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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY CASWELL FILE
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MAY 27 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: TB Project No. 8-0597; EPA ID No. 40810-6.
Terbuthylazine: 28-Day Oral and Dermal Toxicity Studies in Rabbits.

Tox Chem No. 125B

FROM: David G. Van Ormer, Ph.D.
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THRU: Marcia van Gemert, Ph.D.
Head, Section III
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and

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5/27/88

The applicant has submitted, at request of TB, the subject studies, each of which is a repeat of a previously submitted study. The data are acceptable, and the reviews are attached.

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Reviewed by: David G. Van Ormer, Ph.D. DVO 05/12/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. M. van Gemert 5/24/88
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 28-day gavage, rabbit TOX. CHEM. NO.: 125B

TEST MATERIAL: Terbuthylazine MRID NO.: 405148-01

SYNONYM: Belclene 329

STUDY NUMBER: 86178

SPONSOR: Ciba-Geigy Corp.

TESTING FACILITY: Research Dept., Pharmaceuticals Div., Ciba-Geigy

TITLE OF REPORT: Tebuthylazine: 28-Day Oral Toxicity Study in Rabbits

AUTHOR: D.M. Schiavo, J.R. Hazelette and J.D. Green

REPORT ISSUED: February 12, 1987

Summary and Conclusions

Gavage of Terbuthylazine to rabbits (5/sex/group) for 29 days at 0, 0.05, 0.5, and 5.0 mg/kg produced no treatment effects in signs, body weight, food consumption, hematology, serum clinical chemistry, ophthalmoscopic or physical examinations, and gross or microscopic pathological evaluation.

NOEL > 5.0 mg/kg for 29 days

Classification: Supplementary

A. MATERIALS:

1. Test compound: Terbuthylazine (FL 860558), of unstated purity and not described. Purity and stability records are on file with the Sponsor. (A review of HRC No. CBG 345/341/83354 states that terbuthylazine is a white powder and that Batch No. EN 16727, in that study, had a purity greater than 98.5%). In the 3 percent cornstarch vehicle the compound was reported stable in 0.002, 0.02, and 0.2% suspensions for 24 hours at room temperature. Analyses at the beginning and end of the study showed that mean concentrations were within ±10% of target. The analysis for homogeneity is reported to show adequate uniformity.

2. Test animals: Male and female New Zealand White rabbits from H.A.R.E., Inc., Hewitt, New Jersey. Age: 13-15 weeks; Weight: 2.15-2.88 kg (males), 2.24-2.66 kg (females). Certified Purina Rabbit Chow® #5325 and tap water were available ad libitum and both were analyzed for contaminants. The animals were identified with ear tags, and given preliminary examinations, including determinations of baseline ophthalmoscopic, hematologic, and biochemical parameters. The animal room was maintain at 60-70°F with relative humidity at 30-70%, and illuminated with 12 hours per day of continuous artificial light.

B. DOSING AND STATISTICAL TREATMENT:

Each of five rabbits of each sex (assigned randomly to dose groups) received the appropriate single daily gavage (2.5 ml/kg) of either 0, 0.05, 0.5 or 5.0 mg/kg, for the respective groups. Dosing was for at least 29 days.

Statistical analyses involved sequential tests for outliers and homogeneity of variances, followed by Dunnett's test for comparison with control. When required, a data transformation or nonparametric test was performed. Where sample sizes were adequate, pathology data were analyzed by Fisher's exact test for each sex, and for both sexes, by computing the "convolved probabilities" (W. Feller, 1950).

The Report lists eight dates on which quality assurance inspections were performed.

C. PARAMETERS AND THEIR RESULTS:

1. Mortality and Clinical Signs (recorded daily)

Two animals died prior to study termination. One male died 3 hours post-dose on day 4, apparently from intratracheal intubation (test material in lungs). One female of the low-dose group died on day 20. Necropsy revealed shrunken spleen, red lungs, and mottled heart. Another female (control) exhibited head tilt, epistaxis, and inappetence. A high-dose female exhibited rales on day 9.

2. Body Weight and Food Consumption

Both body weight and food consumption for all rabbits were recorded on the ninth day prior to start of dosing, then just prior to dosing on day 1, weekly thereafter, and prior to necropsy. Treatment had no effect on either body weight or food consumption in either sex.

3. Physical/auditory (P/A) and Ophthalmoscopic Examinations

P/A examinations were performed on predose day -4 and on day 25, while eye exams were recorded on predose day -6 and on day 26. The examinations were performed on all rabbits and were unremarkable.

