	0.0	3/6	0.0	0/6
Detection Limit µg/g	6.92		3.19		1.06		2.33		1.09		1.43		1.90		3.03	

^a Mean ± S.D.

^b Number showing ¹⁴C vs number of samples counted

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