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

JAN 25 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Triforine: Evaluation of supplemental data for a chronic feeding toxicity study in dogs

Caswell No. 890AA DP Code: D203580
EPA Chemical No. 107901 MRID No. 432221-02
Submission No. S46505

TO: R. Kendall/V. Dietrich, PM Team 51
Special Review and Re-registration Division (7508W)

FROM: Whang Phang, Ph.D. 1/19/95
Pharmacologist
Tox. Branch II/HED (7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 1/19/95
Section Head
and
Marcia van Gemert, Ph.D. Marcia van Gemert 1/19/95
Branch Chief
Tox. Branch II/HED (7509C)

Introduction

In 1993, Biologic Inc. submitted a chronic toxicity study in dogs with triforine[1]. This study was evaluated. In this study, groups of dogs (4/sex/dose) received triforine at dietary concentrations of 10, 40, 100, and 1000 ppm (0.24, 0.96, 2.48, and 23.05 mg/kg). At 1000 ppm, an increase in the incidence of hemosiderosis in the kupffer cells and in the bone marrow was reported. The study was classified as supplementary because of the inadequate reporting of the histopathology data to support findings of hemosiderosis in bone marrow cells and of erythropoiesis (see the attached Data Evaluation Report (DER) (Tox. Doc. No. 010573)(Attached). The previous reviewer also identified the following shortcomings on the study:

[1] Goburdhun, R. and Greenbough, R.J. (1973) WS24-XX (Triforine): 104-Week oral toxicity study in dogs. Unpublished study conducted by Inveresk Research International, Scotland. Study No. 102AB-437-017. Aug., 19733. Submitted to EPA by Biologic Inc.; EPA MRID No. 423804-10.

1. Stability, concentration (except sporadically), and homogeneity were not reported for the actual dosages.

2. Data on the individual clinical observations were not included.

3. Standard deviations were not included in the summary tables of body weights and food consumption.

4. Animals were 10 months-old.

5. Page 13 of the report was missing.

6. Protocol and Protocol deviations were not submitted.

Evaluation and Discussion

The supplemental data satisfactorily addressed the missing individual animal histopathology data on the incidence of hemosiderosis in bone marrow and the shortcomings 2, 3, and 5 as listed above.

The deficiencies No. 4 and No. 6 could not possibly be rectified, and they did not appear to negatively influence the interpretation of the results of this study. The reason is that this study was conducted prior to the adoption of the current EPA guidelines for toxicity studies (Pesticide Assessment Guidelines: Subdivision F: Human Evaluation: Human and Domestic Animals. EPA-540/9-82-025; October 1982).

The individual animal data of the incidence of hemosiderosis in bone marrow showed that the test article at all dose levels caused hemosiderosis in bone marrow in both male and female dogs. In male dogs the incidence in the 40 and 100 ppm groups was less than that seen in the lowest dose group (10 ppm). Since the incidence of iron stained in the liver and the hemosiderosis seen in bone marrow are related effects, combining these two sets of data seems logical and may provide a clearer understanding of the effects of triforine on the red blood cells (Table 1). The number of animals with both hemosiderosis of bone marrow and the iron stained liver are presented below:

Table 2. The number of animals which had both hemosiderosis of bone marrow and iron stained liver

	0 ppm	10 ppm	40 ppm	100 ppm	1000 ppm
Males	0/4	3/4	1/3	1/3	3/4
Females	0/4	1/4	2/4	2/3	4/4

In males, a dose-related increase in the incidence of hemosiderosis was not present. In females, there appeared to be a dose-related increase in the number of animals with both hemosiderosis of bone marrow and iron stained liver, but one animal in the 100 ppm group was reported to have no slide which introduced uncertainty about the dose-related effect seen in female dogs. It appeared that the test chemical caused hemosiderosis at the lowest dose tested, but this set of data was not robust enough to allow for this conclusion. The data clearly showed that triforine caused hemosiderosis in bone marrow and iron stained liver in male and female dogs at 1000 ppm. At 1000 ppm, a slight increase in erythropoiesis was also reported.