4. Hematology

Sampling occurred predose on days -7, -6 (or -4), and on days 25, 26 or 29. Blood samples from all animals were taken from an auricular artery after approximately 17 hours fasting and prior to dosing (during the dosing period). Parameters included the following, as indicated:

|X|	Hematocrit (HCT)*	|X|	Total plasma protein (TP)
|X|	Hemoglobin (HGB)*	|X|	Leukocyte differential count
|X|	Leukocyte count (WBC)*	|X|	Mean corpuscular HGB (MCH)
|X|	Erythrocyte count (RBC)*	|X|	Mean corpuscular HGB conc.(MCHC)
|X|	Platelet count*	|X|	Mean corpuscular volume (MCV)

The reticulocyte count was determined only on the control and high-dose groups. Results show neither significant trends nor mean values which are outside the stated laboratory historical controls.

5. Clinical Chemistry.

Sampling was by the same protocol as for hematology. Analytes determined are indicated as follows:

	Electrolytes:		Other:
|X|	Calcium*	|X|	Albumin*
|X|	Chloride*	|X|	Blood creatinine*
|X|	Phosphorous*	|X|	Blood urea nitrogen*
|X|	Potassium*	| |	Cholesterol*
|X|	Sodium*	| |	Globulins
	Enzymes	|X|	Glucose*
|X|	Alkaline phosphatase	|X|	Total Bilirubin*
| |	Cholinesterase	|X|	Total Protein*
| |	Creatinine phosphokinase*	| |	Triglycerides
| |	Lactic acid dehydrogenase		
|X|	Serum alanine aminotransferase (also SGPT)*		
|X|	Serum aspartate aminotransferase (also SGOT)*		
|X|	Gamma-glutamyl transferase		

Mean values of the results show no significant tends or data points. Urinalysis was not performed.

6. Necropsy

All rabbits, including the two succumbing prematurely, were subjected to necropsy after at least 29 days of dosing. The tissues indicated below were preserved. The double X indicates organs which also were weighed from each animal. Paired organs were weighed as pairs.

Digestive System	Cardiaovasc./Hemat.	Neurologic
| | Tongue	| | Aorta*	|XX Brain* w/brainstem)
| | Salivary glands*	|XX Heart*	| | Periph. nerve*
| | Esophagus*	| | Bone marrow*	| | Spinal cord (3 levels)*
| | Stomach*	|XX Lymph nodes*	|XX Pituitary*
| | Duodenum*	| | axillary & mesenteric	| | Eyes (optic n.)*
| | Jejunum*	|XX Spleen*	Glandular
| | Ileum*	|XX Thymus*	|XX Adrenals*
| | Cecum*	Urogenital	| | Lacrimal gland
| | Colon*	|XX Kidneys*	| | Mammary gland*
| | Rectum*	| | Urinary bladder*	| | Parathyroids*
|XX Liver*	|XX Testes*	| | Thyroids*
| | Gall bladder*	| | Epididymides	| Other
| | Pancreas*	| | Prostate	| | Bone*
Respiratory	| | Seminal vesicle	| | Skeletal muscle*
| | Trachea*	|XX Ovaries	| | Skin
| | Lung*	| | Uterus*	|X| All gross lesions and masses

Microscopic examinations (all animals) were performed on axillary and mesenteric lymph nodes, kidney, liver, ovary, testis, spleen, thymus, and any organs showing gross lesions or size change.

The organ weights and their ratios to either body weight or brain weight are without treatment effect. Neither do tabulations of gross pathology or histopathology show treatment effect. The Pathology Report states for the two intercurrent deaths that the mid-dose male was judged to have succumbed to improper intubation, and the low-dose female died from undetermined causes.

CASWELL FILE

Reviewed by: David G. Van Ormer, Ph.D. DVO 05-18-88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769C) Marcia Gemert 5/24/88

DATA EVALUATION REPORT

STUDY TYPE: 28-day dermal toxicity-rabbit TOX. CHEM. NO.: 125B

TEST MATERIAL: Terbuthylazine MRID NO.: 405148-02

SYNONYM: Belclene 329

STUDY NUMBER: 2-052-02

SPONSOR: Agricultural Division, Ciba-Geigy Crop.

TESTING FACILITY: Pharmaceuticals Division, Ciba-Geigy Corp.

TITLE OF REPORT: 28-Day Dermal Toxicity Study in Rabbits

AUTHOR: D.M. Schiavo (Director), J.R. Hazelette and J.D. Green

REPORT ISSUED: March 02, 1987

Summary and Conclusions:

The intact skin of male and female rabbits (5/sex/group) received application of Terbuthylazine (under occlusion) at dosages of 0, 0.05, 0.5, or 500 mg/kg/day for six hours during 29 consecutive days. The controls received purified water. The death of one high-dose female was attributed to treatment. Signs at high dose, confined largely to females, included decreased frequency of feces, muscle wasting, hypothermia, and cachexia. Males also produced fewer feces. Animals at high dose throughout the study exhibited significant weight loss and reduced body weight gain. Food consumption at high dose was decreased at all 4 weeks in both sexes. Lack of treatment effect is our interpretation for data on hematology, clinical chemistry, organ weights/ratios, and gross/microscopic pathology (except for the one death at high dose).

