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TXR-6704

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

006704

MAR 28 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Hartz 2 in 1 Plus Long Lasting Collar for Dogs
Cholinergic Effects Study on Bitches and Puppies

TO: Mr. George LaRocca, PM-15
Registration Division (TS-767C)

FROM: Byron T. Backus, Toxicologist Byron T. Backus 03/25/88
Toxicology Branch (TS-769C)

THROUGH: Marcia van Gemert, Ph.D.
Section Head, Review Section III M. van Gemert 3/25/88
Toxicology Branch (TS-769C)

and

Theodore M. Farber, Ph.D., D.A.B.T.
Branch Chief
Toxicology Branch (TS-769C)

EPA Record No. 210551

Project No. 8-0331

Tox. Chem. 217A

EPA Reg. No. 2596-62

Action Requested:

Review a collar exposure study (including cholinesterase activity measurements) involving beagle bitches and their offspring in conjunction with labeling recommendations for this product.

Comments and Recommendations:

1. The study shows that statistically significant plasma cholinesterase inhibition occurred in puppies which wore one or two collars (applied 4 weeks after birth). The greatest mean measured plasma cholinesterase inhibition (about 20-30%) was at 42 days (12 days after these collars were applied).

1

The Agency's experience has been that the greatest degree of cholinesterase inhibition occurs in the week or two following collar application, and this is additional evidence that the collars were responsible for this drop.

2. One of 18 litters in this study was born prematurely, to a dog in the 2-collar group, and all the pups died. The statement is made in the report (p. 14) that "This relates to 5.5% (1 of 18) of all the litters born during the study and is not considered significantly different than the colony experience (4.4%) for losses based on premature litter births." According to the letter of October 27, 1986 (p. 141) the value of 4.4% was percent total litter loss in non-study dogs for this period (2/45 = 4.4%). The only premature litter born during this period was to the dog in the study. The letter also states that in the 6 months preceding this study there were 4 premature litters. However, since the number of litters born during the pre-study period is not reported, we have not been able to calculate the incidence of premature litters. However, we are convinced that it is below 4.4%.

3. There was no evidence of any RBC cholinesterase inhibition in either the bitches or puppies, and there was no evidence of plasma ChE inhibition in the bitches. There was no evidence of any significant dose-related differences in weight gains of the different groups of puppies, particularly when sex ratio differences between the different groups are taken into consideration.

4. After taking into account the findings of this study, as well as the highest level of exposure (2 collars), it is the conclusion of the Toxicology Branch that the Agency's position, as stated in the letter of May 10, 1985 to the registrant, should remain unchanged. As stated in that letter:

> Labeling for this product should be revised to state that pregnant bitches, newborn puppies, and nursing puppies should not wear or otherwise be exposed to this collar. Additionally, it would also be appropriate to add a label statement that additional cholinesterase inhibiting compounds should not be used on dogs wearing this collar.

2

Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus 03/25/88
Section III, Tox. Branch (TS-769C)

Secondary Reviewer: Marcia Van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT I

STUDY TYPE: Cholinesterase - Dog TOX. CHEM. NO.: 217A

ACCESSION NUMBERS: 404366 (5 volumes) MRID NO.: not given

TEST MATERIAL: 14% Tetrachlorvinphos (Rabon) Collar

SYNONYMS: Hartz 2 in 1 Plus Long Lasting Collar for Dogs
(EPA Reg. No. 2596-62)

STUDY NUMBER(S): WEL 156

SPONSOR: Hartz Mountain Corporation

TESTING FACILITY: White Eagle Laboratories
2003 Lower State Road
Doylestown, PA 18901

TITLE OF REPORT: Cholinergic Effects of the Use of a Tetrachlorvinphos Collar on the Bitch Dog and Its Offspring

AUTHOR(S): D'ver, A. & Perlberg, W.

