

ACTIVITIES OF DICHLORVOS OR DISOPHENOL AGAINST THE HOOKWORM (*Uncinaria lucasi*) AND SUCKING LICE OF NORTHERN FUR SEAL PUPS (*Callorhinus ursinus*) ON ST. PAUL ISLAND, ALASKA [□]

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Abstract: One controlled and six critical tests were conducted in July, 1977 with northern fur seal pups (*Callorhinus ursinus*) to determine the efficacies of a single dose of dichlorvos capsules at 29.3 to 32.8 mg/kg, tablets at 10.5 to 11.5 mg/kg, or disophenol at 9.9 mg/kg given subcutaneously against natural infections of adult *Uncinaria lucasi*. In the controlled test, 20 pups were treated and 10 pups were nontreated. Removal of hookworms in this test was 99% for five pups receiving dichlorvos capsules, 99% for five pups receiving dichlorvos tablets, and 77% for 10 pups receiving disophenol. Also, both formulations of dichlorvos and the formulation of disophenol were highly active against natural infestations of two species of sucking lice (*Proechinophthirus fluctus* and *Antarctophthirus callorhini*).

In critical tests with four pups treated with dichlorvos capsules at 28.6 to 30.6 mg/kg, removal of hookworms was uniformly 100%. Disophenol at 9.9 mg/kg removed 100% and <1% of hookworms in two pups, respectively, in critical tests.

INTRODUCTION

Control of hookworms in northern fur seal pups (*Callorhinus ursinus*) by drugs has not been studied extensively. Chemotherapy for adult hookworms in pups may not be practical at this time but knowledge of effective compounds may be useful in special circumstances such as in animals used for research. Also, there may be occasion, such as a catastrophic decline of the seal herd, to reduce mortality in pups due to hookworms. Two anthelmintics, dichlorvos¹ and disophenol,¹¹ both highly effective against hookworms in dogs, were selected for evaluation of activity

against adult *Uncinaria lucasi* in naturally-infected fur seal pups. Observations were also made on activity of the two compounds against natural infestations of *Proechinophthirus fluctus* and *Antarctophthirus callorhini*. Previously, there was indication that disophenol was active against *U. lucasi*⁵ and possibly against lice (pers. obs. by one of us - Keyes) in fur seal pups.

MATERIALS AND METHODS

A total of 36 fur seal pups on St. Paul Island, Alaska, were used in one controlled¹⁰ and six critical^{2,3,10} tests for

[□] The investigation reported in this paper (78-4-34) is published with approval of the Director of the Kentucky Agricultural Experiment Station.

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evaluation of the activity of dichlorvos and disophenol against natural infections of adult hookworms. The controlled test also was used to evaluate activity of the two drugs against natural infestations of lice. Pups used in the tests were collected on either 13 or 18 July 1977 from Northeast Point Rookery where virtually 100% prevalence of hookworms have been found in pups born there over the years. The biggest, thus probably oldest pups were selected; therefore, the pups were a maximum of about four weeks of age because the earliest births are usually in mid-June. Pups were weighed, sexed, and individual doses of drug were calculated for each pup to be treated. Dichlorvos was administered orally with a plastic balling gun. Disophenol was injected subcutaneously into the left axillary region.

For the controlled test, 30 pups were placed in cages, five pups per cage. Five pups (Group Ia) received dichlorvos capsules[□] and dose rates varied from 29.2 to 32.8 mg/kg of body weight. Five other pups (Group Ib) received dichlorvos tablets[□] contained in gelatin capsules and dose rates ranged from 10.5 to 11.5 mg/kg of body weight. Ten pups (Group II) received disophenol,[□] 4.5% solution, at a dose rate of 9.9 mg/kg (0.22 cc/kg) of body weight. For nontreated controls (Group III) 10 pups were used. Fecal samples were collected and examined for hookworm eggs at 0, 24, and 48 h posttreatment. Indications, according to positive fecal samples, were that at least 27 of 30 of the pups were infected with hookworms at time of treatment. All pups were killed at 48 h posttreatment. The object of the controlled test was to treat three groups of pups and not the fourth group. When all pups were killed, numbers of hookworms recovered from pups in the treated and nontreated

groups were tabulated and percent efficacy of treatment was calculated; formula used is given in Table 1.