The previous review (DER) established the NOEL for chronic toxicity in dogs as 100 ppm (2.56 mg/kg) (Tox. Doc. No. 010573) (Attached). The supplemental data hinted that the NOEL might be lower than lowest dose tested (10 ppm), but the entire data package was not robust enough to support this conclusion. Therefore, the LEL and the NOEL values for chronic toxicity in dogs remain unchanged (i.e., 1000 and 100 ppm, respectively).

It should be noted that the table on page 2 of the DER for this study contained an error. The superscript, a, should be located on the males of 100 ppm group because the report indicated that a male died on day 225 of the dosing period not a female.

Since this study was conducted prior to the adoption of the current study guidelines, certain deficiencies of this study could not be rectified. However, the results provided a fair understanding of the chronic toxicity of this chemical in dogs. To require a repeat of this study probably would not yield more information than what is available. Based upon these reasons, the study is upgraded to minimum, and it meets the data requirements for a chronic toxicity study in dogs (83-1b).

TABLE I*. Incidence of IRON Deposits in Bone Marrow and Liver

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Group/Dose Level (mg/kg/day)	Animal No.	Description	IRON STAINED Liver	Hemosiderosis in Bone Marrow (1)	Combined Hemosiderosis in bone marrow + Iron Stain Liver	Combined Incidence
			Qualitative Grading	Numerical Grading	Numerical Grading(2)	
1 (0)	1**	Dead	0	0	0	0/4 ♂
	5	Dead	0	0	0	
	9	Dead	0	+	0	
	4	(Moribund) Slight	+	0	0	
	30	Moderate	++	0	0	0/4 ♀
	6	Moderate	++	0	0	
	7	Severe	+++	0	0	
	8	Moderate	++	0	0	
2 (100)	9**	Slight	+	0	0	3/4 ♂
	10	Slight	+	+	+	
	11	Moderate	++	+	+	
	12	Moderate	++	+	+	
	13**	Slight	+	0	0	1/4 ♀
	14	Moderate	++	0	0	
	15	Slight	+	+	+	
	16	Slight	+	0	0	
3 (300)	17**	Severe	+++	0	0	1/3 ♂
	18	Slight	+	No slide	—	
	19	Slight	+	0	0	
	20	Severe	+++	+	+	
	21**	Slight	+	0	0	2/4 ♀
	22	Slight	+	0	0	
	23	Moderate	++	+	+	
	24	Moderate	++	+	+	
4 (1000)	25**	Slight	+	+	+	1/3 ♂
	26	Dead	+	0	0	
	27*	*	*	Dead	—	
	28	Severe Slight	+	+	+	
	29**	Moderate	++	+	+	2/3 ♀
	30	Dead	0	No slide	—	
	31	Slight	+	0	0	
	32	Slight	+	+	+	
5 (1600)	33**	Severe	+++	+	+	3/4 ♂
	34	Moderate	++	+	+	
	35	Moderate	++	+	+	
	36	Slight	+	0	0	
	37**	Severe	+++	+	+	4/4 ♀
	38	Moderate	++	+	+	
	39	Severe	+++	+	+	
	40	Severe	+++	+	+	

NOTE: * No slide available
** Dog #29 died in Week 25

(1): Only Morse Grading applied received a "slight", were labeled as "+" FOR Hemosiderosis in bone marrow (presented on pages 30 to 31 of the MRED No. 43221-01)

+: Data extracted from the REPORT (MRED No. 43221-01).

(2): "+" represent iron deposits were seen in both bone marrow + Liver.

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MRID No. 423804-10

010573

FINAL

Caswell File No. 590AA.

