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SPECIAL REPORT K-54

Studies on Lindane Residues in Meat and Milk

Kerrville, Texas, Laboratory 1/ 2/ 3/
Agricultural Research Service
United States Department of Agriculture

The public health significance of residues in meat and milk required studies to determine the extent and duration of such residues resulting from the use of lindane. A number of experiments have been conducted at the Kerrville Laboratory for this purpose

PREVIOUSLY REPORTED DATA

In 1950, Special Report K-28[1] was submitted, which described the tests and results obtained when lindane was used as a spray on dairy cows, as a residual spray in dairy barns, and when various amounts were fed to beef cattle. The results of those tests will be summarized briefly.

Residues in Milk

Composite milk samples taken from 5 dairy barns sprayed with a wettable powder formulation and 5 with an emulsifiable formulation, each at a concentration of 0.25% lindane, showed no evidence of contamination.

1/ Conduct of experiments and collection of fat and milk samples under the supervision of R. D. Radeleff and J. B. Jackson, Animal Disease and Parasite Research Division.

2/ Treatment and infestation of test animals under the supervision of O. H. Graham and R. H. Roberts, Entomology Research Division.

3/ Chemical analysis of residues under the supervision of H. V. Claborn, Pesticide Chemicals Division.

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Two lactating Jersey cows that were sprayed with 0.1% lindane, and 1 sprayed with 0.05% lindane, using a wettable powder formulation, eliminated lindane in their milk. The animals treated with 0.1% lindane had 43 and 69 p.p.m. in the butterfat (1.5 and 2.4 p.p.m. on the basis of milk with 3.5% butterfat) the first day after treatment. The amounts eliminated dropped rapidly and were practically negative after 7 days. The animal treated with 0.05% lindane eliminated 31 p.p.m. in butterfat (1.0 p.p.m. in milk at 3.5% butterfat) 1 day after spraying. The level dropped to zero at 7 days, but 15 p.p.m. (0.5 p.p.m. in milk) was found at 14 days post-treatment.

Another Jersey cow was sprayed with 0.05% lindane and fed 100 mg. in the feed just prior to spraying. This animal had 18.2 p.p.m. in the butterfat (0.6 p.p.m. in milk) the day after treatment. At 3 days post-treatment and thereafter, no lindane was detected.

Residues in Meat

In a feeding test, 4 pairs of Herefords (1 steer and 1 heifer per pair) were used. Three separate levels of lindane, 1 p.p.m., 10 p.p.m. and 100 p.p.m. were fed for a period of 12 weeks, with 1 pair of Herefords to a level and 1 control pair. Samples of omental fat were taken before the start of the test, at 2, 6 and 10 weeks during the feeding period, and at 2, 6, 10 and 14 weeks after the feeding of lindane terminated. Analyses indicated that the lindane was rapidly absorbed and stored in the fat. Fat samples taken on the 70th day of feeding contained about the same proportion of lindane as were present in the diet. Because of the limitations of analytical accuracy (± 2 p.p.m.) the 1 p.p.m. level in the diet was not proven to contaminate fat. After lindane feeding ceased, fat samples became negative for lindane as follows: 1 p.p.m. diet, 2 - 4 weeks; 10 p.p.m. diet, 6 - 10 weeks; 100 p.p.m. diet, 10 - 14 weeks.

A number of residue tests have been conducted since Report K-28, which will be described in this report.

STUDIES SINCE 1950

Lindane Residues in the Milk of a Cow Treated with EQ 335

Methods--To determine the residues of lindane appearing in milk following treatment of screw worm wounds with EQ 335, 2 lactating cows were wounded on the left cervical region and screw worm larvae introduced. When the larvae were established, 1 wound was treated with EQ 335, and the other with benzol, according to the recommended procedure. Morning milk samples were collected before treatment and at 5 hours, 1, 2, 5, 7 and 14 days post-treatment, and were analyzed for lindane.

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Results--Table 1 shows the amount of lindane found in the milk. The greatest amount of lindane was found in the milk the first day after treatment. The amount of lindane found gradually diminished over a 7 day period. At the end of this 7 day period, lindane was no longer found in the milk.

Lindane Residues in Range Cattle Treated with 0.075% Lindane

Methods--To determine residues in range cattle after dermal application, 4 yearling cattle were dipped in 0.075% lindane and 4 were sprayed with 0.075% lindane formulated from a 20% emulsifiable concentrate, and 4 were sprayed with 0.075% lindane formulated from a 25% wettable powder. Four yearling steers were used as controls. All of the 16 cattle were biopsied before treatment, and one-half of each group were biopsied at 1, 2, 3, 4, 6 and 8 weeks post-treatment to obtain omental fat samples.