REPORT ISSUED: November 19, 1987

STUDY CLASSIFICATION: Acceptable

CONCLUSIONS:

1. The study shows that there was statistically significant plasma cholinesterase inhibition in puppies which wore one or two collars during the period of 30-90 days after birth. The greatest degree of measured plasma cholinesterase inhibition (approximately 20-30% mean inhibition in both groups) was at 42 days (12 days after these collars were applied). The Agency's experience has been that the greatest degree of cholinesterase inhibition occurs in the week or two following collar application, and this is additional evidence that the collars were responsible for this drop.

2. One of 18 litters in this study was born prematurely, to a dog in the 2-collar group, and all the pups died. The statement is made in the report (p. 14) that "This relates to 5.5% (1 of 18) of all the litters born during the study and is not considered significantly different than the colony experience (4.4%) for losses based on premature litter births." According to the letter of October 24, 1986 (p. 141) the value of 4.4% was percent total litter loss in non-study dogs for this period (2/45 = 4.4%). The only premature litter born during this period was to the dog in the study. The letter also states that in the 6

months preceding this study there were 4 premature litters. However, since the number of litters born during the pre-study period is not reported, we have not been able to calculate the incidence of premature litters. However, it is probably below 4.4%.

3. There was no evidence of any RBC cholinesterase inhibition in either the bitches or puppies, and there was no evidence of plasma ChE inhibition in the bitches. There was no evidence of any significant dose-related differences in weight gains of the different groups of puppies, particularly when sex ratio differences between the different groups are taken into consideration.

A. MATERIALS:

1. Test collar: According to analytical data test collars used contained 14.5% of technical (97.3%) Rabon.

2. Test animals: Species: dog. Breed: Beagle (female). Their weights ranged from 8.0 to 12.3 kgs on day 0 (when collars were applied). These dogs were a variety of ages (2-10 years old) at the start of the study. All had successfully raised at least one previous litter.

006704

B. STUDY DESIGN:

1. Animal assignment - The following bitches were selected from a closed breeding colony and grouped as indicated below:

Animal Number	Year of Birth	Weight (kg) day 0	Number of previous Litters	Page Ref. Day 0		Day of Parturition	Days to Parturition
Controls (placebo collar):							
2283	1976	12.0	11	359	4/28/86	7/07/86	70
2739	1979	8.0	6	383	5/09/86	7/17/86	69
2915	1980	11.5	7	416	5/05/86	7/15/86	71
2967	1981	11.6	4	474	5/14/86	7/21/86	68
3220	1983	9.0	2	535	5/14/86	7/24/86	71
3262	1983	10.1	2*	588	5/05/86	7/14/86	70
1-collar:		*Also one aborted litter					
2390	1977	11.0	10	625	5/14/86	7/21/86	68
2576	1978	12.1	6	674	5/09/86	7/18/86	70
2639	1979	11.8	8	751	4/30/86	7/12/86	73
3261	1983	11.1	2	802	5/05/86	7/11/86	67
3349	1984	10.5	1	862	5/14/86	7/24/86	71
3368	1984	9.3	1	930	5/05/86	7/16/86	72
2-collar:							
2756	1979	10.3	8	988	4/28/86	7/09/86	72
2819	1980	9.3	6	1032	5/14/86	7/26/86	73
2841	1980	12.3	7	1096	5/05/86	7/10/86	66
2894	1980	12.0	5	1157	5/29/86	8/05/86	68
2942	1980	10.5	5	1189	5/09/86	7/17/86	69
3348	1984	12.6	1	1252	5/05/86	7/13/86	69

The following gives survival to weaning of pups born after 1982:

Animal Number	# previous litters after 1982	Total # of pups born after 1982	# of pups born after 1982 surviving to weaning
Controls (placebo collar):			
2283	4	18	11
2739	4	18	11
2915	4	26	19
2967	3	26	14
3220	2	10	7
3262	2	10	7
1-collar:			
2390	4	22	18
2576	3	19	14
2639	4	26	18
3261	2	13	10
3349	1	6	4
3368	1	6	3
2-collar:			
2756	4	23	16
2819	4	32	21
2841	4	31	24
2894	3	17	11
2942	3	17	16
3348	1	6	5

