Chronic Feeding of *S*-[(2-Methoxy-5-oxo-Δ^2-1,3,4-thiadiazolin-4-yl)methyl] *O,O*-Dimethyl Phosphorodithioate (Surpracide) to Rumating Bull Curves

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The physiological effects and the metabolism in the bovine of a new organic phosphate insecticide *S*-[(2-methoxy - 5 - oxo - Δ^2 - 1,3,4 - thiadiazolin 4 - yl)-methyl] *O,O*-dimethyl phosphorodithioate (Supracide) was studied. Ruminating bull calves received Supracide by capsule once daily at the rate of either 2.0, 1.0, 0.5, or 0.0 mg. per kg. of live weight for a 10-week period. Three of five animals receiving 2.0 mg. per kg. succumbed after 12, 33, and 34 days of continuous treatment. Voluntary hay consumption was inversely related ($P < 0.01$) to Supracide intake. Whole blood acetylcholinesterase activity was linearly inhibited ($P < 0.01$) as level of pesticide increased. Analysis of several tissues for Supracide and its oxygen analogue revealed the presence of very low levels (<0.05 p.p.m.) of the parent compound at 2.0 mg. per kg., but not at 1.0 mg. per kg. No evidence for presence of the oxygen analogue was obtained.

Supracide is an organic phosphorus insecticide of J. R. Geigy, S. A., Basle, Switzerland, and has been chemically described (Esser and Müller, 1966) as *O,O*-dimethyl-*S*-[2-methoxy-1,3,4-thiadiazol-5-(4H)onyl-(4)methyl]-dithiophosphate. Experimentally, it has effectively controlled alfalfa weevils (Cassidy *et al.*, 1968b) and orchard and vineyard pests (Gasser *et al.*, 1965). Methods for crop residue determinations have been described utilizing a colorimetric sulfide procedure, gas-liquid and thin-layer chromatography (Eberle *et al.*, 1967; Mattson *et al.*, 1968). Two independent investigators have reported that a large percentage of ring carbonyl-^{14}C was converted to $^{14}CO_2$ by rats (Bull, 1965; Esser and Müller, 1966; Esser *et al.*, 1968) indicating that the compound can be completely degraded metabolically. A significant storage of the radioactive pesticide did not occur when a lactating cow was orally administered 1 mg. per kg. bodyweight daily for five days (Cassidy *et al.*, 1968). Furthermore, labeled studies reveal plants readily metabolize GS-13005 (Bull, 1965; Esser and Müller, 1966) with a large percentage of the radioactivity recovered as $^{14}CO_2$.

The physiological effects and the metabolism in the bovine of Supracide have not been reported, but must be understood to make proper recommendations regarding its use on alfalfa. Therefore, studies reported here were conducted to ascertain the physiological effect of different levels of this organophosphorus compound, when chronically administered to the young ruminating male bovine. Furthermore, selected tissues were examined histologically and analyzed for possible residue accumulation.

EXPERIMENTAL

Animal. Twenty male Holsteins were placed into five outcome groups of four animals each. Animals in each group were of similar body weight. Weights for all animals averaged 145 kg. and ranged from 115 to 220 kg. One calf from each outcome group was then randomly assigned to the following dosage levels of Supracide; 0.0, 0.5, 1.0, or 2.0 mg. per kg. (mg. of Supracide administered per kg. body weight). The maximum treatment level had been determined in a preliminary study mentioned in the results section. The insecticide was given by capsule prior to each morning feeding. Placebos were administered to the control group. Animals were kept in individual tie-chain stalls equipped with automatic waterers. They were offered a 16% crude protein concentrate at 1% of body weight daily. Medium to good quality grass hay was offered *ad libitum*. Individual consumption records were maintained. Animals that were adversely affected by the pesticide were permitted additional concentrate if they refused to eat hay. This was to provide adequate energy consumption during stress. Calves were weighed at seven-day intervals throughout the study. Height of withers and heart girth circumference were periodically recorded.