DATA EVALUATION RECORD

TRITICONAZ

Study Type: Chronic Feeding in Dogs

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer: Pia Lindstrom, DVM — Date: 8/24/93

Independent Reviewer: William Mahaffey, Ph.D. — Date: 8/26/93

QA/QC Manager: Sharon Segal, Ph.D. — Date: 8/26/93

Contract Number: 68D10075
Work Assignment Number: 2-68
Clement Number: 193
Project Officer: Caroline Gordon

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Guideline Series 83-1: Chronic Toxicity

EPA Reviewer: Alberto Protzel, Ph.D.
Review Section III, Toxicology Branch II/HED

Signature:
Date: 9/8/93

EPA Section Head: James Rowe, Ph.D.
Review Section III, Toxicology Branch II/HED

Signature: James N. Rowe
Date: 9/9/93

DATA EVALUATION RECORD

STUDY TYPE: Chronic Feeding Study (Dog); Guideline Series 83-1

EPA IDENTIFICATION NUMBERS

TOX CHEM NUMBER: 890 AA

MRID NUMBER: 423804-10

TEST MATERIAL: N,N'-[1,4-piperazine diylbis(2,2,2-trichloro-ethylidene)]-bis-[formamide]

SYNONYMS: WS24-XX; Triforine; CME/4770; Funginex

SPONSOR: Shell Forschung GmbH, Schwabenheim, Germany

STUDY NUMBER: 102AS-437-017

TESTING FACILITY: Inveresk Research International, Musselburgh, Scotland

TITLE OF REPORT: WS24-XX (Triforine) 104 Week Oral Toxicity Study in Dogs

AUTHORS: R. Coburndum and R.J. Greenhough

REPORT ISSUED: August 1973

CONCLUSIONS: Triforine was administered in the diet to groups of four beagle dogs/sex for 104 weeks at dosage levels of 0, 10, 40, 100, or 1000 ppm (approximately 0, 0.24, 0.96, 2.48, and 23.05 mg/kg/day, respectively). Chronic toxicity was reported at 1000 ppm as manifested by [redacted] data [redacted] and minor increases in erythropoiesis. Based on these results, the NOEL and LOEL were 100 and 1000 ppm, respectively (2.56 and 23.05 mg/kg/day).

CORE CLASSIFICATION: Core Supplementary Data. This study does not meet the minimum requirements set forth under EPA FIFRA Guideline Series 83-1 for a chronic toxicity study in dogs. Inadequate reporting of the histopathology data (to support findings of hemosiderosis and increases in erythropoiesis) constitutes a major deficiency in this study.

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A. MATERIALS

Test Compound

Purity: 96.6%
Description: Not reported
Stability: Not reported
Batch number: T 3/70
Received: August 22, 1971

Vehicle: None was used. The test material was administered in the diet.

Test Animals

Species: Dog
Strain: Beagle
Source: C.H. Boehringer Sohn, Rhein, Germany
Age: 10 Months at start of study
Weight: Males--11.0-14.8 kg at start of study
Females--8.7-13.2 kg at start of study

B. STUDY DESIGN

Animal Assignment

Animals were acclimated to laboratory conditions for 8 weeks. During this time, they were vaccinated against distemper, hepatitis, leptospirosis, and parvovirus and were also dewormed twice. Animals were assigned by sex to the following test groups:

Test group	Dosage level (ppm)	Number of Animals Males	Females
I	0	4	4
II	10	4	4
III	40	4	4
IV	100	4	4[a]
V	1000	4	4

[a]One female died on day 223 of the dosing period.

The study authors did not indicate how randomization of the animals was achieved in the test groups.

Animal Husbandry

Animals were housed individually. Environmental conditions were not reported.

Dosage Rationale

Dosages were selected based on the results of two subchronic feeding studies in beagle dogs (study numbers not provided). The exact dosage levels used in the first subchronic study were not reported but the study authors stated that dosages ranging from 3500 to 30,000 ppm were associated with significant reductions in erythrocytes and hematocrit values. At 3500 ppm, reduced hemoglobin values and increased reticulocyte counts were observed. At dosage levels ≥10,000 ppm, increased levels of alkaline phosphatase, bilirubin, and cholesterol were observed. Histopathological changes (steep-like fatty infiltrations in myocardial fibers and liver cells and siderosis of the Kupffer cells) were also observed at all dosage levels. A NOEL was not established.