5

2. Collars were applied on or about day 7 (there were 2 baseline cholinesterase measurements on bitches, on days 0 and 5), and were kept on during pregnancy, parturition and nursing. Collars were removed from bitches when their pups were weaned (on or about day 125). Collars were placed on each of the puppies when they were four weeks old (those with a mother in the control group received a placebo collar; those with a mother in the 1X group received a single Rabon collar; and those from mothers of the 2X group received two Rabon collars). Collars were removed from pups on or about day 90 (62 days after they were placed on them). In each case, the amount of material cut off from the collars was weighed, and analyses were made on both the material which was cut off as well as the used collars to determine how much Rabon the dogs had been exposed to.

3. There is no indication as to what the diet consisted of, its availability, nor is there any indication that food consumption was measured.

4. Statistics - Student's t test was used to compare group mean weights (bitches and puppies), as well as group means for plasma and RBC cholinesterase activities.

5. There is a Good Laboratory Practice Statement on p. 3. The signatures on it are those of the co-authors. Appendix L (vol. 1, p. 352-353) contains two signed quality assurance statements. The first is from Research Pathology Services, Inc, which conducted an audit of 33% of the raw ChE measurements. The second is signed by a M. Lytwyn, Ph.D., but there is no indication of this individual's title or affiliation.

C. METHODS AND RESULTS:

1. Observations

Bitches and their litters were observed daily. The following were observed and recorded with respect to litters: number of pups, numbers of each sex, number born dead, physical deformities, number of pups in each litter dying before weaning, and veterinary treatment of any individual pups.

Results: There is no indication that any collar-related effects were noted. The statement is made on p. 10 that: "The collars performed normally and were well tolerated by the dogs; they

did not cause any observable irritation to any of the animals during the test."

The following individual litter findings are reported:

Animal Number	Total number of pups born		Number of pups born dead		No. of pups dying before day 104		Number of pups surviving to day 104	
	M	F	M	F	M	F	M	F
Controls								
2283	0	1	0	0	0	0	0	1
2739	1	2	0	0	0	1	1	1
2915	2	5	1	0	0	0	1	5
2967	7	1	0	0	3	0	4	1
3220	6	0	0	0	1	0	5	0
3262	2	1	0	0	0	0	2	1
1-collar:								
2390	3	2	0	0	0	0	3	2
2576	6	3	0	0	0	1	6	2
2639	4	4*	2	1*	2	0	0	3*
3261	4	3	0	0	1	0	3	3
3349	6	3	0	1	1	1	5	1
3368†	2†	4†	0	0	0	0	2†	4†
2-collar:								
2756	1	3	0	0	0	1	1	2
2819	2	6	0	1	1	0	1	5
2841	3	4	0	0	0	1	3	3
2894	2	3	1¶	0	1	3	0	0
2942	3	4	0	0	0	0	3	4
3348	2	4	0	0	0	0	2	4

*According to information on p. 751 there were 2 males and 0 females "DOA" ("dead on arrival") at birth. On p. 751 it is stated that 4 males and 4 females were born in this litter, but on p. 26 only 3 females are listed. On p. 32 it is stated that there were 3 DOA so from this it is assumed that one female was born DOA.

†Records for the litter of 3368 are not given on p. 26. However, they can be found on p. 946.

¶Listing on p. 1157 indicates 2 males, 3 females born, but no DOA. On page 33 it is indicated 4 were born live, with one DOA.

