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

004646

9/9/85

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 3.0% Chlorpyrifos Cat Collar

TO Mr. Jay Ellenberger (PM-12)
Registration Division (TS-767)

FROM: Byron T. Backus
Toxicology Branch
HED (TS-769)

THROUGH: Clint Skinner, Ph.D., Head
Review Section III
and
Ted Farber, Ph.D., Chief
Toxicology Branch

Chemical no. #219AA

EPA File Symbol: 8220-GO

Registrant: Carter-Wallace, Inc.
Lambert Kay Division
Half Acre Road, P.O. Box 418
Cranbury, NJ 08512

Action Requested:

The Registration Division has requested reviews of two cat cholinesterase studies submitted as supporting data for a 3% chlorpyrifos cat collar.

Background:

The proposed label includes the statement "Kills fleas up to 13 months."

There are no chlorpyrifos-containing collars registered for use on cats.

Comments and Recommendations:

1. Both the 1X and 5X studies have been classified as supplementary for reasons which are specified in the attached

data evaluation reports. The 5X study can be upgraded to minimum with additional information as to how the collar was applied to (worn by) the cats and some data as to the rate of release of the active ingredient. The rate of release data should be based on measurements from a number of collars and should be presented both as the individual measurements and as averages.

2. Although the proposed label has a claim for 13 month efficacy, both the 1X and 5X studies lasted only 3 months. Normally for a product of this type we have required the study duration to be the greater of 90 days or one-half the maximum period of claimed efficacy. In this case the study should have been for 6 1/2 months.

3. The 5X study adequately demonstrates that considerable plasma ChE inhibition occurs as a result of exposure to 5 collars. After correcting for control values, mean plasma ChE activity in exposed cats was between 14.6 and 44.6% of pre-exposure baseline levels. In half the cats plasma ChE had recovered to pre-exposure levels 14 days after the collars were removed.

4. The maximum possible measured RBC ChE depression in any one individual cat occurred on day 69, when one cat (#4898) had approximately 73% of its baseline activity. However, given the usual uncertainties regarding any single individual measurement, and the fact that exposed cats (including #4898) often had RBC ChE activities above baseline readings, it is doubtful whether any significant RBC ChE inhibition occurred.

5. It is the understanding of this reviewer that the product would be initially marketed through veterinarians so as to more fully assess potential adverse incidents.

6. If the additional information is submitted to upgrade the 5X study, then the Toxicology Branch would have no objections to the registration of this product, provided all claims are reduced to no more than 6 months and provided that, for at least the first year that this product is actively marketed it will only be available through veterinarians.

Data Evaluation Reports (attached):

Boyd, J. P. Blood cholinesterase activity in cats treated with a single single 3% Chlorpyrifos collar. Project number 20-170-683. Unpublished study conducted by P.A.C.E. International, Suite 251, 2925 LBJ Freeway, Dallas, 75234. Dated 05/30/85.

Boyd, J. P. Blood cholinesterase activity in cats wearing five pet collars containing 3% Chlorpyrifos. Project report no. 20-207-1184. Unpublished study conducted by P.A.C.E. International, Suite 251, 2925 LBJ Freeway, Dallas, 75234. Dated 06/14/85.

Data Evaluation Report (I)

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Compound:

Cat collar containing 3% Dursban (Chlorpyrifos)

Study type:

Cholinesterase (collar exposure) - cat

Citation:

Boyd, J. P. Blood cholinesterase activity in cats treated with a single Chlorpyrifos collar. Project report no. 20-170-683; study conducted by P.A.C.E. International, Suite 251, 2925 LBJ Freeway, Dallas, TX 75234. Dated 05/30/85. Received at EPA 7/02/85; in Acc. 258494.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Approved by:

Clint Skinner, Ph.D.
Section Head
Review Section III
Toxicology Branch

Core Classification: Supplementary

Toxicity Category: N/A

Comments and Conclusions:

1. Since on days 3, 30 and 57 the exposed cats had approximately one-half their baseline plasma ChE activity, this indicates no more than 50% plasma ChE depression in cats wearing one collar.

2. The reported values for plasma ChE on day 101, and probably day 14 as well, have to be regarded as suspect as they are simply too low for the controls. Because the readings made on day 101 are suspect, this reviewer does not accept the report statement that plasma ChE had "returned to baseline by day 101."

3. The values obtained for RBC ChE activities were low. The submitted reference paper by Pickering and Martin notes (p. 193) that "the erythrocytes of the cat have negligible activity" and (on p. 194) "the erythrocyte activity is too low to measure accurately by a variant of the Michel method."