Results--Table 2 shows the amount of lindane found.

Residues persisted in range cattle for 6 to 8 weeks. Lindane was present as a residue longer in the animals sprayed with the emulsion formulation.

Three of the 4 cattle dipped in 0.075% emulsified lindane were poisoned, 1 severely. However, these 3 recovered without treatment. None of the sprayed cattle were affected.

Lindane Residues in Sheep and Goats Treated with 0.025% Lindane

Methods--Four sheep and 4 goats in short fleece were dipped in 0.025% lindane formulated from 25% wettable powder. Four sheep and 4 goats were used as controls. All the sheep and goats were biopsied before treatment and one-half of each group were biopsied at 2, 4, 6, 8, 10 and 12 weeks post-treatment.

Results--Table 3 shows the amount of lindane found. Residues persisted through the tenth week, but were absent at the twelfth week. No symptoms of poisoning were noted.

CONCLUSIONS

The residues presented in this report, and in the earlier K-28, indicate that lindane is stored in meat and eliminated in milk when animals are fed or sprayed with various concentrations and formulations.

The apparently longer time required for disappearance of residues observed in the new series as against that observed in the studies reported in Special Report K-28 must be considered against the analytical methods employed. The Schechter-Hornstein method employed in the recent studies is several-fold more sensitive than the earlier method, and was capable of detecting the smaller residues for a longer period of time.

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References

1. 1950 Cooperative Tests with Lindane. Special Report K-28, USDA, pp. 1 - 24.

   A. Wells, R. W. and C. Condron. Tests with Lindane Applied as an Animal Spray to Dairy Cattle and as a Residual Spray in Dairy Barns.

   B. Condron, C. Laboratory Tests on the Residual Effects of Lindane Sprays.

   C. Radeleff, R. D., C. Condron and G. W. Eddy. Effects of Various Levels of Lindane in the Feed of Beef Cattle.

September 26, 1958

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Table 1. Parts Per Million of Lindane in the Milk of a Cow Treated with EQ 335. (3% lindane wound dressing)

Time after treatment	P. P. M. Lindane Butterfat	4% Milk
5 hours	0.2	0.008
1 day	0.83	0.033
2 days	0.48	0.019
5 days	0.21	0.008
7 days	0	0
14 days	0	0

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Table 2. Parts Per Million of Lindane in the Fat of Cattle Dipped or Sprayed with 0.075% Concentration of the Insecticide.

Animal Number	Treatment	P.P.M. Lindane in Fat weeks after treatment 1	2	3	4	6	8
11	Emulsion Dip	23.0		4.9		1.4	
12	" "	20.6		5.9		1.0	
13	" "		14.2		1.5		0
14	" "		10.4		2.0		0
21	Emulsion Spray	20.0		5.9		1.4	
22	" "	14.6		3.4		0.4	
23	" "		5.9		1.5		0.5
24	" "		6.4		0.9		0.1
31	Suspension Spray	10.4		1.3		0.0	
32	" "	6.8		1.6		0.3	
33	" "		6.4		1.7		0
34	" "		9.3		1.1		0

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Table 3. Parts Per Million of Lindane in the Fat of Sheep and Goats Dipped in 0.025% Concentration of the Insecticide

Animal Number	P.P.M. Lindane in Fat weeks after [illegible]					
	2	4	[illegible]	[illegible]	10	12
Goats						
4682	3.20		0.15		0.14	
4684		1.56		0.15		0
4686		1.15		0.22		-*
4687	2.14		0.40		0.23	
Sheep						
4688	4.30		0.37		0.27	
4692		1.50		0.12		0
4694		2.00		0.40		0
4695	4.15		0.81		0.27	

* Fat sample not available

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH DIVISION
Beltsville, Maryland

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July 1, 1959

Dr. T. Walter Reed
California Spray-Chemical Corp.
P. O. Box 118
Moorestown, New Jersey

Dear Dr. Reed:

Enclosed is a copy of a letter from Mr. H. V. Claborn to Mr. S. A. Hall which supplies the information requested by Dr. R. L. Gardner. Also enclosed is a copy of Special Report K-57. You will note that Mr. Claborn checked his work very carefully and we feel that there is no doubt about its validity.

If there is any further question, please feel free to call on us.