Group	Total number of pups born		Number of pups born dead		No. of pups dying before day 104		Number of pups surviving to day 104	
	M	F	M	F	M	F	M	F
Placebo	18	10	1	0	4	1	13	9
1-collar	25	19	2	2	4	2	19	15
2-collars	13	24	1	1	2	5	10	18

There were no significant differences between groups with respect to overall pup survival rates. From p. 13:

	# Pups Born	# Pups Survived	% Survived
Placebo	28	22	78.6
1-collar	44	34	77.3
2-collars	37	28	75.7

The overall pup survival rate for the colony during the time period the study took place is reported as 67.9% (p. 14). This is misleading. The letter of October 24, 1986 (p. 141) states that the death rate for April 1-June 30 was 32.1%, "about average." During the period of the study, non-study puppies had a death rate of 14.3% and study puppies had a death rate of 22.9%. It is stated that both rates were somewhat lower than normal as the poorer bitches were being rested because of a weak market, with a resultant upward shift in survival rates. The study animals were "second best bitches" (this is contradictory to the cover sheets for each of the bitches which consistently contain the notation: "This Dog is Graded #1.").

It is noted that all mortalities in the placebo group occurred before day 30, while 2 mortalities in the 1-collar and 2 in the 2-collar groups were after day 30. However, these numbers are too low for any significance to be attached to them.

The living pups of bitch 2894 (2-collar group) were all runts, and all died within 8 days of birth; the comment is made (p. 33) that "Entire Litter appears Premature." On p. 14 the statement is made that: "For the entire study, one premature litter occurred (2 collar group) in which all five puppies were lost. This relates to 5.5% (1 of 18) of all the litters born during the study and is not considered significantly different than the colony experience (4.4%) for losses based on premature litter births." The citation for this statement is given as a letter of October 24 1986 (p. 141).

According to the letter of October 24, 1986 one out of 18 study litters (= 5.5%) showed complete litter loss, while in the non-study dogs 2 of 45 (= 4.4%) showed complete litter loss. However, these were apparently not premature litters, as the statement is made that "the only premature litter occurred in the study." In the six months prior to the study, four premature litters were born. The incidence is not reported, but it was certainly lower than 4.4%.

One pup in the one-collar group (bitch 2576) was hydrocephalic, and was euthanized 50 days after it was born. The incidence of hydrocephalus in this colony over the 4-year period ending June 1987 is reported (p. 15) as 1 in every 246 births.

2. Weights

Bitch weights:

Individual bitches were weighed on days 0, 35, 65, 95, 125 and 140. Parturition occurred in each case between days 65 and 95, and weaning occurred (and collars were removed) on or about day 125.

Results: There were no significant differences at any time with respect to mean bitch weights or mean weight changes from day 0.

The following is calculated from data in table B2, p. 23:
Mean weight changes (kg) from day 0:

	Day 35	Day 65	Day 95	Day 125	Day 140
Placebo group	0.0	2.9	0.8	0.9	0.1
1 collar group	0.3	4.1	-0.4	0.7	-0.4
2 collar group	-0.1	2.8	-0.5	0.4	-0.6

Note: collars were removed from individual dogs (and weaning took place) on or about day 125.

Pup weights: Pups were weighed at birth, and on days 14, 30, 42, 60, 90 and 104.

Results: The following (from table B3, p. 24) gives the mean pup weights (in kg) for each exposure group on these days:

	Day						
	0	14	30	42	60	90	104
Placebo group	0.283	0.676	1.265	1.797	2.564	4.666	5.483
1 collar group	0.294	0.666	1.102*	1.657	2.457	4.336	5.019
2 collar group	0.276	0.661	1.161	1.709	2.487	4.322	5.053

*Reported as statistically significant at $p \leq 0.05$ (p. 29).

It is uncertain whether the t-test was used to determine whether a mean weight value for exposed pups was significantly different or significantly lower than the corresponding control value.