Critical tests were done with six pups treated with either dichlorvos or disophenol. Dichlorvos capsules were given orally to each of four pups at dose rates varying from 28.6 to 30.6 mg/kg. Disophenol, 4.5% solution, was administered subcutaneously at a dose rate of 9.9 mg/kg to two pups. The critical tests entailed placing each pup in an individual cage, treating it, and collecting all feces passed for 36 to 84 h posttreatment to recover adult hookworms. Heavy brown paper was placed under the cage of each pup and feces deposited each 12 h period was washed from the paper into a jar and preserved with 10% formalin. Preserved feces was washed through a 100 mesh sieve and the residue examined for hookworms under a dissecting microscope at about 10× magnification. The pups were examined at necropsy at the close of the fecal collection period and numbers of hookworms remaining in each pup were added to the numbers passed in the feces, to calculate the percent removed by each drug. Fecal samples were collected and examined for hookworm eggs at 0 and 36, 72 or 84 h posttreatment. All pups had hookworm eggs in the feces at time of treatment. Of the dichlorvos-treated pups, one (No. 32) died at 36 h posttreatment and the other three were killed at 72 h posttreatment. The two disophenol-treated pups were killed at 84 h posttreatment.

Techniques for collecting fecal samples, examination for hookworm eggs, and for necropsy of pups in the two tests to recover residual hookworms, were as previously reported.⁹

Additional data from the controlled test were obtained on activity of the two

[□] Task Dog Anthelmintic, Shell Chemical Co., Houston, Texas 77001, USA.

[□] Task Tabs, Shell Chemical Co., Houston, Texas 77001, USA.

[□] D.N.P., American Cyanamid Co., Princeton, New Jersey 08540, USA.

drugs against natural infestations of *P. fluctus* and *A. callorhini*. After the pups were killed and eviscerated, all bodies were placed on cardboard on the laboratory floor by group. Each pup in each group was examined cursorily several times for lice. Then each pup was examined individually on cardboard by combing the entire pelage together with all nonhaired portions, including the openings of nostrils and ears, with forceps. After several examinations, skins were removed, placed in individual plastic bags, kept at room temperature, and re-examined about every four hours. An exception to the technique was for pup Nos. 14, 15, and 16 in Group II where skins were not removed but otherwise examination was the same as for other pups. Total examination period for the pups was for about 48 h postmortem. Activities of the two drugs against the lice were calculated using formula given in Table 4. Identification of the stages and species of lice was done with the aid of published descriptions^{6,8}

RESULTS

Data on hookworms recovered at necropsy of pups in the controlled test have been summarized (Table 1).

At necropsy, adult hookworms were recovered from only one of five pups treated with dichlorvos capsules (Group Ia), three of five pups treated with dichlorvos tablets (Group Ib), all 10 pups treated with disophenol (Group II), and all 10 nontreated control pups (Group III). The number of hookworms varied from 3, 1 to 8, 1 to 144, and 8 to 731 in infected pups in Groups Ia, Ib, II, and III, respectively. Residual hookworms recovered at necropsy appeared to be in good physical condition. Removal of hookworms was 99%, 99%, and 77% for Groups Ia, Ib, and II, respectively.

Feces from 27 of the 30 pups in the controlled test was positive for hookworm eggs at least one of the three times that samples were examined.

Three pups (Nos. 1, 4, and 5) treated with dichlorvos capsules had feces negative for hookworm eggs each of the three sampling periods. The other two pups (Nos. 3 and 10) treated with dichlorvos capsules had feces positive for hookworm eggs only at the time of treatment. Of fecal samples collected at necropsy, none was positive for the pups treated with dichlorvos capsules, 1 of 5 was positive (pup No. 6) for pups treated with dichlorvos tablets, 9 of 10 were positive (all except pup No. 13) for pups treated with disophenol, and all of 10 were positive for nontreated control pups.

Results of the four critical tests on the activity of dichlorvos capsules against adult hookworms are summarized (Table 2). The drug removed 100% of the hookworms from all four pups. Most of the hookworms were passed in feces of the pups by 12 h posttreatment and all hookworms were eliminated by 24 h for two pups, 36 h for one pup, and 48 h for the other pup. None of the pups had feces positive for hookworm eggs at necropsy.

Pup No. 32, harboring the highest number of hookworms in pups in critical tests, died 36 h posttreatment. Cause of death of the pup was not definitely diagnosed although the heavy hookworm burden probably contributed to death. This pup had a seemingly large volume of milk in its stomach at time of treatment and an estimated aggregate total of 250 cc was vomited during three regurgitations over 36 h posttreatment.

