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Response of Rats to Chronic Ingestion of Diphacinone

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Diphacinone, 2-(diphenylacetyl)-1H-indene-1,3(2H)-dione, is a potent hypoprothrombinemic (anticoagulant) agent which has been utilized as a rodenticide since the 1950's (MARTIN and WORTHING 1977; GATES 1957). However, examination of published data on diphacinone reveals considerable variation in acute toxicity for rats and little information on chronic toxicity. Reported acute oral LD_{50}'s, for example, range from 1.9 mg/kg (GAINES 1969) to 17 mg/kg (FENTLEY and LARTH 1959). CORRELL et al. (1952) reported an acute oral LD_{50} of 3 mg/kg and a 14-day chronic oral LD_{50} of > 0.1 mg/kg per day. To our knowledge, no definitive chronic toxicity studies on this compound have been reported.

The purpose of this work was to determine the effects on albino laboratory rats of long-term feeding on low levels of diphacinone. Specifically, the work was designed as a dose level range-finding study preparatory to a 2-year or lifetime chronic toxicity investigation. In addition to mortality and clinical symptoms of intoxication, we investigated various physiological indicators to determine if any pathological effects developed in surviving animals.

MATERIALS AND METHODS

Rats

Sprague-Dawley rats (Charles River Breeding Laboratories*), weighing between 100-150 g at the start of the test, were identified by numbered ear tags, individually caged, and acclimated to laboratory conditions for 7 days before testing. Lighting and temperature in the test laboratory were automatically controlled and maintained (i.e., 12 h light/dark cycle and 23-26° C). Food and water were available *ad libitum*; however, food consisted only of the test foods with no alternatives offered.

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* Reference to commercial products or companies does not imply endorsement by the Federal Government or any of its agencies.

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Test foods

Test foods were prepared with a twice recrystallized technical grade diphacinone obtained from the Motomco Chemical Company. The carrier was Purina Lab Chow in powdered form. Diphacinone was dissolved in acetone and thoroughly mixed into a measured amount of the carrier to form a 10-ppm premix. The acetone was evaporated off and all test food concentrations were subsequently derived by geometrical dilution.

The control food was prepared in the same manner except that diphacinone was omitted.

Procedure

The investigation involved two separate feeding studies, one of 90 days' duration and a later 21-day study. The second study was conducted as a consequence of results obtained in the first. The objective of the second study was to define a dose level where mortality was clearly evident since this was not accomplished in the first study. Essentially, the procedures followed were the same for each study. In the 90-day study, 16 animals (8 M and 8 F) were fed one of six concentrations (0.5, 0.25, 0.125, 0.0625, 0.0313, and 0 ppm) of diphacinone for a total of 96 rats. In the 21-day study, 4 animals (2 M and 2 F) were fed one of seven diphacinone concentrations (4, 2, 1, 0.5, 0.25, 0.125, and 0 ppm) for a total of 28 rats. Each animal was randomly assigned to a concentration, weighed immediately before start of the test, and weekly thereafter. Food consumption was determined by weigh-back three times each week. Feeding continued for the duration of each study (i.e., 90 and 21 days, respectively). Visual checks for mortality, hemorrhage, or gross behavioral changes (e.g., loss of alertness) as clinical signs of intoxication were made daily, but animals were handled only during weighing.

90-day study: Of the original 96 animals, 12 (1 M and 1 F from each concentration level) were sacrificed at 30 days and 12 more at 60 days. Gross necropsy examinations and prothrombin clotting time determinations were performed on each to determine if any treatment effect was evident at these two time periods. A contractual agreement was made with Elars BioResearch Laboratories, Fort Collins, Colorado, to make pathological examinations and blood chemistry tests on the remaining 72 animals. The authors conducted the prothrombin tests according to the method of Quick (MONNHOUSE 1961). At the end of the 90-day period, all 72 animals were examined for gross pathology and 70 were included in the blood tests and prothrombin determinations. The 2 rats excluded from blood examinations had died during the study and blood collection was not feasible. The 70 surviving animals were anesthetized with chloroform, and blood was taken from the brachial artery of each for prothrombin clotting time measurements, hematology, and clinical chemistry determinations. The animals were then euthanized and necropsies were performed. Blood from

two males and two females, randomly selected from each treatment group, was used for hematology and clinical chemistry evaluations.