The statement is made (p. 12) that: "puppies in the larger litters were smaller, irrespective of treatment group." This is not supported by the data. The following is a listing of mean pup weight at birth and number of live pups in each litter at birth:

Bitch	Number of live pups in litter	Group	Birth Weight
3368	6	1 collar	0.370
3348	6	2 collar	0.359
3262	3	placebo	0.345
2283	1	placebo	0.332
3349	8	1 collar	0.325
2756	4	2 collar	0.325
3220	6	placebo	0.304
2390	5	1 collar	0.295
2576	9	1 collar	0.295
2915	6	placebo	0.282
2942	7	2 collar	0.274
2819	7	2 collar	0.270
2739	3	placebo	0.261
2967	8	placebo	0.247
2841	7	2 collar	0.244
3261	7	1 collar	0.236
2639	4	1 collar	0.233
2894	4	2 collar	0.171
	101		

9

Overall, there was a mean of 5.61 liveborn pups/litter. The nine litters with highest mean birth weights had a mean of 5.33 pups/litter. If the value for #2283 (only one pup) is removed then the other eight highest litters had a mean of 5.88 pups per litter, and the remaining nine litters had a mean of 5.89 (6.13 if the value for #2894, with 4 premature liveborns, is thrown out) pups/litter.

Although no weight differences appear to exist for days 0 and 14, they do appear to be present for days 30-104.

After examining the data, it is the conclusion of this reviewer that the weight differences are accounted for by sex ratio differences in each set of litters, the males tending to be heavier than females after day 30. The following means are from only those pups which survived to 104 days:

Male pups: Group	# of Pups	Day 0	14	30	42	60	90	104
Placebo	13	0.297	0.738	1.309	1.937	2.71	5.02	5.82
1 collar	19	0.313	0.711	1.133	1.794	2.69	4.73*	5.47
2 collar	10	0.308	0.746	1.310	1.970	2.87	5.04	6.06

*value for 3368-1 (6.15 kg) seems too high on this date, particularly as it is reported as 5.54 kg for day 104. Without 3368-1 the mean is 4.65.

Female pups: Group	# of Pups	Day 0	14	30	42	60	90	104
Placebo	9	0.286	0.611	1.202	1.594	2.35	4.15	5.00
1 collar	15	0.293	0.633	1.100	1.570	2.28	3.84	4.45
2 collar	18	0.285	0.626	1.077	1.586	2.30	3.92	4.49

Note: There are no individual weights for the pups in the litter of #3368 on page 26; however, these individual weights are provided on page 946.

The collars were removed from the pups when they were 90 days old. At that time the mean pup weight for the controls was 4.666 kg; for the one-collar pups was 4.336 kg; and for the 2-collar pups was 4.322. The greater value for the controls (as compared with the 2-collar animals) appears to have been because there was a greater proportion of males (with heavier mean body weights than females) in controls (13/22 pups) than the 2-collar group (10/28).

3. Cholinesterase activities:

Cholinesterase (RBC and plasma) activities were measured using the Boehringer Mannheim reagent kit (catalogue no. 124117).

Bitch cholinesterase activities:

These were measured (in mu/ml) for days 0 and 5 (pre-collar measurements), and then at days 7, 19, 35, 65, 95, 125 and 140.

Results:

There were no significant differences at any time between the different groups with respect to RBC and plasma ChE activities.

Puppy cholinesterase actitivities:

Both RBC and plasma ChE activities (in mu/ml) were measured on or about days 14, 30, 42, 60, 90 and 104 (after birth). It is stated on p. 9 that collars were put on puppies when they were 4 weeks of age. From the individual observation sheets, these collars were removed when the puppies were about 90 days old.