Blood. Blood, drawn from the jugular vein at one and two weeks prior to the treatment period, and at weekly intervals during pesticide administration, was analyzed for hemoglobin, red and white cells, and differential cell counts. Heparinized blood, similarly sampled, was assayed for acetyl cholinesterase activity after the method of Winteringham and Disney (1964). Statistical analyses were determined on the average of all values taken during treatment and were expressed as a percentage of the preliminary observations. Periodically glutamic-oxalacetic and glutamic-pyruvic transaminase activities were determined after Reitman and Frankel (1957). Both enzymes become elevated in serum as a result of cellular necrosis and may be used in the diagnosis of heptocellular damage and myocarditis (Nydick *et al.*, 1955; Ostraw *et al.*, 1955; Wroblewski and LaDue, 1955a,b).

Tissue. All animals that died during the experiment and two animals from each treatment group slaughtered at the end of 10 weeks were necropsied to establish gross alterations associated with the phosphate treatment. In addition, one animal which survived the 2 mg. per kg. treatment was slaughtered five weeks post-treatment. Weights of principal vital organs were recorded. Lungs, heart, spleen, liver, kidney, and intestinal tissues were examined histologically. Samples of longissimus dorsi, liver, heart, kidney, brain, spleen, and omental, perirenal, and tail head fat were preserved by freezing until analysis for Supracide and its oxygen analogue, GS-13007.

Methods for tissue extraction, cleanup, and quantitation of Supracide and GS-13007 were modifications of those described by Cassidy *et al.* (1968a). Acetonitrile extracts

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Table I. Effect of Supracide Treatment on Growth, Feed Consumption, Certain Blood Enzymes, and Blood Composition

	Level of Supracide				
	2.0	1.0	0.5	0.0	Significance
	Treatment av. + s.e.				
Hay consumption (% of body weight)	1.61 ± 0.10	1.82 ± 0.08	2.06 ± 0.04	2.07 ± 0.11	Linear ($P < 0.01$)
Body weight gain (kg./day)	0.56 ± 0.07	0.60 ± 0.03	0.74 ± 0.08	0.70 ± 0.05	
Heart girth increase (mm./day)	1.04 ± 0.28	1.53 ± 0.22	1.60 ± 0.45	1.89 ± 0.39	
Withers height increase (mm./day)	1.50 ± 0.51	1.00 ± 0.16	1.07 ± 0.07	1.04 ± 0.22	
Blood cholinesterase (μmoles hr./ml.)	14.7 ± 1.3	36.6 ± 2.0	43.5 ± 3.0	47.1 ± 3.2	Linear ($P < 0.01$)
Glutamic-oxalacetic transaminase (Sigma units)	45.5 ± 0.7	47.9 ± 4.3	42.5 ± 5.3	47.0 ± 5.6	
Hemoglobin (g./100 cc.)	11.0 ± 0.17	10.3 ± 0.12	10.4 ± 0.13	10.6 ± 0.17	($P < 0.01$)
Hematocrit (%)	33.0 ± 0.61	30.2 ± 0.35	32.2 ± 0.60	32.3 ± 0.51	($P < 0.01$)
White blood cells ($\times 10^3$)	13.9 ± 0.62	12.9 ± 0.57	11.8 ± 0.43	10.9 ± 0.49	
Differential cell count (%)					
Monocytes	67.3 ± 0.83	4.8 ± 0.43	5.1 ± 0.54	4.2 ± 0.46	
Lymphocytes	62.4 ± 2.4	67.7 ± 2.8	66.8 ± 2.2	67.5 ± 2.8	
Neutrophils	30.3 ± 8.0	26.2 ± 7.0	26.9 ± 6.5	27.4 ± 6.3	

were washed with three portions of petroleum ether prior to the addition of water and partition of Supracide into petroleum ether. Hexane was substituted for petroleum ether on florisil chromatography. Samples were analyzed in duplicate and recoveries of pesticide determined on each run by comparing similar tissues from control animals with control tissues that were fortified with known quantities of both compounds. Recoveries varied between tissues but usually exceeded 70%.