In the second subchronic study, animals received dietary concentrations of 100, 600, or 3500 ppm. Iron deposits were detected in the spleen, liver, and bone marrow among animals at 600 and 3500 ppm. Significant reductions in hemoglobin, red blood cell count, hematocrit, total proteins, albumin, and globulin and increased relative spleen weight were observed at 3500 ppm. The NOEL was 100 ppm.

Dosage Preparation

Diets were prepared weekly by mixing the test substance with the standard diet and 900 mL of water. This mixture was ground for 20 minutes and then mixed in a Turbulle mixer.

Analyses for test-article concentrations, for the highest dose only, were reported in association with treatment dates of approximately 9 months (5/15/71) and 17 months (1/23/73 and 1/30/73). It is unclear if these dates corresponded to dates of diet preparation or diet analyses. Reported values for 5/15/71, 1/23/73, and 1/30/73 for nominal 1000 ppm were 934, 947, and 947 ppm, respectively. Additionally, test-article concentrations, were analyzed at the end of the study at all treatment levels. Results were reported for prepared diet prior to mixing with meat (nominal: 15, 60, 150, and 1500 ppm) and presumably for prepared diet after mixing with meat (nominal 10, 40, 100, and 1000 ppm). The results for nominal 15, 60, 150, and 1500 ppm (no meat) were 15.3, 61.0, 146.3, and 1468.7 ppm, respectively. The results for nominal 10, 40, 100, and 1000 ppm (with meat) were reported to be 10.4, 39.8, 102.8, and 979.9 ppm, respectively. The authors did not specify if the results obtained after adding the meat were obtained right after mixing with the meat or after 4 weeks of storage. No analytical data for lower doses during treatment were available.

The authors also provided data on samples from a different study (at 15, 60, 150, and 1500 ppm) which was completed in December 1969. In this study, analyses for stability (after 7, 14, 21, and 28 days) revealed mean values from 79% to 103% of nominals with coefficients of variation ranging from 1.2% to 14.9%. Analyses for concentration and homogeneity revealed mean values from 95% to 109% of nominals with coefficients of variation ranging from 1.0% to 12.4%.

Food and Water Consumption

Animals received 300 g of food (200 g standardized powd... ...s-futterfur Hunde 42H' [Jacob ZAHN II] with 100 g 'Leta-Pu... ...ls dried beef [source not stated]) daily throughout the study. ...ter was available ad libitum except during the urine collection.

Statistics

The following procedures were used in analyzing hematology, clinical chemistry, organ weights and body weights: F-max test for homogeneity of variance; ANOVA and Student's t-test for homogeneous data; and Kruskal-Wallis test for heterogeneous data. Organ weight data were also analyzed by covariance with body weight.

Compliance

- A signed Statement of No Data Confidentiality Claims, dated June 25, 1992, was provided.
- A signed Statement, dated June 25, 1992, indicating that the study was not in compliance with EPA GLPs, was provided. The study was completed prior to the date that GLP standards were issued.
- A signed Statement of Quality Assurance, dated March 1, 1990, was provided.

C. RESULTS AND DISCUSSION

Observations

Animals were observed at regular intervals throughout the day for clinical signs and morbidity and twice a day for mortality.

Results: No compound-related mortalities or clinical signs were observed in either sex at any dosage level. One female at 100 ppm died on day 225. Prior to death, a purulent nasal discharge was observed. Necropsy revealed acute pneumonia as the cause of death. Incidental clinical signs, observed in all groups, included liquid feces, vomiting, estrous, salivation, mild trembling, and moderate ataxia. Data for individual clinical signs were not presented.

Body Weights

Body weight data were recorded weekly throughout the study.

Results: No compound-related effects on body weight were observed in either sex at any dosage level. A summary of body weight data at selected intervals is presented in Table 1. Total weight gains for weeks 0-104 were similar in all groups.

Food Consumption and Compound Intake

Food consumption data were recorded daily throughout the study and group mean food consumption was calculated weekly. Food efficiency was not reported.