Critical test data on activity of disophenol against adult *U. lucasi* in two pups are given (Table 3). For pup No. 35, only 2 of 352 (<1%) hookworms were passed in the feces. However, 100% of 58 hookworms were passed in the feces of pup No. 36. Hookworms were not found in feces of either pup after 48 h posttreatment. Some of the hookworms found in the feces from pup No. 36, at 36 and 48 h after treatment, were in a deteriorated condition. Hookworm eggs were found in feces of pup No. 35 but not pup No. 36 at necropsy.

TABLE 1. Worms recovered at necropsy of 30 fur seal pups in a controlled test of activities of dichlorvos or disophenol against natural infections of adult hookworms (*Uncinaria lucasi*).

No.	Pup Sex	Wt. (kg)	No. of hookworms present		Pup		Wt. (kg)	No. of hookworms present	
			♂	♀	No.	Sex		♂	♀
Group Ia (Treated with dichlorvos capsules)*									
1	♂	8.4	0	0	2	♂	7.7	0	2
3	♂	8.3	0	0	6	♀	8.1	0	8
4	♂	8.8	0	3	7	♂	8.1	0	1
5	♂	9.3	0	0	8	♂	7.4	0	0
10	♂	8.3	0	0	9	♂	8.0	0	0
Group Total (% removal)			0	3	Group Total (% removal)		0	11	11 (99)
Group II (Treated with disophenol)***									
11	♂	6.8	87	57	21	♂	8.6	31	57
12	♂	8.7	77	10	22	♂	8.3	72	62
13	♂	8.7	6	1	23	♂	9.2	16	12
14	♂	8.4	8	11	24	♂	8.3	0	40
15	♂	8.3	29	26	25	♂	8.0	62	87
16	♂	7.9	21	23	26	♂	8.8	1	7
17	♂	8.0	0	1	27	♂	8.4	44	92
18	♀	7.6	2	2	28	♀	7.7	138	159
19	♀	7.3	11	7	29	♂	7.7	334	397
20	♀	7.9	3	9	30	♂	7.6	42	60
Group Total (% removal)			244	147	Group Total		740	973	1,713

* Administered intraorally at dose rates of 29.3 to 32.8 mg/kg.

** Administered intraorally at dose rates of 10.5 to 11.5 mg/kg.

*** Administered by subcutaneous injection at dose rate of 9.9 mg/kg.

% removal = $\frac{\text{Total No. of hookworms in nontreated group minus total No. of hookworms in treated group}}{\text{Total No. of hookworms in nontreated group}} \times 100$

TABLE 2. Data on four fur seal pups in critical tests of dichlorvos capsules,* against natural infections of adult hookworms (*Uncinaria lucasi*).

No.	Sex	Pup		Time posttreatment (hours)	No. of hookworms passed in feces		Pup		Time posttreatment (hours)	No. of hookworms passed in feces			
		Wt. (kg)	Sex		♂	♀	Wt. (kg)	Sex		♂	♀	Total	
31	♂	7.9		12	7	11	18	32	♀	7.7	340	363	703
				24	1	7	8				3	13	16
				36	0	0	0				3	2	5
				48	0	0	0				—	—	—
				60	0	0	0				—	—	—
				72***	0	0	0				—	—	—
Total No.:		Passed in feces			8	18	26	Total No.:		Passed in feces			724
		Found at necropsy			0	0	0			Found at necropsy			0
		Present			8	18	26			Present			346
		(% removal)					(100)			(% removal)			(100)
33	♀	6.7		12	13	16	29	34	♂	8.1	73	84	157
				24	3	4	7				1	1	2
				36	0	0	0				0	0	0
				48	1	1	2				0	0	0
				60	0	0	0				0	0	0
				72	0	0	0				0	0	0
Total No.:		Passed in feces			17	21	38	Total No.:		Passed in feces			159
		Found at necropsy			0	0	0			Found at necropsy			0
		Present			17	21	38			Present			74
		(% removal)					(100)			(% removal)			(100)

* Administered intraorally at dose rates of 28.6 to 30.6 mg/kg.

**Pup No. 32 dead at 36 hours posttreatment.

***Time of termination of experiment.

TABLE 3. Data on two fur seal pups in critical tests of disophenol* against natural infections of adult hookworms (*Uncinaria lucasi*).