Samples were quantitated in a Micro-Tek GLC fitted with a Dohrmann microcoulometric sulfur specific cell No. T-300. A 120- × 0.64-cm. borosilicate glass column packed with 3% SE 30 and 0.3% EPON 1001 supported on Gas Chrom Q was maintained in an oven at 200° C. Nitrogen carrier flowed at 90 ml. per minute. Temperature of injection port, transfer line, and electric furnace were respectively 240°, 260°, and 775° C. The sensitivity of the method for both Supracide and the oxygen analogue was greater than 0.01 p.p.m.

Where appropriate, data were statistically analyzed according to Snedecor (1956).

RESULTS AND DISCUSSION

Preliminary studies were conducted to ascertain the appropriate levels for chronic administration of Supracide. Initially, an animal received daily 8 mg. per kg. of body weight of Supracide. Within an hour after the third administration, the animal displayed characteristic symptoms associated with ingestion of anticholinesterase compounds and succumbed a few hours later.

A second animal received 2 mg. per kg. of body weight for seven days with no observed physiological effects. On day eight, the dose was increased to 4 mg. per kg. On day 10, cholinesterase activity had declined to 8% of the initial levels and typical symptoms developed followed by death. From these data, a high dosage of 2 mg. per kg. of body weight was established for the main study.

Two of five animals receiving 2 mg. per kg. survived the 10-week treatment period. Animal 1056 lived 33 days and 1025, 34 days after initiation of treatment. Animal 1048 was more rapidly affected by treatment and was near death at 12 days when treatment was discontinued. Cholinesterase remained below 30% of normal for three weeks. By the fourth week, activity had doubled that of the previous three weeks. After 37 days off treatment, 1048 was again given daily 2 mg. per kg. and died after 12 days. Animal 1034, a survivor, was unable to stand between the fourth and seventh week of treatment. Beyond seven weeks, he regained muscle control and began walking, eating, and behaving quite normally. Animal 1071 was able to stand and walk throughout the treatment period; however, considerable incoordination was observed in the front legs. No such problems were observed in animals of the other treatment groups.

Voluntary hay consumption (Table I) based on the total treatment period declined linearly ($P < 0.01$) with Supracide administration. For the 2 mg. per kg. group, the decrease in hay consumption seemed to follow increased blood cholinesterase inhibition. Concentrate was readily accepted by calves in this group, when contrasted to hay consumption.

Body weight gains were generally decreased with increased levels of pesticide administration but the large within-treatment variation precluded statistical significance. Neither was growth, as determined by increased heart girth and height of withers, significantly altered by treatment. In the group receiving the highest level of pesticide, accurate measurements related to growth could not be obtained because several animals were prostrate or succumbed relatively early in the study. Animal and experimental error accounted for the differences observed between the three remaining treatment groups. Blood acetyl cholinesterase was linearly inhibited ($P < 0.01$) ($b = 39.76\%$ inhibition when based on preliminary values for each 1 mg. per kg. increment of Supracide). Actual cholinesterase averages are shown in Table I. The average cholinesterase activity as influenced by time on treatment is shown in Figure 1. Individual cholinesterase values had declined nearly to zero for a few days before death in animals receiving 2 mg. per kg.

Glutamic-oxalacetic transaminase was not affected by treatment (Table I). Glutamic-pyruvic transaminase activity is very weak in bovine blood and did not increase with pesticide treatment.

Hemoglobin and hematocrit increased slightly, but significantly ($P < 0.01$) when pesticide consumption increased from 1.0 to 2.0 mg. per kg. (Table I). White blood cells also increased, although not significantly, with level of treatment.

