Effects of a Herbicide, 2-Ethyl Hexyl Ester of 2,4-D on Magnesium: Calcium Ratios and Blood Urea Nitrogen Levels in Sheep and Cattle

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Weed Rhap[R] [1] (2,4-Dichlorophenoxyacetic acid, 2-ethyl hexyl ester), a low volatile 2,4-D ester, controls many weeds in wheat, corn, barley, oats, flax, sorghum and other grain crops. It may also be used to kill and control weeds and small brush in rangeland, grass pastures, fence rows, ditch banks, roadways, right-of-ways as well as general weed control. It would be possible, therefore, for livestock to graze in areas where the vegetation has been treated.

Several herbicide compounds were used for toxicological studies, and a few produced an enlargement or congestion of either the thyroids or parathyroids or both, and the kidneys (4). Weed Rhap was one of these compounds. It was reasonable, therefore, to expect that a lowering of the calcium levels would result since hypocalcemia is one of the most constant and characteristic features of diminished parathyroid function. The biological roles of calcium (Ca) and magnesium (Mg) are already well established and quantitated by a reliable method of analysis (3).

Materials and Methods

Test Animals. Seven yearling sheep and 3 yearling cattle were used. One sheep was a control, 3 were dosed daily with the regular formulation (69.4% EC), 2 were dosed with technical material, and 1 sheep was dosed with the formulation inerts. Two yearling cattle were dosed with regular formulation and the third was a control. Sheep and cattle were given a 250 mg/kg dose daily. Not enough technical and inert material was available for daily dosing of the cattle.

Chemical Analysis. Blood samples were taken from the jugular vein daily for 3 days before initiating oral exposures. Samples were taken daily after the trials began. Fresh blood was heparinized and the plasma used for the Ca and Mg determinations by atomic absorption spectrophotometry (2).

Tissue biopsies for Ca and Mg determinations were taken before the experiment, as well as at the time of greatest ratio changes in the plasma and at necropsy. Samples of muscle tissue (0.5 gram)

[1] Weed Rhap[R] and other test materials used in this study were furnished by Hercules Incorporated, Wilmington, Delaware.

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 1, 1970, published by Springer-Verlag New York, Inc.

TABLE I

Effects of Repeated Oral Doses (250 mg/kg) of a 2-Ethyl Hexyl Ester of 2,4-D Compound and Its Major Components to Cattle and Sheep

Animal and treatment	No. of Doses To produce ill effects	No. of Doses Total administered	Final biopsy After	Results and remarks
Cattle[a]				
EC[b]	3	14	9 doses	Died after 14 doses
EC[b]	3	9	9 doses	Survived
Control	----	----	9 days	----
Sheep				
EC[b]	3	17	17 doses	Died after last dose and biopsy
EC[b]	4	50	50 doses	Killed after last dose and biopsy
EC[b]	12	56	56 doses	Survived
Tech.[c]	14	29	29 doses	Died after last dose and biopsy
Tech.[c]	13	56	56 doses	Survived
Inerts[d]	----	56	56 doses	Survived
Control	----	----	56 days	----

(a) Insufficient quantities of technical material and inerts for cattle trials.

(b) Emulsifiable concentrate (69.47%) formulated by Hercules Inc., Wilmington, Delaware, as Weed Rhap®.

(c) Technical material as 2-ethyl hexyl ester of 2,4-D.

(d) Inerts (30.53%) in EC formulation.

were dry-ashed at 600°C for 18 hours. The ash was digested with 5 ml of HCl then dilutions were made with a 1:100 dilution of lanthanum chloride. Determinations of Ca and Mg were made by atomic absorption spectrophotometry (1). All samples, both tissue and plasma, were run in duplicate.

Results and Discussion

Toxicologic. Preliminary screening trials with this herbicidal formulation were carried out on yearling cattle and sheep. The purpose was to determine the toxic effects of daily oral encapsulated doses (10 or less) administered at various dose levels. Dosages were calculated on milligrams of technical content of the formulation to kilograms of body weight (mg/kg) of each test animal. At the 100 mg/kg level, no apparent ill effects (NIE) followed a dosing regimen to a yearling heifer and wether; two additional sheep showed partial anorexia during the trials and 7 and 8% weight loss from their pre-exposure condition.

Dosages at 250 mg/kg in 5 additional animals resulted in death to 3 following 6 or 8 doses. Swollen, blood-engorged thyroids and inflammation of the kidneys were prominent post mortem observations. The results to the 2 remaining animals, one of each species, were loss of 5% or more of initial weight following 10 daily doses. The variation in severity was considered to be due to individual tolerance.

To investigate the observed deleterious effects to cattle and sheep that were severely or fatally affected, a study was initiated to relate their occurrence to physiological functions. Table I summarizes the toxicologic results of randomly selected cattle and sheep to repeated oral exposures of the commercial formulation, the technical material, or the inerts of the formulation.

Biochemical.

Cattle: The results are reported as change in ratios of Mg to Ca (i.e., pre-experiment Mg 2 mg/100 ml:Ca 12 mg/100 ml, hence a ratio of 1:6; post-experiment Mg 3 mg/100 ml:Ca 9 mg/100 ml, hence a ratio of 1:3 or a 50% ratio decrease).

Plasma Ratios: The control yearling showed a decrease of 11%, and the two treated yearlings averaged a 42% decrease. Not enough technical and inerts were available to carry out this phase as mentioned previously (Table II).