Results: No compound-related effects on food consumption were observed in either sex at any dosage level. Nearly all males consumed the 300 g of food with which they were provided. A few females consistently failed to eat all of their food. Mean test material intake for weeks 1-104 were 0.23, 0.93, 2.39, and 22.50 mg/kg/day in males and 0.25, 0.99, 2.56, and 23.60 mg/kg/day in females at 10, 40, 100, and 1000 ppm, respectively.

Ophthalmological Examinations

The fundus of the eyes of all animals was examined prior to study initiation and during weeks 13, 26, 52, 78, and 104 using a Kowa Fundus Camera. Prior to examination, pupils were dilated with 1-2 drops of Roche mydriatic agent.

Results: No compound-related lesions were observed in either sex at any dosage level. Incidental findings included one male at 10 ppm with discoloration of the retina at all eye examinations; one female at 100 ppm and one male at 1000 ppm with keratitis with vascularization and pigmentation during week 104; and one male at 1000 ppm with an intra-retinal hemorrhage during week 92.

Hematology and Clinical Chemistry

Hematology and clinical chemistry parameters were determined at the start of the study and during weeks 6, 13, 26, 52, 78, and 104. The study authors did not indicate from where the blood was collected or if the animals were fasted prior to collection. The following checked (X) parameters were determined.

Hematology

X Hematocrit (HCT)*	X Mean cell hemoglobin (MCH)
X Hemoglobin (HGB)*	X Mean cell hemoglobin concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean cell volume
X Leukocyte count (WBC)*	X Thrombocytes*
X Leukocyte differential count	X Prothrombin time
X Lymphocyte count	X Blood sedimentation rate
X Monocyte count	X Osmotic resistance
X Reticulocyte count	X Siderocytes

*Recommended by Subdivision F (October 1982) Guidelines

In addition, femoral bone marrow smears, taken at necropsy, were evaluated.

Results: No significant compound-related effects were observed on any hematological endpoint. Incidental differences from control were

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noted. However, no consistent dosage-, sex-, or time-related pattern was noted for any endpoint. The study authors reported a "...trend for a dose-related reduction in red blood cell parameters (Hb, RBC, and HCT) and associated cell indices in the treated groups during week 6...". The reviewers note, however, that even though there was a generally dose-related decrease in Hb, RBC, and HCT at 6 weeks, especially in females, there were no statistically significant pairwise decreases and all mean values were within 5% of controls with the exception of Hb in females at 100 and 1000 ppm, which decreased by 10% and 14%, respectively. At 6 weeks, the individual animal values for high-dosage females (but not males) were all below pretest values. They were, however, similar to pretest values at week 13 and thereafter. Reticulocyte counts were not effected. A slight increase was noted in treated males at week 13; however, no consistent time- or dosage-related pattern was observed.

Bone marrow smears demonstrated a shift towards erythropoiesis in the granulopoietic-erythropoietic ratio at the high-dosage level in two males and three females. Additionally, the mitotic index in the erythropoietic system of one high-dose female was also above normal limits.

Clinical chemistry

Electrolytes	Other
X Calcium[a]	X Albumin[a]
X Chloride[a]	X Albumin/globulin ratio
Magnesium	X Creatinine[a]
X Inorganic phosphate[a]	X Blood urea nitrogen[a]
X Potassium[a]	X Total cholesterol[a]
X Sodium	X Globulins
X Iron	X Glucose
	X Total bilirubin[a]
Enzymes	X Total protein[a]
	X Carbon dioxide
X Alkaline phosphatase (ALP)	
Cholinesterase	
Creatine kinase[a]	
X Alanine aminotransferase (ALT/SGPT)[a]	
X Aspartate aminotransferase (AST/SGOT)[a]	

[a] Recommended by Subdivision F (October 1982) Guidelines

Dosing: No significant compound-related effects were observed in clinical chemistry parameters. Although bilirubin levels were increased with statistical significance versus controls for weeks 13, 26, and 104 in males and weeks 13 and 104 in females, there was no clear dosage- or time-related trend. Individual values in treated groups were generally within the range of pretest values (0.04-0.11 mg/dL). Although mean cholesterol levels tended to be higher than controls in both males and females receiving 1000 ppm, at most intervals, the increases were slight, rarely were statistically significant, and there was no trend over time. Most values for

individual animals were within the range of pretest values which were 108-224 mg/dL. These increases in bilirubin and cholesterol may have been compound related; however, they were not biologically significant since they were within the normal ranges for this species (as reported by the study authors; no historical control data were submitted). For all other endpoints, no consistent dosage-, sex-, or time-related pattern was noted.