Pup		Wt. (kg)	Time posttreatment (hours)	No. of hookworms passed in feces		Pup		Wt. (kg)	Time posttreatment (hours)	No. of hookworms passed in feces		
No.	Sex			♂	♀	No.	Sex			♂	♀	Total
35	♂	9.3	12	0	0	0	0	6.1	12	2	5	7
			24	0	0	0	0		24	0	0	0
			36	0	0	0	0		36	36	13	49
			48	1	1	2	2		48	1	1	2
			60	0	0	0	0		60	0	0	0
			72	0	0	0	0		72	0	0	0
			84**	0	0	0	0		84	0	0	0
Total No.: Passed in feces				1	1	2				Total No.: Passed in feces		58
Found at necropsy				204	146	350				Found at necropsy		0
Present				205	147	352				Present		39
(% removal)						(<1)				(% removal)		(100)

*Administered by subcutaneous injection at dose rate of 9.9 mg/kg.

**Time of termination of experiment.

In the critical tests, pups treated with dichlorvos had bowel movements much more often than pups receiving disophenol.

Data on recovery of lice at necropsy of the 30 pups in the controlled test are recorded (Table 4). Nymph or adult stages of *P. fluctus* were not found on pups treated with dichlorvos, capsules or tablets, or disophenol; a total of 203 nymph but no adult *P. fluctus* were recovered from the nontreated pups. Both nymph and adult *A. callorhini* were detected on all three groups of treated pups and the nontreated control pups. The total number of nymph and adult *A. callorhini* recovered from dichlorvos capsule-treated pups, dichlorvos tablet-treated pups, and disophenol-treated pups was 14, 20, and 11, respectively; however, a total of 265 *A. callorhini* were found on nontreated pups. Removals of both species of lice were 97%, 96%, and 98%, for the three treated groups, Ia, Ib, and II, respectively.

DISCUSSION

Data from the controlled and critical tests indicated overall excellent activity of dichlorvos but generally poorer and variable activity of disophenol against adult *U. lucasi*.

In the controlled test, dichlorvos capsules and dichlorvos tablets removed 99% of adult hookworms. Of the five pups treated with dichlorvos capsules in the controlled test, only one was infected at necropsy, and it had only three worms. Even though verification of hookworm infection was not made, by finding eggs in feces, of three pups at time of treatment with dichlorvos capsules, infection was assumed because of the high prevalence in other pups collected in same area of Northeast Point Rookery. Obtaining enough feces for proper examination for eggs was a problem in some cases. Although the dichlorvos tablets showed excellent activity, three of five of the pups retained a few hookworms at necropsy.

The excellent efficacy of dichlorvos capsules in the controlled test was further supported in the four critical tests where all hookworms were removed by this formulation using similar dose rates. Efficacy of dichlorvos possibly should be investigated further as to most effective dosage because even the lowest dose rates of both formulations evaluated was quite good. The dichlorvos tablet formulation has been reported to be highly effective against *Uncinaria stenocephala* in canine puppies.⁴ Probably it would be advisable to treat several more fur seal pups with a broader range of dosages of both formulations of dichlorvos to determine minimal effective dose rates and possible differences in margins of safety. Also, such factors as heavy and light infections of hookworms and presence or absence of large quantities of milk in the stomach should be considered in evaluation of efficacy and toxicity of dichlorvos. It should be noted that one pup (No. 32), with a large quantity of milk in its stomach and treated with dichlorvos capsules, died. Hookworm infection probably was the cause of death, but the possibility of direct or indirect deleterious effects from the drug could not be ruled out.

Disophenol showed poorer removal activity in the controlled test than did dichlorvos. Removal of hookworms by disophenol was 77% but all 10 pups in this treated group harbored hookworms at necropsy. Possibly if the controlled test had proceeded for more than 48 h, a greater number of hookworms would have been removed in the disophenol treated pups. However, in the two critical tests with disophenol, no hookworms were passed by either pup between 48 and 84 h posttreatment. Also, it should be noted that hookworms remaining in the pups, after treatment, in the controlled test were in good condition while at least some passed at 36 and 48 h posttreatment of one pup in the critical test, where activity was 100%, were deteriorated. Results of disophenol treatment of pups

TABLE 4. Data at necropsy of 30 fur seal pups in a controlled test of activities of dichlorvos or disophenol against natural infestations of two species of lice (*Proechinophirus fluctus* and *Antarctophirus callorhini*).

