Effects of Some Organophosphorus Insecticides on Vitamin E and Other Blood Constituents and on the Apparent Inducement of Diarrhea in Neonatal Calves

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Signs of intoxication due to an excessive exposure to an organophosphorus (O.P.) insecticide may be dyspnea, weakness, excessive salivation, muscular stiffness, decreased cholinesterase (ChE) activity, and diarrhea (14). However, an exposure to a smaller quantity of the same chemical (but greater than the "no effect level") may produce none or a few of these signs but be responsible for undesirable biologic responses. The consequences of these responses may be directly associated with growth retardation and inefficiency of feed utilization resulting in economic loss to the stockman (3, 6, 7).

Generally, tocopherol levels of the plasma appear to be related to dietary intake and defects in intestinal absorption of fat. Therefore, this study was initiated using plasma tocopherol and ChE as indicators of O.P insecticide toxicosis. Additionally, it seemed appropriate from previous studies that the effect on free fatty acids, carotene, and hematocrit values would have value (2, 5, 8, 9, 12, 16, 17, 19).

When neonatal calves are moved to a new environment and given a substitute diet, many stress factors become paramount. Physical, bacteriological, dietary, and other radical changes can have a profound influence on a study involving neonatal calves (4, 13, 18).

Therefore, in order to differentiate changes caused by O.P. insecticides from other etiologic factors, all test results are compared to untreated controls which were subjected to the same environmental stresses as the test animals.

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[1] R. T. McCarty, deceased.

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Bulletin of Environmental Contamination & Toxicology, Vol. 8, No. 5, 1972 © by Springer-Verlag New York Inc.

Materials and Methods

Test Animals. Twenty-nine unweaned calves 3 to 10 days old from beef and dairy stock were used for this study. Calf weights ranged from 27.7 to 59.5 kg., and principals and controls were selected randomly. All calves were kept under the same environmental conditions and given the same diets: bucket-fed milk, rufis[3], and grain.

Organophosphorus Compounds.

1. Formulation of 25% wettable powder 0,0 diethyl 0-(3-chloro-4-methyl-2-OXO-2H-1-benzopyran-7-yl)phosphorothioate[4], administered as a drench at 25 mg./kg. level.

2. Formulation mixture of technical 0,0-diethyl-s(and-0)-[2(ethylthio)ethyl] phosphorothioates[5] administered in gelatin capsule at 0.5, 1.0, and 1.5 mg./kg. levels on day 1, 3, and 4, respectively.

3. Formulation of 75% wettable powder 2-chloro-1(2,4,5-trichlorophenyl)vinyl dimethylphosphate[6] administered in gelatin capsule at 50 mg./kg. level.

4. Formulation of 25% wettable powder S-(((P-chlorophenyl)-thio)methyl)0, 0-dimethyl phosphorodithioate[7], administered in gelatin capsule at 1 mg./kg. level.

5. Formulation of 25% emulsifiable concentrate phosphorodithioic acid, O,O-diethyl ester, S-ester with N,N-diallyl-2-mercapto acetamide[8] administered as 0.1% and 0.25% sprayed in quantities of 1 gallon per calf.

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[3] Dried rumen bacteria with soluble nutrient base, Poul-an Laboratories, Inc., Kansas City, Missouri.

[4] Coumaphos, Chemagro Corp., Kansas City, Mo. 64120.

[5] Demeton, Chemagro Corp., Kansas City, Mo. 64120.

[6] Rabon®, Shell Chemical Co., San Ramon, Calif. 94583.

[7] Trithion®, Stauffer Chemical Co., Mountain View, Calif. 94040.

[8] CP 18978, Monsanto Chemical Co., St. Louis, Mo. 63166.

6. Formulation of 25% emulsifiable concentrate phosphorothioic acid, O,O-dimethyl ester, O-ester with 5-chlorosalicylonitrile[9] administered as 0.25% and 0.50% sprayed in quantities of 1 gallon per calf.

7. Formulation of 25% emulsifiable concentrate phosphorothioic acid, O,O-diethyl,S-(3,4,4-trifluoro-3-butenyl)= ester[10] administered as 0.5% sprayed in quantities of 1 gallon per calf.