21-day study: Since mortality did not occur at the highest treatment level (0.5 ppm) of the 90-day study and only two deaths occurred at lower levels, the 21-day study was designed. In this study, three higher concentrations (i.e., 1, 2, and 4 ppm) of diphacinone were used to determine a point at which mortality might be expected. Gross necropsies were performed on all animals and prothrombin determinations were conducted on those euthanized at the end of the study. Hematology and clinical chemistry analyses were not performed in this test.

RESULTS AND DISCUSSION

90-day study: No abnormalities were evident at necropsy of the 12 animals sacrificed at 30 days nor the 12 sacrificed at 60 days. Prothrombin clotting times of the animals that received diphacinone had a mean of 13.2 s, SD 0.8. Those that consumed untreated food had a mean of 13.2 s, SD 0.5.

Table 1 summarizes the results of this trial on the 72 remaining animals. Two animals died during the test, a male at 0.25 ppm and a male at 0.0625 ppm. Post-mortem examination revealed subdural hemorrhage as the probable cause of death. Both of these animals and 10 others on diphacinone exhibited a pinkish eye discharge and some hair loss around the eyes. These symptoms were seen in four rats on the 0.125 ppm treatment level and two rats on each of the other levels. The control animals did not exhibit these symptoms. Prothrombin clotting times for the 10 rats had a mean of 12.8 s, SD 1.5. Prothrombin times for the 70 animals alive at the end of the study were examined for treatment effect by analysis of variance. The prothrombin times of each treatment were not significantly different (P = 0.38).

TABLE 1. Prothrombin clotting times and mortality associated with ingestion of diphacinone in the diet during 90-day feeding test.

Diphacinone concentration in diet (ppm)	Mean prothrombin clotting times (± SD)	Mortality # deaths/ # animals tested	Time to death (days)
0.5	13.0 ± 1.8	0/12	
0.25	13.4 ± 2.5*	1/12	17
0.125	12.5 ± 1.3	0/12	
0.0625	12.8 ± 0.5*	1/12	20
0.0313	12.5 ± 1.0	0/12	
0.0	13.6 ± 1.6	0/12	

* Based on 11 animals.

Hematology results are given in Table 2; clinical chemistry results in Table 3. Values are similar for animals of all groups; however, fibrinogen levels in the 0.5 ppm group are lower than the others.

In summary, the animals appeared generally healthy and in good condition, irrespective of dosage regimen, with the exception of the 2 that died with indications of subdural hemorrhage and the 10 that had the symptoms associated with the eye area.

21-day study: All animals in the 4-ppm and 2-ppm dose-level groups succumbed to diphacinone poisoning (Table 4). Post-mortem examinations revealed massive internal hemorrhage, primarily in the thoracic or abdominal areas. Animals in the other groups (1, 0.5, 0.25, 0.125 and 0 ppm) did not exhibit any clinical symptoms of intoxication during the test period. The eye discharge, noted in several animals during the 90-day test, was not manifest during this trial. Gross necropsy did not reveal any abnormalities with the exception of one rat in the 0.5-ppm group which had some hemorrhage in the thymus. We analyzed prothrombin clotting times for the 20 rats examined at the end of the 21-day study using analysis of variance. The prothrombin times of each treatment were not significantly different ($P = 0.15$).

General

Body weight increase from start to finish of a test and food consumption were analyzed by a two-factor (treatment and sex) analysis of variance in both the 21- and 90-day studies. The 4-ppm and 2-ppm levels were excluded from the 21-day analyses. Food consumption was analyzed using mean weekly grams of food eaten per kilogram of body weight. The body weights used to standardize food consumption were mean weights for the length of the study and were transformed to the 0.75 power to correct for metabolic size (BRODY 1945). A summary of body weight gain and food consumption means is given in Table 5.

In the 90-day study, body weight gain differences among treatment groups were not significant ($P = 0.13$) nor was food consumption ($P = 0.19$). In the 21-day study there was a significant body weight gain difference among treatments ($P = 0.05$). However, this difference only occurred with the 0.25-ppm level differing from the other three diphacinone levels but not the control. Food consumption for the 21 days was not significantly different among treatments ($P = 0.13$). Males on the 90-day study gained more weight than females ($P < 0.01$) but ate less ($P = < 0.01$). In the 21-day study males gained more weight than females ($P < 0.01$) and ate more ($P < 0.01$).