Sincerely yours,

W. E. Westlake

W. E. Westlake, Head
Analytical Section
Pesticide Chemicals Research
Branch

Enclosures

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH DIVISION
P. O. BOX 232
KERRVILLE, TEXAS

June 26, 1959

TO : Mr. S. A. Hall, Chief, Pesticide Chemicals Research Branch, Beltsville, Maryland

FROM : H. V. Claborn, Analytical Section, Kerrville, Texas

SUBJECT: Validation for Analytical Method for Lindane

I have a letter dated June 22 from Dr. Lindquist on the above subject, a copy of which was sent to you.

If Dr. McFarland had been given a copy of our Special Report K-57, I believe he would have had all the information he needed. I am enclosing a copy of Special Report K-57 and also a table of recovery data, which should give him all the information requested.

Since Dr. McFarland cannot understand the low residues in hog fat it probably should be pointed out that the hogs were sprayed and not dipped as were the sheep and goats. In all our residue studies we have found residues to be lower in hogs than in cattle, sheep, or goats, and it does not appear unusual to me that a spray application to a hog, which has such a light coat, to cause lower residues than would result from dipping a sheep or goat. Regardless of any logical explanation for the results we are absolutely sure the lindane residues in the samples were as reported. The method was working perfectly, the samples were analyzed with extreme care, adequate samples were used for the needed sensitivity, and a control sample was run with each group (4) of test samples.

If more information is needed, we would be happy to supply it.

Enclosures:

1. Special Report K-57.
2. Table.

cc: Dr. A. W. Lindquist
Dr. R. C. Bushland

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Table 1. Percent recoveries when known amounts of lindane were added to fatty tissues.

Fat Used	Amount Added (Micrograms)	Recovery (Percent)
Beef	10	94
"	30	91
"	50	92
"	70	98
"	30	97
"	50	94
"	70	92
Chicken	30	98
"	50	96
"	70	95
"	20	88
Hog	20	98
"	30	98
Sheep	30	96
Goat	30	98

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SPECIAL REPORT K-57

Studies on Lindane Residues in Hogs

Kerrville, Texas, Laboratory 1/ 2/
Agricultural Research Service
United States Department of Agriculture

Lindane is recommended and widely used for the control of ectoparasites of hogs, but no data were available on the extent of its storage in the tissues. Tests were, therefore, conducted to determine the amounts of lindane stored and the length of time such residues would remain in the fat of hogs as a result of one application of a spray containing 0.06 percent lindane.

Methods:--A total of 14 hogs was used in the tests--two served as controls and 12 were sprayed once with 0.06 percent lindane. One group of six was sprayed with 0.06 percent lindane emulsion made from a 20 percent emulsifiable concentrate and the other with 0.06 percent suspension made from a 25 percent wettable powder.

The original plan was to slaughter one hog from each group at 1, 2, 4, 6, 8, and 10 weeks after treatment. Also at the first and tenth weeks' post-treatment slaughter, one control hog was to be sacrificed. However, sampling was discontinued after 6 weeks because residues were zero at that interval after treatment.

The Schechter-Hornstein Colorimetric Method 3/ was used for analyzing the fat samples. In order to get low and constant blanks and satisfactory recoveries, it was necessary to modify the method as described below.

1/ Treatment, slaughter and collection of tissue samples were supervised by R. H. Roberts, Entomology Research Division.

2/ Chemical analyses of lindane residues were made under the supervision of H. V. Claborn, Pesticide Chemicals Research Laboratories.

3/ Schechter, Milton S., and Irvin Hornstein. 1952. Colorimetric determination of benzene hexachloride. Anal. Chem. 24, 544.

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The modified method gave recoveries of 98 percent when known amounts of lindane were added to hog fat. It was sensitive to ± 0.1 p.p.m. on a 10 gram sample and ± 0.04 on a 50 gram sample.

Procedure for Analysis of Hog Fat:--Weigh a 10 gram sample of fat into a Waring blendor and blend for two minutes with 150 ml. of distilled chloroform. Add 15 grams anhydrous sodium sulfate and blend for three minutes longer. Add 1 gram of Filter-Cel, mix, and decant into a 400 ml. Erlenmeyer flask. Wash the blendor, beaker, and filter paper with about 125 ml. of chloroform. When less than 0.1 p.p.m. of lindane is expected use 50 gram samples with proportionally larger amounts of solvents and reagents.