Materials:

Cats were obtained from the city-maintained animal shelter in Dallas, TX. Weights varied from 3 to 10 lbs at the start of the study. Some cats had short hair, others had long. All are described as being "mature," defined (p. 7) as being over 18 months of age.

Test material: beige colored pet collars containing 3% Chlorpyrifos, measuring 3/8" wide x 1/8" thick x 15" long with a metal buckle, each weighing 17 g prior to fitting on the cat. Identified as lot C, manufactured May, 1983.

Procedure:

Cats were vaccinated for rhinotracheitis, calici and panleukopenia upon arrival. They were caged individually.

Following a 10-14 day acclimation period the cats were separated into two groups. Three female cats served as controls; the others (3M, 3F) wore one collar for what was probably a 101 day period. The cats were observed on a daily basis from day -32 through 101.

Individual non-fasted 1.5 ml blood samples were taken at 8:00 A.M. on days -32, -25, 0, 1, 3, 7, 14, 30, 57 and 101. Samples were taken from the cephalic vein of each animal, and were placed in a heparin coated vacutainer which were transported on ice to Launey Medical and Surgical Clinic, 9528 Webbs Chapel Road, Dallas, TX 75220, "where they were analyzed following the Modified Method of Michel" for both plasma and RBC ChE activities. Samples were assayed within 2-4 hours after being taken.

Results:

There were no signs or possible symptoms of cholinesterase inhibition in the daily observations. Individual cats either maintained their initial weights or made slight gains.

Cholinesterase measurements:

There was a considerable amount of variation in mean measured plasma ChE activities during the course of this study:

	Day -32	Day -25	Day 0	Day 1	Day 3	Day 7	Day 14	Day 30	Day 57	Day 101
Controls	0.52	0.59	0.32	0.47	0.54	0.32	0.15	0.43	0.33	0.05
Treated	0.46	0.55	0.36	0.39	0.28	0.18	0.15	0.28	0.26	0.05

Plasma ChE (from table 3, p. 9):

Percent changes in mean plasma ChE activities from base levels:

	Day 1	Day 3	Day 7	Day 14	Day 30	Day 57	Day 101
Controls (3 cats)	- 1.75	+12.60	-33.99	-68.10	-11.56	-31.40	-89.57
Treated (6 cats)	-11.42	-37.76	-60.67	-66.72	-38.59	-42.20	-89.24
Mean difference between controls and exposed cats:	- 9.67	-50.36	-26.38	+ 1.38	-27.03	-10.80	+ 0.33
95% C.L. Lower	+26.11	-30.28	-12.88	+10.84	+ 8.68	+ 2.16	+ 4.75
Upper	-45.45	-70.44	-39.88	- 8.08	-62.74	-23.76	- 4.09

RBC ChE (from table 5, p. 11):

Percent changes in mean RBC ChE activities from base levels:

	Day 1	Day 3	Day 7	Day 14	Day 30	Day 57	Day 101
Controls (3 cats)	- 7.70	-27.99	+13.37	+62.82	-39.63	+ 8.71	+ 2.89
Treated (6 cats)	+2'.99	+38.43	+89.59	+92.13	+22.92	+ 3.47	+ 5.79
Mean difference between controls and exposed cats:	+29.69	+66.42	+76.22	+29.31	+62.55	- 5.24	+ 2.9
95% C.L.							
Lower	+98.87	+148.65	+157.01	+96.71	+207.31	+61.93	+61.88
Upper	-39.49	-15.81	- 4.57	-38.09	-82.21	-72.41	-56.08

Discussion:

Among the conclusions of this study is the statement (p. 5) that there was a depression of plasma ChE in cats wearing one collar "that returned to baseline by day 101."

It is the conclusion of this reviewer that the mean percentage differences between controls and exposed cats were to some extent a function of the mean control values. This can be observed from the following:

Day	Mean for control cats	Mean for exposed cats	Exposed in terms of controls	Difference of means as reported
101	0.05	0.05	1.00	+0.33
14	0.15	0.15	1.00	+1.38
7	0.32	0.18	0.563	-26.38
57	0.33	0.26	0.788	-10.80
30	0.43	0.28	0.651	-27.03
3	0.54	0.28	0.519	-50.36

The tendency was that the lower the control mean, the less difference there was in the amount of inhibition as reported in terms of the baseline.

The reported values for day 101, and possibly day 14 as well, have to be regarded as suspect. The values for day 101 are simply too low and therefore cannot be used to demonstrate that plasma ChE had returned to

baseline on this date. However, since on days 3, 30 and 57 the exposed cats showed approximately one-half of their baseline plasma ChE activity, this indicates no more than 50% plasma ChE depression in cats wearing one collar.