Urinalysis

Urinalysis was performed on all animals prior to treatment and during weeks 6, 13, 26, 52, 78, and 104 . Urine samples were collected over a 24-hour period while the animals were housed in metabolism cages. The following checked (X) parameters were determined.

	Volume[c]		Ketones[a]
	Appearance[c]	X	Acetone
X	pH[c]	X	Bilirubin[c]
X	Specific gravity[c]	X	Sediment (microscopic)
X	Protein	X	Occult blood[c]
X	Glucose[c]		

[a] Recommended by Subdivision F (October 1982) Guidelines

Results: No compound-related effects were observed for any urinalysis endpoint in either sex at any dosage level.

Sacrifice and Pathology

All animals were subjected to gross pathological examination. The method of sacrifice comprised deep anesthesia followed by exsanguination via the carotid arteries. Gross examination consisted of external and internal examinations including a recorded description of all gross lesions. The following checked (X) tissues were preserved in 10% calcium-buffered formalin for histological examination. Additional sections of adrenals, kidneys, heart, and liver were stained with fat red 7 b and the Perlin blue method was used for detection of iron in the heart, lung, liver, kidneys, and spleen. In addition, the double checked (XX) organs were weighed.

Digestive system	Glandular system	Urinary system
X Tongue	XX Adrenals[a]	XX Kidneys
X Salivary gl.[a]	Lacrimal	X Urinary bladder
X Esophagus[a]	X Mammary[a]	XX Testes (with epididymides)
X Stomach[a]	XX Thyroids[a]	XX Prostate gland
X Duodenum[a]	.. Parathyroids[a]	Seminal vesicles
X Jejunum[a]	Harderian	XX Ovaries
X Ileum[a]		Uterus
X Cecum[a]		Cervix
X Colon[a]		Renal pelvis
X Rectum		Vagina
XX Liver[a]		
X Pancreas[a]		
X Gall bladder		

Cardiovascular and hematologic system	Respiratory system
X Aortic arch	X Trachea[a]
XX Heart[a]	XX Lungs[a]
X Bone marrow[a]	Larynx
X Lymph nodes[a]	Pharynx
XX Spleen	
X Thymus	

Nervous system	Other
XX Brain w/stem	X Skeletal muscle[a]
X Peripheral nerve[a]	X Skin[a]
X Spinal cord	X All gross lesions
XX Pituitary[a]	Bone[a]

[a]Recommended by Subdivision F (October 1981) Guidelines.

Females

Organ weights: No compound-related effects on unadjusted organ weights or weights adjusted by covariance with body weights were observed in either sex at any dosage level.

Gross pathology: No compound-related gross findings were observed in either sex at any dosage level. Incidental findings included hematocyst of the mesenteric, hematoma on the edge of the spleen, and parasitic changes.

Microscopic pathology: The authors stated that compound-related histopathological findings were observed at 1000 ppm. The authors stated that the effects were manifested as increased iron content of Kupffer cells in the liver of animals receiving 1000 ppm and siderosis of the bone marrow in the animals at 1000 ppm. However, histopathology data in support of these assertions were not present in the summary tables or in the individual histopathology sheets. Therefore, they can not be verified. A summary of the incidence of Kupffer cell

siderosis, as extracted from a text table, is presented in Table 2. Severity of this effect was increased at 1000 ppm.