8. Formulation of 25% emulsifiable concentrate phosphorodithioic acid, O,O dimethyl,S-[2-methyl-1,3-oxathiolan-2-yl)methyl)] ester[11] administered as 0.05% sprayed in quantities of 1 gallon per calf.

9. Formulation of 20% emulsifiable concentrate phosphoric acid, dimethyl ester, ester with 2-chloro-N-ethylcrotonamide[12] administered in gelatin capsule at 10 mg./kg. level.

10. Formulation of technical phosphoric acid, dimethyl ester, ester with 2-chloro-N-methylcrotonamide[13] administered in gelatin capsule at 5 mg./kg. level.

11. Formulation of 50% emulsifiable concentrate phosphorodithioic acid, O,O-dimethyl ester, S-ester with-N-isopropyl-2-mercapto-N-methoxyacetamide[14] administered in gelatin capsule at 10 mg. and 25 mg./kg. levels.

Chemical Analysis.

Blood samples were collected 3 consecutive days before the test, 6 hours after exposure to the insecticide, and daily for the remainder of the observation time.

Determinations for tocopherol (total, free, and esterfied) and carotene were conducted by the Gianguido Rindi method (15), cholinesterase by a modified method of Michel (11), free fatty acids by Donald Kvam method (10), and hematocrit levels by Andujar method (1).

Results

A. Coumaphos

The 6 calves dosed with coumaphos showed maximum decreases during the trials in total, free, and ester tocopherol to 21, 20, and 0%, respectively, of their pre-exposure levels. Fourteen days after exposure the trial was terminated, and the averages had recovered to 72, 99, and 28% of their pre-exposure levels (Table 1). At varying times during the postexposure period, 5 of the calves developed diarrhea of which 4 showed no

9 Bayer 54203, Chemagro Corp., Kansas City, Mo. 64120
10 Stauffer 8963, Stauffer Chemical Co. Mountain View, Calif. 94040.
11 Stauffer B9625
12 C-776, Ciba Agrochemical Co., Vero Beach, Fla. 32960
13 C-768, Ciba Agrochemical Co., Vero Beach, Fla. 32960
14 C- 2428, Ciba Agrochemical Co., Vero Beach, Fla. 32960

detectable level of tocopherol coincidentally with the onset of diarrhea. ChE activity reached a mean of 24% at the lowest level postexposure, returning to 48% of the activity on the 14th day (Table 2). Diarrhea and depressed ChE activity were the only signs of O.P. toxicosis. A mean decrease of 18% in PCV was found (which is usually counterindicated in diarrhea cases), could be indicative of red cell destruction. Although controls had a decrease, it was not of the magnitude of the coumaphos-exposed calves (Table 3). This was also true of the fatty acid and carotene levels, and these levels did not return to pre-exposure levels as did the controls (Tables 2 and 3).

B. Demeton

The multiple exposures of the 5 demeton-dosed calves caused the ChE activity to decrease to 9% of the pre-exposure level. However, the decreases were not as rapid as those of the single-dosed coumaphos calves (Table 2). Maximum decreases in total, free, and ester tocopherol to 57, 24, and 23%, respectively, were shown during the postexposure period. The averages had recovered to 91, 148, and 78%, respectively, by the 14th day. During the postexposure period of 3 of the 5 calves developed diarrhea, had lower tocopherol, carotene, and PCV levels with slower recoveries than the other 2 exposed calves which had no diarrhea. One of these calves (which had no diarrhea) had only ChE depression and the other had most of the O.P. toxicosis signs except diarrhea. PCV levels in all 5 calves decreased just as the coumaphos-dosed calves and did not return to pre-exposure levels (Table 3).

C. Various O.P. insecticide compounds

1. Oral Capsule

All seven of the orally-dosed calves developed diarrhea and, at sometime during the postexposure period, had tocopherol and carotene levels lower than the controls. They did not return to pre-exposure levels as did the controls (Table 1).

Calf 16 was the only one not exposed at a level high enough to significantly lower the ChE, and no other signs of toxicosis were observed in calves 12, 14, 16, and 18. Calves 13, 15, and 17 showed all the classical signs of O.P. poisoning with only one [15] surviving.