Although the values obtained for RBC ChE activities were low, the submitted reference paper by Pickering and Martin notes (p. 193) that "the erythrocytes of the cat have negligible activity" and (on p. 194) "the erythrocyte activity is too low to measure accurately by a variant of the Michel method."

In conclusion, this particular study demonstrates that at the 1 collar level there is a maximum of about 50% plasma ChE depression. No conclusions can be drawn as to whether or not there was recovery in plasma ChE activity by day 101, as the values obtained from blood taken on that date are suspect. The RBC ChE activities were measured by a method which has been reported in the literature as not giving accurate results.

This study is therefore classified as supplementary.

Data Evaluation Report (II)

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Compound:

Cat collar containing 3% Dursban (Chlorpyrifos)

Study type:

Cholinesterase (collar exposure) - cat

Citation:

Boyd, J. P. Blood cholinesterase activity in cats wearing five pet collars containing 3% Chlorpyrifos. Project report no. 20-207-1184; study conducted by P.A.C.E. International, Suite 251, 2925 LBJ Freeway, Dallas, TX 75234. Dated 06/14/85. Received at EPA 7/02/85; in Acc. 258494.

Reviewed by:

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Approved by:

Clint Skinner, Ph.D.
Section Head
Review Section III
Toxicology Branch

Clint Skinner 9-9-85

Core Classification: Supplementary

Comments and Conclusions:

1. In order to upgrade this study additional information should be provided. This should include i) some indication as to how the five collars were applied to (or worn by) the cats, and ii) data regarding release rates of chlorpyrifos from these five collars.

2. The study lasted 3 months, while the proposed label has a flea control claim of 13 months. Normally, we have asked for studies lasting either 90 days or half the maximum specified exposure period, whichever is longer. With a 13 month claim the duration of the study should have been 6 1/2 months.

3. The study adequately demonstrates that considerable plasma ChE inhibition occurs as a result of exposure to 5 collars. After correcting for control changes, mean plasma ChE activity in the exposed cats ran between 14.6 and 44.6 pre-exposure baseline levels. In half the cats plasma ChE had recovered to pre-exposure levels 14 days after the collars were removed.

4. The maximum possible measured RBC ChE depression was in cat 4898 on day 69, at approximately 27% inhibition. However, given the usual uncertainty regarding individual measurements, and the fact that exposed cats (including #4898) often had RBC ChE activities above baseline readings, it is doubtful whether any significant RBC ChE inhibition really occurred.

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Materials:

Cats were obtained from the city-maintained animal shelter in Dallas, TX. Weights varied from 4 - 10 lbs at the start of the study. Hair was either short or long. All are described as "mature" in the table on p. 9 (where "mature" is defined as being 18 months of age or older). However, the statement is made on p. 4: "The age was monitored only to the point of assuring the Study Director that no kittens (under 3 months of age) were involved in the study."

Test material: beige colored pet collars containing 3% Chlorpyrifos, measuring 3/8" wide x 1/8" thick x 15" long with a metal buckle, each weighing 17 g prior to fitting on the cat. Identified as Cat formulation C, manufactured November, 1983.

Procedure:

Cats were vaccinated for rhinotracheitis, calici and panleukopenia upon arrival. They were caged individually.

Following a 10-14 day acclimation period the cats were separated into two groups on the basis of "size, hair coat, demeanor and sex." Four cats (1M, 3F) served as controls; the others (6F) wore (in some unspecified fashion) 5 collars for a period of 91 days. The cats were observed on a daily basis from day -14 through 105.

Individual non-fasted 1-2 ml blood samples were taken at 8:00 A.M. on days -14, -7, 0, 1, 3, 7, 14, 30, 69, 91 (when collars were removed) and 105. Blood samples were taken from the cephalic vein of each cat, placed in 2 ml sodium-heparin coated vacutainer tubes, and were transported in a cooler to 8 Medical Parkway, Dallas, "where they were prepared for air shipment to Vetpath Labs, One Malcolm Ave., Teterboro, NJ 07608," where ChE activities were determined.

Cholinesterase activities (both RBC and plasma) were measured using a variation of the Ellman Method.