The results of these tests define factors that should be considered in designing a long-term chronic toxicity feeding study with diphacinone. Mortality appears to occur at concentrations above 1 ppm. Sporadic isolated mortality can occur at dose levels which are lower than 1 ppm as evidenced by the deaths at 0.25 and 0.0625 ppm. There is some indication of possible treatment

TABLE 2. Results of hematology analyses--90-day diphacinone toxicity trial.

Treatment level	Sample number	PCV ( )	Hemoglobin (g/100 ml)	Total protein (g/100 ml)	Fibrinogen (mg/100 ml)	WBC ($X10^3$)	Differential leukocyte count (%) SEGS	LYMPHS	MONOS	EOSINO	BASO
0.5 ppm	1 F	57	17.6	8.1	<100	5,000	15	85			
	2 F	47	15.9	7.9	<100	3,500	33	51	15	1	
	3 M	53	17.0	7.7	<100	8,600	9	79	11	1	
	4 M	52	17.6	7.9	<100	4,000	13	70	16	1	
0.25 ppm	1 F	40	13.6	7.5	200	2,400	22	76	1	1	
	2 F	44	14.8	7.3	300	3,500	15	78	7		
	3 M	49	15.9	7.3	<100	11,900	11	83	6		
	4 M	54	16.4	7.3	200	6,100	11	88	1		
0.125 ppm	1 F	50	16.4	8.1	400	10,400	15	76	8	1	
	2 F	51	16.4	8.3	400	8,600	28	70	1	1	
	3 M	51	17.6	7.3	200	7,000	13	87			
	4 M	49	15.9	7.5	400	6,400	16	84			
0.0625 ppm	1 F	48	15.4	7.1	<100	5,900	23	76	1		
	2 F	48	15.0	8.3	300	12,900	10	88	2		
	3 M	49	15.4	7.3	300	9,200	13	84	2	1	
	4 M	47	15.0	6.9	200	6,000	25	74	1		
0.0313 ppm	1 F	49	15.9	7.5	<100	9,100	16	83	1		
	2 F	47	15.0	7.7	200	8,800	35	64	1		
	3 M	47	14.8	7.3	300	8,900	35	65			
	4 M	45	15.0	7.3	400	6,100	76	22	1	1	
0 ppm	1 F	52	17.0	8.5	400	6,900	16	78	3	3	
	2 F	50	16.4	9.2	100	8,000	15	72	13		
	3 M	52	17.0	7.8	400	20,600	2	98			
	4 M	48	15.4	7.5	100	8,300	25	75			

TABLE 3. Results of clinical biochemistry analysis--90-day diphacinone toxicity trial.

Treatment level	Sample number	Glucose (mg/100 ml)	BUN (mg/ 100 ml)	Globulin (g/100 ml)	Protein (g/100 ml)	Chloride (meq/l)	Cholesterol (mg/100 ml)	K (meq/l)	NA (meq/l)
0.5 ppm	1 F	273	20	3.4	7.8	100	96	10.2	141
	2 F	332	24	3.7	8.2	98	102	10.0	141
	3 M	132	18	2.9	6.5	100	66	8.8	141
	4 M	100	18	2.7	6.2	101	70	9.5	141
0.25 ppm	1 F	125	16	2.8	6.4	102	81	6.7	142
	2 F	130	21	3.1	6.8	105	75	8.5	140
	3 M	147	13	2.9	6.2	108	84	6.4	140
	4 M	127	16	3.6	5.8	103	86	10.9	139
0.125 ppm	1 F	137	17	3.0	6.5	102	75	10.7	140
	2 F	112	18	4.0	6.8	100	[illegible]	11.8	139
	3 M	295	18	3.6	6.6	99	87	11.3	140
	4 M	165	17	2.8	6.5	99	70	9.4	139
0.0625 ppm	1 F	179	22	3.2	6.6	106	93	6.5	140
	2 F	118	16	2.9	6.5	105	97	7.7	139
	3 M	460	18	3.4	6.9	97	72	11.2	143
	4 M	115	19	2.9	6.4	100	105	9.6	139
0.0313 ppm	1 F	310	19	3.3	7.4	95	95	11.8	138
	2 F	103	24	2.9	7.0	107	85	7.6	139
	3 M	100	21	2.6	6.4	102	82	9.4	139
	4 M	107	24	2.6	6.3	105	75	9.6	139
0 ppm	1 F	107	19	3.1	6.6	101	70	9.6	138
	2 F	158	18	2.9	7.0	99	79	9.4	138
	3 M	210	20	3.1	7.0	98	83	9.6	138
	4 M	129	20	2.6	6.2	99	90	10.5	138

TABLE 3. Continued.