Distill off the chloroform to approximately 200 ml.; then transfer to a 500 ml. separatory funnel. Add 8 ml. of a 1:1 mixture of sulfuric acid--fuming sulfuric acid. Shake gently and let stand for the sulfonated fatty layer to separate to the top. Drain the chloroform into another 500 ml. separatory funnel. Wash the fatty layer twice with 35 ml. of chloroform and add to the other chloroform extract. Discard the funnel containing the fatty layer. Extract the chloroform three times with 35 ml. portions of the sulfuric acid mixture. After the last extraction, allow the acid to drain down and then separate as cleanly as possible. Pour the chloroform from the top of the separatory funnel into a clean 500 ml. separatory funnel. Add 50 ml. of 5 percent sodium bicarbonate to the separatory funnel, stopper, invert and swirl several times with the stopcork open. Close the stopcork and shake vigorously, releasing the pressure frequently. Let stand for 10 minutes for a reasonably clear separation and then filter the chloroform layer through a 5 cm. plug of cotton packed in a glass crucible holder, into a 500 ml. Erlenmeyer flask. Wash the bicarbonate layer with 30 ml. of chloroform and filter into the Erlenmeyer flask.

Distill off the chloroform through a Snyder column to a volume of 10 ml. Transfer the residue to a 125 ml. Erlenmeyer flask, using 50 ml. of acetic acid to make the transfer. Add a glass bead and attach a Snyder column to the flask. Suspend a thermometer into the Snyder column so that the bulb is just below the top of the column. Evaporate the solvent until the temperature reaches the boiling point of acetic acid. Remove the Snyder column and let cool to room temperature.

Add three grams of malonic acid and four grams of 50 mesh zinc to the flask. Attach it to the Schecter-Hornstein still and follow the method as described. A sample of control fat should be run with each group of samples analyzed. This sample is used as a blank and subtracted from the test samples. If done this way, the blank will include any contamination of the reagents and any contamination from the air at the time the analysis was carried out.

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Results:—The lindane residues found in the omental fat are shown in Table I. The values are reported as read in the laboratory but it should be pointed out that the sensitivity of the method used for detecting the highest levels at one week after treatment was 0.1 p.p.m. while the zero levels (at six weeks after treatment) were obtained by a method sensitive to 0.04 p.p.m.

It is concluded that treatment of hogs with 0.06 percent lindane will result in residues of less than 1 p.p.m. at one week after treatment and that all detectable residues disappear in four to six weeks.

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Table 1.—Lindane residues found in the omental fat of hogs sprayed with 0.06 percent lindane.

Animal No.	Formulation	P.P.M. Lindane (weeks post-treatment)			
		1	2	4	6
3	W.P.	0.47			
5			0.30		
4				0.14	
6					
12	E.C.	.66			
14			.31		
10				.00	
11					

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SECTION D

Supplementary Petition for the Establishment of Tolerance for Lindane on Raw Agricultural Commodities.

The results of tests on the amount of residue remaining, including a description of the analytical method used.

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SECTION D

Full reports of investigations with respect to the amount of residue remaining, including the description of the analytical method, were presented at the hearings held in 1950 under the authority of the Secretary of Health, Education and Welfare, and reported in full in the Federal Register (1). The data presented at that time and the official transcripts of the hearings as they pertain to lindane are made a portion of this petition.

Additional information is contained in Supplementary Petition for the establishment of tolerance for lindane on raw agricultural commodities submitted September 14, 1955. This supplementary petition is incorporated in this petition by reference.

1. Federal Register, pp. 1473-1508, March 11, 1955.

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SECTION D

RESIDUES OF LINDANE ON RAW AGRICULTURAL COMMODITIES

The use of lindane-containing sprays directly on to the domesticated animals to control ectoparasites may result in deposition of the insecticide in the body fat. In cattle, Claborn (1) has shown that 0.03% lindane applied dermally results in less than 2.5 parts per million in the fat.

A 1.0% lindane dust to poultry (2) resulted in deposition of approximately 11 parts per million in the fat seven days following application. Residue values decreased rapidly. Dusting with 0.25% lindane resulted in two parts per million deposition in body fat.

These data indicate that dermal applications of lindane to domestic animals for the control of ectoparaties may result in residues occurring in the fat; these residues will not exceed the proposed tolerance requested in this petition.

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SECTION D

BIBLIOGRAPHY:

1. Claborn, H. V. Unpublished data. U.S.D.A., Kerrville, Texas.

2. Letter - Donald E. Cooperrider, The University of Georgia, Athens, Georgia, November 16, 1953.

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The following data was submitted by Dr. H. V. Claborn, U.S.D.A., Kerrville, Texas.

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Table 1. Parts per million of insecticides found in the fat of calves sprayed one time with 0.5 percent DDT, 0.5 percent TDE, 0.5 percent methoxychlor, and 0.03 percent lindane.