Pup No.	No. of lice recovered																							
	<i>P. fluctus</i>						<i>A. callorhini</i>						<i>P. fluctus</i>						<i>A. callorhini</i>					
	Nymph (Stage)			Adult			Nymph (Stage)			Adult			Nymph (Stage)			Adult			Nymph (Stage)			Adult		
	1	2	3	♂	♀	Total	1	2	3	♂	♀	Total	1	2	3	♂	♀	Total	1	2	3	♂	♀	Total
Group Ia (treated with dichlorvos capsules)																								
1	0	0	0	0	0	0	1	0	0	1	2	2	2	0	0	0	0	0	0	0	0	0	2	6
3	0	0	0	0	0	0	0	0	0	2	2	2	2	0	0	0	0	0	0	0	0	0	1	1
4	0	0	0	0	0	0	3	0	1	4	4	4	4	0	0	0	0	0	0	0	0	0	1	1
5	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	3	2	2	7
10	0	0	0	0	0	0	1	3	0	1	5	5	5	0	0	0	0	0	0	0	0	0	1	1
Group Total (%)	0	0	0	0	0	0	2	6	0	6	14	14	14	0	0	0	0	0	0	11	2	6	20	
Removal (%)																								(96)
Group Ib (treated with dichlorvos tablets)																								
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	6
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	7
Group Total (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Removal (%)																								(97)
Group II (treated with disophenol)																								
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	20
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	112
14**	0	0	0	0	0	0	0	0	0	2	0	1	3	3	1	3	0	0	0	4	0	3	11	7
15**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	2	4	10
16**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	9
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	1	0	2
Group Total (%)	0	0	0	0	0	0	0	0	0	2	0	2	2	0	0	0	0	0	8	0	0	5	7	17
Removal (%)																								(96)
Group III (Nontreated)																								
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
22	29	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	2	11	21	35	147
23	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	3	11	7	29
24	8	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	2	4	10
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	17
26	10	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	1	0	3
27	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	5	7	1	17
Group Total (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	5	7	1	25
Removal (%)																								(96)

TABLE 4. (continued)

18	0	0	0	0	0	0	0	0	0	1	1	1	1	3	10	4	19	110
19	0	0	0	0	0	0	2	0	2	4	4	0	4	9	7	18	36	53
20	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	14
Group Total	0	0	0	0	0	0	2	6	0	3	11	11	203	62	79	94	265	468
(%)																		
Removal	(98)																	

*Treatment specifics given in footnotes in Table 1.

**Pup Nos. 14, 15, and 16 examined for about 48 hours postmortem but not skinned as were other pups.

$$\% \text{ Removal} = \frac{\text{Total No. of both species of lice in nontreated group minus total No. of both species of lice in treated group} \times 100}{\text{Total No. of both species of lice in nontreated group}}$$

in the critical tests indicated erratic activity because all hookworms were removed from one pup and only 2 of 352 were removed from the other pup.

Removal of hookworms by disophenol in the controlled test would have been much less if one pup (No. 29) in the nontreated group had not had such a high number (731) of hookworms, because removal efficacies in treated groups are related to numbers of hookworms in the control group. For instance, if the number of hookworms in pup No. 29 were not used in calculating removal activity of disophenol-treated pups, only 60% efficacy would have been obtained.

Research on activity of disophenol against hookworms in dogs indicates that the dose rate of 7.5 mg/kg is adequate for removal of species of *Ancylostoma* but that 10.0 mg/kg is necessary for high activity against *U. stenocephala*.¹¹ Apparently, doses higher than 10.0 mg/kg are necessary for complete removal of *U. lucasi* in fur seal pups; any further tests should include dose rates above 10.0 mg/kg to establish minimal effective dose rate and safety level.

Both dichlorvos and disophenol, in the controlled test, appeared to be highly active against two species of sucking lice, *P. fluctus* and *A. callorhini*, found on fur seal pups. Nymph but not adult *P. fluctus* were present on nontreated pups but no specimens of this species were found on any treated pups. Only a few nymph and adult *A. callorhini* were found on treated pups, whereas numerous specimens were detected on nontreated pups. Because pups were not caged separately during the test and not isolated individually at the moment they were killed, the numbers of lice found on each pup probably are not accurate. Also, the visual technique used in recovery of lice in this study is not as accurate as the Cook method.⁷ However, the overall group data appear to give a valid indication of drug activity against lice for two

reasons: 1. the same essential method of lice recovery for all pups in the test was used and 2. the purpose of a controlled test, as originally designed for evaluation of compounds against helminths, is based on group and not individual data.

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Received for publication 9 March 1978