D. STUDY AUTHOR'S CONCLUSIONS

Triforine was administered in the diet to beagle dogs at dosage levels of 1, 10, 40, 100, or 1000 ppm for 104 weeks. Chronic toxicity was observed at 1000 ppm as evidenced by increased Kupffer cell hemosiderosis, siderosis of the bone marrow, and minor increases in erythropoiesis. The absence of any consistent reductions in red blood cell parameters indicated that the effect was mild. The NOEL and LOEL were 100 and 1000 ppm, respectively.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

The animals may have tolerated a higher dose of triforine as evidenced by unaffected body weights and no clinical signs. Body weight in all groups of males, including controls, tended to decrease during the first 26 weeks of the study compared to body weight at initiation. This is not unusual for mature dogs (10-months-old). Younger dogs (4-6-months-old) should have been used in this study (guideline recommendation). The reviewers agree with the study authors that no compound-related toxicity was observed in clinical signs, food consumption, body weight, urinalysis, organ weights, and gross findings. Sporadic deviations from control in these parameters were within the normal variation for this species and strain of dogs. Slight effects were observed in selected hematology and clinical chemistry parameters. However, these effects do not appear to be biologically significant and were frequently within the historical control range (as reported by the study authors; historical control data were not provided). Compound-related histopathological findings were observed. Both individual pathology sheets and summary pathology tables were provided. However, these sources (Table 26 and Appendix 33, CBI) did not support the reported findings of increased hemosiderosis in the bone marrow or in the Kupffer cells of the liver. This major reporting deficiency is the basis for classifying the study as Supplementary. In addition, the following deviations from EPA Guidelines were noted:

- Stability, concentration (assays sporadically), and homogeneity were not reported for the actual dosages in this study. Missing Supplemental Data.
- Data on individual clinical observations were not submitted. — O.K.
- Standard deviations were not included in the summary tables of body weights and food consumption. Submitted O.K.
- Animals were 10-months-old at initiation of the study. Therefore, body weights (particularly in males) were near their maximum and body weight gain data in this situation are not a good effect indicator.

- Page 13 of the report is missing.
- Protocol and protocol deviations were not submitted.

GLPs were not completely followed as the study was conducted before this requirement, which may explain why the data reporting is insufficient.

Based on the above deficiencies, this study was classified as Core Supplementary Data.

TABLE 1. Mean Body Weight (kg) at Representative Intervals in Dogs Fed Trifocine for 104 Weeks[a,b]

Study Week	Dietary Level (ppm) 0	10	40	160	1000
			Males		
1	12.1	12.2	12.9	12.0	12.7
13	11.7	12.2	12.5	11.9	12.5
26	12.2	12.3	12.6	12.0	12.9
52	12.6	12.8	13.1	12.5	13.2
78	12.2	12.7	12.8	11.9	13.2
104	12.9	13.3	13.3	12.5	13.9
Mean weight gain Weeks 0-104	0.6	0.1	0.5	-0.7	0.9
			Females		
1	10.8	10.9	10.9	10.9	11.2
13	10.6	10.7	11.1	10.8	11.6
26	11.0	11.1	11.3	11.1	11.8
52	11.9	12.0	11.9	12.2	13.0
78	12.6	12.6	11.5	12.6	13.6
104	13.5	13.6	12.3	12.8	14.2
Mean weight gain Weeks 0-104	2.6	2.9	1.4	1.9	3.1

[a] Data were extracted from Study No. MRID 417-017, Table 2.

[b] Standard deviations were not provided.

TABLE 2. Incidence of Kupffer Cell Hemosiderosis in Dogs Fed Trifocrine for 104 weeks[a,b]

Degree of Siderosis	Dietary level (ppm)				
	0	10	25	100	1000
0	3/8	---	---	---	---
+	1/8	4/8	4/8	2/8[c]	1/8
++	3/8	2/8	2/8	1/8[c]	3/8
+++	1/8	---	2/8	---	4/8

[a]Data were extracted from Study No. 16520-437-017, p. 36

[b]The study authors did not provide individual or summary data to support this nor did they define the various degrees of severity.

[c]Degree of siderosis

[d]Only six animals available; one female died and one infest was not examined.