Weeks after treatment	Number of samples	Insecticides (p.p.m)*			
		DDT	TDE	Methoxychlor	Lindane
2	4	11.2	11.0	2.8	2.5
6	4	8.1	5.2	1.7	2.5
10	4	5.3	3.9	0	
16	4	2.3	1.1		
22	4	2.8	0.7		
27	4	1.7	0.5		

* Figures are averages of fat analyses from 4 calves given same treatment.

Table 2. Parts per million of insecticides found in the fat of calves sprayed six times at 3-week intervals with 0.5 percent DDT, 0.5 percent TDE, 0.5 percent methoxychlor, and 0.03 percent lindane.

Weeks after treatment	Number of Samples	Insecticides (p.p.m.)*			
		DDT	TDE	Methoxychlor	Lindane
3 wks. after 1st	3	18.0	13.2	1.5	2.5
3 wks. after 2nd	3	31.2	32.7	1.5	2.5
3 wks. after 4th	3	32.8	36.5	.8	2.5
3 wks. after 6th	3	35.2	28.4	2.4	2.5
12 wks. after 6th	3	7.7	13.6	0	
24 wks. after 6th	3	4.7	1.3		
36 wks. after 6th	3	2.2	0.8		

*Results are averages of fat analyses from 3 calves given same treatment.

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SECTION B

Supplementary Petition for the Establishment of Tolerance for Lindane on Raw Agricultural Commodities.

The amount, frequency and time of application of the pesticide chemical, Lindane.

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SECTION B

The full reports covering the amount, frequency and time of application of the pesticide chemical, lindane, were presented at the hearings held in 1950 under the authority of the Secretary of Health, Education and Welfare, and reported in full in the Federal Register (1). The data presented at that time and the official transcripts of the hearings as they pertain to lindane are made a portion of this petition.

Additional information is contained in Supplementary Petition for the establishment of tolerance for lindane on raw agricultural commodities submitted September 14, 1955. This supplementary petition is incorporated in this petition by reference.

1. Federal Register, pp. 1473-1508, March 11, 1955.

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SECTION B

SUMMARY

From a review of these data submitted in the 1950 hearings and supplementary petition dated September 14, 1955, it is submitted that the following generalizations may be drawn:

(1) That lindane is insecticidally effective against a wide variety of insect pathogens on a number of plants and animals.

(2) That lindane has been extensively tested by research and extension entomologists in many state and federal experiment stations and has quite consistently given effective control of the insects for which it is currently recommended.

(3) That as a result of the performance in experimental trials and in grower usage, lindane has found a prominent place in the recommendations of many federal and state agricultural advisors.

(4) The application of lindane at the recommended concentrations controls lice, ticks, mange, [illegible]ss, stable flies, and mosquitoes on cattle; keds, lice, mange and fleece worms of sheep and goats; lice and mange of swine, and mites, lice, fowl ticks and bedbugs of poultry.

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ISOTOX SPRAY 200
20% gamma isomer of benzene hexachloride from lindane.

The forage crops are deleted and the animal uses modified as listed below.

USES ON ANIMALS

BEEF CATTLE, SHEEP, HOGS AND HORSES, Mange (Psoroptic and Sarcoptic) or Barn Itch, Scab or Scabies - 1 pint per 100 gallons - For most effective treatment, apply 2 sprays one week apart. Sheep Ticks (Keds) on Sheep - 1 pint ISOTOX SPRAY 200 and 3 lbs. ORTHO DDT 50 WETTABLE in 100 gals. water. Spray along back and around ears, spraying against wool. Spray in fall. In some areas, a repeat treatment after shearing is also advisable. About 350 lbs. pressure is desirable. Do not use on lactating dairy animals.

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PROPOSED LABEL COPY

ISOTOX 25 WETTABLE
25% gamma isomer of benzene hexachloride from lindane.

The forage crops are deleted and the animal uses modified as listed below.

USES ON ANIMALS

BEEF CATTLE, SHEEP, HOGS AND HORSES: Mange (Psoroptic and Sarcoptic) or Barn Itch, Scab or Scabies - 1½ lbs./100 gals. - For most effective treatment, apply 2 sprays one week apart. Lice, Ticks, Horn Flies, Stable Flies, Mosquitoes - 1 lb. per 100 gals. Sheep Ticks (Keds) on Sheep - 1 lb. ISOTOX 25 Wettable and 3 lbs. ORTHO DDT 50 WETTABLE in 100 gals. water. Spray along back and around ears, spraying against wool. Spray in fall. In some areas, a repeat treatment after shearing is also advisable. About 350 lbs. pressure is desirable. Do not use on lactating dairy animals.

Mange (Psoroptic and Sarcoptic) - Dairy Cattle: This is a therapeutic use, consult your local veterinarian.