The following group means (in mu/ml) have been calculated using only data from puppies which survived to 104 days:

Males:

	Day					
RBC ChE:	14	30	42	60	90	104
placebo	2770.2	2802.6	2951.9	2435.4	2516.4	2845.6
1-collar	2771.1	2678.7	2558.6	2649.1	2612.2	3022.3
2-collar	2772.9	2499.1	2625.5	2653.6	2625.5	3004.6
Plasma ChE:						
placebo	2011.5	1978.0	2075.2	1668.1	1929.4	2036.6
1-collar	2036.9	1802.7	1619.8	1640.8	1960.1	2312.5
2-collar	1982.5	1766.9	1564.1	1424.4	1727.6	2184.6

Females:

RBC ChE:	14	30	42	60	90	104
placebo	3026.4	2831.4	2964.0	3276.0	2691.0	3143.4
1-collar	2863.2	2692.7	2657.6	3083.8	2687.7	3164.0
2-collar	2765.1	2757.3	2722.2	2893.8	2698.8	3003.0
Plasma ChE:						
placebo	2212.9	2086.1	2017.1	1984.3	1978.9	2418.8
1-collar	2155.1	1829.7	1635.7	1880.9	2027.3	2271.5
2-collar	2132.1	1874.3	1749.9	1631.2	1828.7	2308.4

The mean plasma ChE activity for puppies (combined females and males) in 1 and 2-collar litters is reported as being significantly lower than control values at days 30 and 42, and, for 2-collar puppies only, at 60 days. There was no evidence of a significant reduction in RBC ChE activity. From p. 44:

Plasma ChE

vs. Placebo:	14	30	42	60	90	104
1 Collar	ns	S-	S-	ns	ns	ns
2 Collar	ns	S-	S-	S-	ns	ns

vs. Day 14:

Placebo	-	ns	ns	S-	ns	ns
1 Collar	-	S-	S-	S-	ns	S+
2 Collar	-	S-	S-	S-	S-	S+

The significantly elevated level (relative to day 14 readings) at day 104 for the 1-collar and 2-collar groups 2 weeks after removal of the collars is similar to what has been seen in other studies involving dog collars with cholinesterase inhibitors.

While most of the puppies in the 1 and 2-collar group had only something like 20-30% plasma ChE inhibition, others showed more. In the case of pup 2819-1 (of the 2-collar group) which died on or about day 45, the plasma ChE activity (see p. 1056) on day 42 was measured as 891.54 mu/ml; which was about a 55% drop from its baseline activity on day 14 (2007.72).

4. Exposure to Rabon:

Collars were analyzed for Rabon content both before and after they were worn by the dogs. From these values (as well as the weight of the collar as placed on each dog) individual Rabon exposures over the study period were calculated:

Results:

The following dosage ranges (in g/kg) for Rabon are reported: (p. 94-114):

Group	low value	high value
Placebo bitches	0	0
1-collar bitches	0.17	0.23
2-collar bitches	0.35	0.55
Placebo puppies	0	0
1-collar puppies	0.39	0.74
2-collar puppies	0.81	1.48

D. DISCUSSION:

This study was submitted in partial response to an Agency letter of May 10, 1985, which stated that: "Labeling for this product should be revised to state that pregnant bitches, newborn puppies, and nursing puppies should not wear or otherwise be exposed to this collar. Additionally, it would also be appropriate to add a label statement that additional cholinesterase inhibiting compounds should not be used on dogs wearing this collar."

There were no indications of effects on plasma ChE activity in the bitches, or on weight gains or RBC ChE activity in either bitches or pups.

However, the birth of a premature litter in one of the 6 females of the 2-collar group is not reassuring as to the safety of this product, although it cannot be conclusively stated that this occurred because of exposure to the collar. Also, this reviewer has interpreted the cholinesterase data as indicating that the product is causing at least a low level (about 20-30%) of plasma cholinesterase inhibition in puppies at the normal use level in the 2-week period after collar application. While the argument is made (p. 16) that a mean depression should exceed 25-30% of a baseline measurement to be truly meaningful, it is noted that there were some instances involving individual puppies where this was exceeded. It is also noted that this a comparatively stressful period of life.

In short, this safety study utilized a fairly limited number of dogs, they were all of one breed, and the bitches were all (presumably) in good health. Given these limitations, as well as the reported results of this study, the Toxicology Branch sees no reason to change the Agency's position as previously stated to the registrant in the letter of May 10, 1985.