2. Dermal Spray

Calves 23 and 24 had no signs of O.P. toxicosis except a depressed ChE activity. Both tocopherol and carotene levels decreased and did not return to pre-exposure levels. Calves 18, 20, and 22 developed diarrhea, and depressed ChE activity. The tocopherol and carotene levels were lowered and did not return to pre-exposure levels. Calves 20 and 22 manifested classical signs of toxicosis and were fatally poisoned.

TABLE 1

Tocopherol Levels in Calves Exposed to Organophosphorus Compounds

Calf No.	Compound	Dosage	Days	Tocopherol: Total Pre-treatment µg/100cc	Total % of Pre-treatment Low	Total % of Pre-treatment Last Day	Free Pre-treatment µg/100cc	Free % of Pre-treatment Low	Free % of Pre-treatment Last Day	Ester Pre-treatment µg/100cc	Ester % of Pre-treatment Low	Ester % of Pre-treatment Last Day
DRENCH												
1-6	Coumaphos	25 mg/kg	14	177* 77-378	21* 0-89	72* 0-205	111* 17-321	20* 0-94	99* 0-271	68* 54-98	0* 0-0	29* 0-89
ORAL CAPSULE												
7-11	Demeton	0.5, 1.0, 1.5 mg/kg	14	289** 141-516	57** 18-106	91** 63-160	171** 45-384	24** 0-56	148** 25-500	117** 96-139	23** 0-34	78** 0-152
12	Rabon®	50 mg/kg	14	285	24	26	188	0	0	97	0	77
13	Ciba C-776	10 mg/kg	14†	175	0	0	35	0	0	140	0	0
14	Ciba C-768	5 mg/kg	14	170	15	21	25	0	0	145	0	24
15	Ciba C-768	10 mg/kg	14	340	41	143	375	25	198	65	51	246
16	Ciba C-2428	10 mg/kg	14	190	18	92	188	11	93	2	0	0
17	Ciba C-2428	25 mg/kg	5†	200	50	50	138	19	19	62	37	119
18	Trithion®	1 mg/kg	7	345	33	68	274	27	57	71	0	113
DERMAL SPRAY												
19	Monsanto CP 18978	0.1%	7	125	28	28	100	35	35	25	0	0
20	Monsanto CP 18978	0.25%	6†	155	16	16	138	14	14	17	0	29
21	Bayer 54203	0.25%	7	85	88	88	0	0	0	85	18	65
22	Bayer 54203	0.50%	6†	125	0	0	125	0	0	0	0	0
23	Stauffer 8963	0.50%	7	375	31	37	313	27	44	62	0	0
24	Stauffer B9625	0.05%	7	605	31	40	555	34	41	50	0	30
25-29	Untreated Controls			172** 115-315	72** 53-95	127** 96-147	83** 30-170	33** 0-81	221** 171-264	89** 43-155	25** 0-60	173** 133-256

* Upper datum is average percentage of 6 animals; lower data are the minimal-maximal values. **Upper datum is average percentage of 5 animals; lower data are the minimal-maximal values. † Died.

TABLE 2

Carotene and Cholinesterase Levels in Calves Exposed to Organophosphorus Compounds

Calf No.	Compound	Dosage	Pre-treatment Weight kg.	Carotene			Cholinesterase		
				Pre-treatment µg/100cc	% of Pre-treatment Value		Pre-treatment ΔpH	% of Pre-treatment Value	
					Low	Last Day		Low	Last Day
DRENCH									
1-6	Coumaphos	25 mg/kg	51.4* 43.6-59.5	32* 21-40	36* 28-43	52* 38-70	.33* .24-.42	24* 18-36	48* 39-64
ORAL CAPSULE									
7-11	Demeton	0.5, 1.0, 1.5 mg/kg	48.6** 36.8-61.4	25** 7-66	67** 53-100	135** 53-265	.33** .28-.39	9** 0-18	39** 33-48
12	Rabon®	50 mg/kg	34.1	66	39	39	.46	22	32
13	Ciba C-776	10 mg/kg	27.7	43	14	21	.50	32	42
14	Ciba C-768	5 mg/kg	39.1	30	47	47	.55	38	53
15	Ciba C-768	10 mg/kg	30.5	63	24	24	.36	19	42
16	Ciba C-2428	10 mg/kg	44.5	26	54	62	.40	90	100
17	Ciba C-2428	25 mg/kg	35.0	54	43	43	.40	38	43
18	Trithion®	1 mg/kg	38.6	99	43	49	.43	23	23
DERMAL SPRAY									
19	Monsanto CP 18978	0.1%	45.5	29	34	69	.51	41	43
20	Monsanto CP 18978	0.25%	34.5	35	40	40	.42	12	57
21	Bayer 54203	0.25%	39.1	25	80	80	.42	36	36
22	Bayer 54203	0.50%	41.8	59	34	34	.31	19	35
23	Stauffer 8963	0.50%	....	81	31	31	.44	52	61
24	Stauffer B9625	0.05%	....	100	31	31	.35	66	71
25-29	Untreated Contols		45.5** 38.6-61.4	35** 13-72	72** 55-90	156** 108-185	.33** .25-.44	89** 80-100	111** 94-145