Results:

One control and 2 exposed cats each lost about 10% of their initial weight during the study; all other cats either maintained or gained weight. One exposed cat (#4875) vomited material containing a considerable amount of hair on day 14; another (#4898) had diarrhea on days 20, 21 and 24:

Percent changes in mean plasma ChE activities from baseline levels (from table 3, p. 11):

	Day 1	Day 3	Day 7	Day 14	Day 30	Day 69	Day 91	Day 105
Controls (4 cats)	- 8.1	- 6.8	+ 8.6	+ 9.1	+15.4	-15.7	+ 6.1	- 4.5
Exposed (6 cats)	+ 2.9	-62.3	-65.1	-57.5	-70.0	-73.1	-69.1	-25.6
Mean difference between controls and exposed dogs:	+11.0	-55.4	-73.6	-66.6	-85.4	-57.4	-75.2	-19.1
95% confidence limits:								
Lower	- 3.2	-62.2	-102.5	-88.1	-95.7	-70.5	-98.0	-60.4
Upper	+25.3	-48.6	-44.8	-45.1	-75.1	-44.3	-52.4	+22.1

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The "recovery" on day 105 was not spread evenly over all subjects. Two of the cats which had worn collars still showed very substantial plasma ChE depression in terms of their baseline averages, while three showed essentially complete recovery:

Cat #	Baseline plasma ChE activity	Plasma ChE activity day 91	Activity day 91 in terms of baseline	Plasma ChE activity day 105	Activity day 105 in terms of baseline
0401	1.37	0.40	0.291	0.50	0.365
0402	1.27	0.70	0.551	0.90	0.709
1990	1.37	0.20	0.146	0.60	0.438
4870	1.17	0.40	0.342	1.10	0.940
4875	0.90	0.30	0.333	0.90	1.000
4898	1.07	0.20	0.187	1.20	1.121

There was no indication from the mean measurements of any RBC ChE inhibition.

RBC ChE (from table 5, p. 13):

Percent changes in mean RBC ChE activities from base levels:

	Day 1	Day 3	Day 7	Day 14	Day 30	Day 69	Day 91	Day 105
Controls (4 cats)	-30.5	+ 0.3	+ 5.5	-11.4	+ 3.7	- 5.9	-20.6	-26.5
Treated (6 cats)	-14.9	+ 7.5	+ 8.6	- 9.1	+12.1	+ 2.4	- 8.1	-26.6
Mean difference between controls and exposed cats:	+15.6	+ 7.2	+ 3.1	+ 2.4	+ 8.4	+ 8.3	+12.5	- 0.1
95% C.L.								
Lower	-13.5	-11.1	-10.1	- 9.7	- 3.5	-11.9	- 8.5	-26.9
Upper	+44.6	+25.5	+16.4	+14.4	+20.2	+28.5	+33.5	+26.7

It is noteworthy that the two cats which showed possible symptoms of ChE inhibition (#4875, which vomited on day 14, and #4898 with diarrhea days 20-24) showed on day 14 the least RBC ChE activity both in terms of absolute values and percentages of baseline values:

Cat #	Baseline RBC ChE activity	RBC ChE activity day 14	RBC ChE on day 14 in terms of baseline
0401	2.54	2.46	0.969
0402	2.87	2.58	0.899
1990	2.99	2.83	0.946
4870	2.71	2.83	1.044
4875	2.67	2.21	0.828
4898	3.04	2.34	0.768

Discussion:

The study adequately demonstrates that considerable plasma ChE depression occurs as a result of exposure to 5 collars. Mean plasma ChE activity in the collared cats ran between 14.6 and 44.6% of control values after correction for differences in baseline activities. The

corresponding levels of plasma ChE inhibition are 85.4 and 55.4%.

Half the cats showed essentially complete recovery to baseline levels for plasma ChE 14 days after the collars were removed. Two cats still showed considerable plasma ChE inhibition (although some recovery had occurred) while the remaining animal showed some (but not complete) recovery.

If RBC ChE depression occurred it was comparatively low. The maximum possible RBC ChE depression measured for an individual cat on day 14 was about 23% with respect to baseline values, and this would be reduced by normalizing using control data (controls showed an average RBC ChE depression on 11.4% on day 14 with respect to their baseline values).

Although the two cats showing possible symptoms (vomiting, diarrhea) of ChE inhibition had the lowest RBC ChE activities of the group on day 14, it is doubtful (since most of the RBC ChE activity was still present) that these signs were actually related to exposure.

The major problem that this reviewer has with this study is that it lasted only 3 months, and the label claim (for flea control) is for 13 months. Normally, we have asked for studies lasting either 90 days or half the anticipated exposure period, whichever is longer. In this case, and for this label claim, the duration of the study would have had to be for 6 1/2 months.

Minor problems are that no information is presented as to how the 5 collars were applied to individual cats, and there are no data as to chlorpyrifos release rates (which would give some indication as to amount of active ingredient these cats were exposed to).