Treatment level	Sample number	BILI (mg/ 100 ml)	Alk. Phos. (units)	SGOT (units)	SGPT (units)	CPK (IU/l)	P (mg/ 100 ml)	LDH (units)	Ca (meq/l)	Creatinine (mg/100 ml)
0.5 ppm	1 F	0.8	45	196	152	612	12.0	1050	13.6	1.2
	2 F	1.4	43	223	157	512	13.0	1250	14.8	1.3
	3 M	0.4	53	189	125	585	8.4	1400	10.8	1.0
	4 M	0.6	48	181	100	643	7.5	1325	10.3	0.9
0.25 ppm	1 F	0.4	65	137	85	452	7.8	975	9.8	0.8
	2 F	0.4	100	146	96	514	7.7	1075	10.6	1.0
	3 M	0.2	120	116	83	394	7.0	800	9.4	0.8
	4 M	0.3	48	150	95	504	8.7	1025	11.4	1.0
0.125 ppm	1 F	0.4	33	173	105	615	8.4	1325	9.6	1.0
	2 F	0.5	46	177	110	728	9.7	1390	11.2	0.8
	3 M	0.5	55	182	95	982	9.9	1075	14.7	0.3
	4 M	0.3	42	139	76	512	7.8	1060	12.2	0.8
0.0625 ppm	1 F	0.5	61	154	100	590	7.5	900	13.3	1.0
	2 F	0.3	51	147	115	515	8.4	960	12.2	0.8
	3 M	0.6	4	137	92	692	11.0	600	4.7	1.6
	4 M	0.5	150	138	115	559	8.6	1025	12.8	0.9
0.0313 ppm	1 F	0.6	41	195	140	713	9.8	975	13.7	1.4
	2 F	0.8	68	146	121	428	6.5	1125	13.2	0.9
	3 M	0.6	95	186	130	611	8.9	1400	12.4	1.0
	4 M	0.8	73	194	148	692	9.2	1550	13.0	1.1
0 ppm	1 F	0.4	31	172	107	548	7.2	1325	13.0	0.9
	2 F	0.4	23	183	107	656	8.7	1310	13.5	1.0
	3 M	0.8	85	218	124	662	9.6	1275	14.8	1.1
	4 M	0.5	50	183	120	664	9.6	1375	13.4	1.0

TABLE 4. Prothrombin clotting times and mortality associated with ingestion of diphacinone in the diet during 21-day feeding test.

Diphacinone concentration in diet (ppm)	Mean prothrombin clotting times (± SD)	Mortality # deaths/ # animals tested	Time to death (days)
4.0		4/4	3, 4, 6, 7
2.0		4/4	5, 5, 7, 14
1.0	13.9 ± 1.9	0/4	
0.5	12.7 ± 0.4	0/4	
0.25	13.6 ± 0.6	0/4	
0.125	11.3 ± 2.3	0/4	
0.0	14.0 ± 0.6	0/4	

TABLE 5. Body weight and food consumption associated with ingestion of diphacinone in the diet.

Study	Treatment (ppm)	Sex	Body weight gain (mean grams)	Food consumption (mean g/kg body weight$^{.75}$/week)
90-day	0.5		300a	375a
	0.25		269a	363a
	0.125		257a	356a
	0.0625		274a	369a
	0.0313		296a	374a
	0		287	372a
		M	373a	360a
		F	193b	376b
21-day	1.0		128a	479a
	0.5		130a	466a
	0.25		103b	473a
	0.125		129a	494a
	0		120a,b	483a
		M	163a	497a
		F	81b	461b

Means having the same letter are not significantly different ($P > 0.05$).

effects below 1 ppm as demonstrated in gross pathology and blood chemistry findings. Such variation in response to the lethal effects of diphacinone has been observed in studies on other species such as wild Norway rats--R. norvegicus, roof rats--R. rattus, and house mice--Mus musculus (BENTLEY and LARTH 1959). SAVARIE et al. (1979) experienced similar results in studies with coyotes--Canis latrans, and ELIAS et al. (1978) observed this phenomenon in domestic cattle.

The authors recommend that a long-term chronic toxicity feeding study with diphacinone include chemical levels in the feed starting below 0.0313 ppm and including 4 ppm as the high level. This study provides a starting point for future research concerning the chronic toxicity of diphacinone.

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