*Upper datum is average percentage of 6 animals; lower data are the minimal-maximal values. **Upper datum is average percentage of 5 animals; lower data are the minimal-maximal values.

TABLE 3

Hematocrit Values and Fatty Acid Levels in Calves Exposed to Organophosphorus Compounds

Calf No.	Compound	Dosage	Pre-treatment Weight kg.	Hematocrit			Free Fatty Acids		
				Pre-treatment mm	% of Pre-treatment Value		Pre-treatment µg/100cc	% of Pre-treatment Value	
					Low	Last Day		Low	Last Day
DRENCH									
1-6	Coumaphos	25 mg/kg	51.4* 43.6-59.5	28* 21-36	82* 71-96	89* 71-103	.48* .45-.54	50* 40-60	88* 58-116
7-11	Demeton	0.5, 1.0, 1.5 mg/kg	48.6** 36.8-61.4	29** 25-35	83** 66-97	86** 66-100	....	....	....
25-29	Untreated Controls		45.5** 38.6-61.4	31** 28-37	94** 81-113	103** 94-119	.53** .35-.66	66** 51-78	145** 116-161

*Upper datum is average percentage of 6 animals; lower data are the minimal-maximal values. **Upper datum is average percentage of 5 animals; lower data are the minimal-maximal values.

Calf 21 was the only treated calf whose tocopherol and carotene levels were not affected more than the control calves. However, upon increasing the dose, a zero tocopherol, 66% decrease in carotene, and 81% loss in ChE activity was observed (Calf 22).

D. Controls

Two of the 5 control calves developed diarrhea and showed notable reductions in tocopherol and fatty acid levels, but recovered rapidly. The lower levels occurred coincidentally with the onset of diarrhea which has previously been reported (16, 17).

Discussion

Calv s dosed with O.P. insecticides at levels high enough to cause significant ChE depression, and occasionally diarrhea, also caused tocopherol, carotene, PCV, and fatty acid levels to decrease. With no other signs of toxicosis and feed consumption relatively unchanged, these levels did not return to pre-exposure normals. This would indicate an inefficiency of feed utilization related to defects in intestinal absorption. These lower levels would be expected and were observed when calves showed other signs of toxicosis and, therefore, consumed less feed.

The lower levels of tocopherol, carotene, PCV, and fatty acid were also noted in control calves (to a lesser degree) when diarrhea of unspecified etiology affected them. However, the controls not only recovered rapidly, but increased significantly during the 2-week test period.

A statistical comparison was not possible because of the limited number of calves in each group.

Summary

Tocopherol, cholinesterase, fatty acids, carotene, and hematocrit levels were determined during a 1- and 2-week-period following exposure to organophosphorus insecticides. Depletions of total tocopherol, occurring coincidentally with the onset of diarrhea in most cases, were observed when neonatal calves were exposed to a significant level of the insecticides in contrast to that of controls. Inability of the exposed calves to return to pretreatment levels of fatty acids, carotene, and hematocrits was notable when tocopherol levels remained below 70 µg/100 ml. for several days. This finding would be expected if the antioxidant activities of tocopherol were not replaced.

Acknowledgements

The authors thank Drs. John W. Safford of the Montana Livestock Sanitary Board, Helena, Montana 59601, and Karl F. Swingle of the Veterans Administration Hospital, 5901 East Seventh St., Long Beach, California 90801, for their review and suggestions in the writing of the manuscript.

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