Tissue Ratios: The average for the EC-treated yearlings showed a 4.5% ratio decrease, while the control yearling showed none (Table III).

Blood Urea Nitrogen (BUN): One of the yearlings receiving the EC formulation showed an elevated BUN level of 40 mg/100 ml (Table II).

TABLE II

Effects of a 2-Ethyl Hexyl Ester of 2,4-D and Components at a Dosage Level of 250 mg/kg on BUN and Plasma Magnesium:Calcium Ratios in Cattle and Sheep

Animal and treatment	Days on test	Pre-treatment ratio[a]	Greatest ratio decrease[a]	Percent decrease	Day of greatest decrease	BUN[b] Pre-treatment	BUN[b] Maximum
Cattle[c]							
EC[d]	14	4.22	1.61	62	14	4	40
EC[d]	15	4.04	3.15	22	9	2	9
Control	13	4.62	4.10	11	4	1	3
Sheep							
EC[d]	50	4.13	2.78	33	13	13	24
EC[d]	17	4.00	1.85	54	16	14	42
EC[d]	56	4.08	2.59	37	29	13	28
Tech.[e]	29	4.00	1.96	51	29	13	75
Tech.[e]	56	4.08	3.09	24	29	16	30
Inerts[f]	56	4.20	3.03	28	29	11	29
Control	56	3.92	3.39	14	43	12	26

(a) Figure in column is the ratio of calcium to magnesium which is always 1 (*i.e.*, 1:4.66).

(b) Blood urea nitrogen in mg/100 ml of plasma.

(c) Insufficient quantities of technical material and inerts for cattle trials.

(d) Emulsifiable concentrate (69.47%) formulated by Hercules Inc., Wilmington, Delaware, as Weed Rhap®.

(e) Technical material as 2-ethyl hexyl ester of 2,4-D.

(f) Inerts (30.53%) in EC formulation.

TABLE III

Effects of the 2-Ethyl Hexyl Ester of 2,4-D and Components at a Dose Level of 250 mg/kg on Muscle Magnesium:Calcium Ratios in Cattle and Sheep

Animal and treatment	Day of biopsy	Day of necropsy	Pre-treatment ratio[a]	Biopsy ratio[a]	Necropsy ratio[a]	Percent greatest decrease
Cattle[b]						
EC[c]	9	14	0.218	0.200	0.200	9
EC[c]	9	----	0.210	0.231	----	0
Control	9	----	0.222	0.223	----	0
Sheep						
EC[c]	50	----	0.195	0.172	----	12
EC[c]	17	17.25	0.311	0.275	0.245	21
EC[c]	56	----	0.212	0.156	----	26
Tech.[d]	28	29	0.231	0.229	0.203	12
Tech.[d]	56	----	0.188	0.198	----	0
Inerts[e]	56	----	0.203	0.163	----	20
Control	56	----	0.195	0.167	----	14

(a) Figure in column is the ratio of calcium to magnesium which is always 1 (i.e., 1:4.66).

(b) Insufficient quantities of technical material and inerts for cattle trials.

(c) Emulsifiable concentrate (69.47%) formulated by Hercules, Inc., Wilmington, Delaware, as Weed Rhap®.

(d) Technical material as 2-ethyl hexyl ester of 2,4-D.

(e) Inerts (30.53%) in EC formulation.

Sheep: The results are presented as previously described for cattle.

Plasma Ratios: The control animal showed a ratio decrease of 14%, and the sheep dosed with EC averaged a ratio decrease of 41%. Technical-dosed sheep decreased 38%, and the inert formulations ratio dropped 28% (Table II).

Tissue Ratios: The average ratio of EC-dosed sheep and the inert-dosed sheep both decreased 20%.

The technical-dozed and control sheep had a decrease of 6 and 14%, respectively (Table III).

Blood Urea Nitrogen: One of the EC-dosed sheep and 1 treated with the technical material showed elevated BUN levels of 42 and 75 mg/100 ml, respectively (Table II).

Weed Rhap^{R}, as the EC formulation, technical material and even the inerts, at a 250 mg/kg dose level, produced reduced Mg:Ca ratios in sheep plasma greater than was shown by the control. The inert materials and EC formulation also had this reduced ratio in the muscle tissue more than did the control or the technical material. If the test animals died too soon after treatment was initiated, this decrease in tissue ratio, of course, is not observed. One of the sheep receiving the EC formulation and 1 receiving technical material had kidney damage in addition to the greatest plasma ratio change. No explanation is offered for the survival of other animals in these 2 groups, or for their not showing significant ratio change or kidney damage.

Both the yearling cattle receiving the EC showed much greater ratio changes than did their control. The heifer that died had almost three times the ratio change than the other treated yearling and also was the only one to show kidney damage.

It would appear the inert materials had little or no effect on the ratio change or the BUN levels.

The ratio changes found do indicate an impaired production or regulation of both magnesium and calcium by the commercial formulation and technical material of the herbicide compound.

Summary

The herbicide, 2,4-Dichlorophenoxyacetic acid, 2-ethyl hexyl ester (Weed Rhap^{R}), produced a decrease in plasma calcium and increase in plasma magnesium, giving a significant ratio change in the plasma of the 2 sheep and yearling heifer that died. Each of these had increased blood urea nitrogen (BUN) levels, and the pathological examinations at necropsy showed kidney damage and swollen, blood-engorged thyroids. The formulation of the dose in

sheep either as emulsifiable concentrate (EC) or as technical material, showed no effect difference.

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