Gross and histological examination of animals which succumbed owing to Supracide administration revealed acute pyogenic hemorrhagic pneumonia and pulmonary congestion, hyperemia, and hemorrhage. These abnormalities were not observed in animals sacrificed from other treatment groups. Proliferative change was noted in splenic corpuscles. No meaningful treatment effects were observed in the differential

Table II. Analysis of Tissues for Supracide and the Oxygen Analog from Bull Calves Administered Supracide[a]

Animal No.	Omental Fat	Perirenal Fat	Brain	Heart	Kidney	Spleen	Liver	Skeletal Muscle	Remarks
	P.P.M. of Wet Tissue								
2 mg. per kg.									
1034	0.04	0.0	0.04	0.0	0.0	0.0	0.0	0.0	Survived treatment
1025	...	...	...	0.03	0.04	...	0.0	...	Succumbed 34 days treatment
1048	0.73	0.03	0.03	0.03	0.02	0.0	0.0	0.0	Succumbed 14 days on treatment
1071	0.0	0.02	0.0	0.0	...	0.0	0.0	0.0	Survived treatment; slaughtered 5-wk. post-treatment
1 mg. per kg.									
1022	0.0	...	...	0.0	0.0	...	...	...	Survived treatment
1036	0.0	...	0.0	...	0.0	0.0	0.0	0.0	Survived treatment

[a] No oxygen analog was selected.

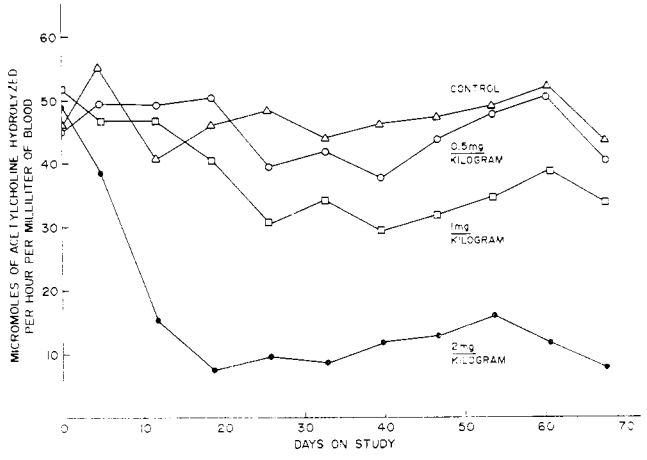

Figure 1. Average weekly acetyl cholinesterase activity in the blood of bull calves at four treatment levels of Supracide. Points represent an average of five animals except at the high level, where animals died after various lengths of treatment as described in the results section.

cell counts (Table I). Differential counts were within the values reported by Frandson (1965), and assumed to be in the normal range.

The average percentage of organ weights to total body weight (expressed as per cent $\times 10^2$) for the 2 mg. per kg. animals compared to animals in the other treatment groups was 140, 108; 50, 43; 185, 152; 40, 32; and 24, 19 respectively for lungs, heart, liver, kidneys, and brain. The increased ratio of organ to body weights probably is not real. Body weight included fill of the digestive tract. Recent studies (Jahn *et al.*, 1969) have shown that body fill accounts for 10 to 25% of the ruminants live weight. Since the 2 mg. per kg. group had fasted or consumed very small quantities of hay for several days before death or slaughter, a 15% correction of body weight owing to lack of body fill is reasonable. If this correction were made there would be little or no difference in organ body weight ratio between treatments.

Data on analysis of several tissues for Supracide and its oxygen analogue, GS-13007, are shown in Table II. Very small quantities of the parent compound were found in some tissues of animals that succumbed on treatment. No trace of the pesticide was found in tissues from animals administered 1 mg. per kg. of body weight. Therefore, tissues from animals receiving 0.5 mg. per kg. were not analyzed. The rate of intake by cattle receiving 1 mg. per kg. of body weight of Supracide would be equal to approximately 50 p.p.m. in the hay. Alfalfa treated with minimum effective levels of Supracide for control of the alfalfa weevil is unlikely to exceed 50 p.p.m. of the pesticide beyond 24 to 48 hours after spraying the crop (Polan *et al.* 1969).

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