Most animals at high dose showed grade 1 (slight) erythema.

NOEL = 0.5 mg/kg/day (29 days)
LEL = 500.0 mg/kg/day
Fewer feces, muscle wasting, hypothermia, cachexia, weight loss, and reduced food consumption. One female death

Classification: Guideline

A. MATERIALS

1. Test Material

Terbuthylazine (FL 860558) a neat powder of unstated purity was stored at room temperature. Data on purity and stability are on file with the Sponsor, and are not presented in the Study. (A prior TB review states that Terbuthylazine from Batch No. EN 16727 is a white powder of purity greater than 98.5%). The test material was weighed undiluted and (just prior to application) moistened with purified tap water to make a paste.

2. Test Animals

The male and female New Zealand White rabbits, age approx. 13 weeks, were from H.A.R.E., Inc., Hewitt, N.J. Weight for males was 2.0 to 2.6 kg, and for females was 2.2 to 2.7 kg. Certified Purina Rabbit Chow^{R} #5325 (contaminant levels specified on label) and tap water were available ad libitum. Both feed and water were periodically analyzed for contaminants. The animals were identified with ear tags, and given preliminary assessment of "baseline values" for body weights and for ophthalmoscopic, hematologic, and biochemical parameters. The animal room was maintained at 60-70^{o}F with relative humidity at 30-70%, and illumination on a 12/12-hour light/dark cycle.

B. TREATMENT SCHEDULE

The flank and back of each rabbit was clipped free of hair prior to dosing. Application of Terbuthylazine was for a daily six-hour period, under an occlusive gauze dressing, to approximately 130 cm^{2} of intact skin. This area represented 5 to 10% of total body surface (to accord with the SOP of the SEF). Each animal then was fitted with an Elizabethan collar. After the six-hour dosing period each treatment site was gently washed with tap water.

Dosages of 0, 0.05, 0.5, and 500.0 mg/kg were applied daily to respective dose groups of 5 rabbits per sex. Dosage was calculated from the most recent body weight.

C. PARAMETERS and STATISTICS

Parameters were recorded for each rabbit except where noted. Appearance and behavior were observed daily during the predose period, also prior to and within 30 minutes of each dosing, and prior to sacrifice.

Body weights were recorded predose on test day -8, just prior to dosing on test day 1, then weekly thereafter, and prior to necropsy. Food consumption was measured daily from test day -8 to 1, and weekly thereafter. The animals received physical, auditory, and eye examinations on test day -6 or -7 and on day 28 or 29. Dermal observations (Draize et al., 1944; see p. 24 attached) were recorded prior to dosing and approximately 30 minutes after the end of each dosing period (after washing) and prior to sacrifice.

Blood samples for hematology and clinical chemistry were obtained once on test days -10, -9, or -7, and on test days 28, 29, 30, or 31.

Hematology parameters were as follows:

|X|	Hematocrit (HCT)*	| |	Total plasma protein (TP)
|X|	Hemoglobin (HGB)*	|X|	Leukocyte differential count
|X|	Leukocyte count (WBC)*	| |	Mean corpuscular HGB (MCH)
|X|	Erythrocyte count (RBC)*	| |	Mean corpuscular HGB conc. (MCHC)
|X|	Platelet count*	| |	Mean corpuscular volume (MCV)
|X|	Prothrombin time	|X|	Reticulocyte count

Reticulocytes were recorded for the control and high-dose animals only.

Clinical Chemistry parameters recorded are checked (X) as follows:

	Electrolytes:		Other:
|X|	Calcium*	|X|	Albumin*
|X|	Chloride*	|X|	Blood creatinine*
| |	Magnesium*	|X|	Blood urea nitrogen*
|X|	Phosphorus*	| |	Cholesterol*
|X|	Potassium*	| |	Globulins
|X|	Sodium*	|X|	Glucose*
	Enzymes	|X|	Total Bilirubin*
|X|	Alkaline phosphatase	|X|	Total Protein*
| |	Cholinesterase	| |	Triglycerides
| |	Creatinine phosphokinase*		
| |	Lactic acid dehydrogenase		
|X|	Serum alanine aminotransferase (also SGPT)*		
|X|	Serum aspartate aminotransferase (also SGOT)*		
|X|	Gamma-glutamyl Transferase		

Urinalysis was not performed.

Necropsy after exsanguination was conducted on all surviving rabbits on day 30 or 31, and included one high-dose rabbit succumbing during study. The tissues checked (X) below were preserved for histological examination. The

organs indicated by (XX) were also weighed.

Digestive system	Cardiovasc./Hemat.	Neurologic
| | Tongue	| | Aorta*	|XX Brain*(w/brainstem)
| | Salivary glands*	|XX Heart*	| | Periph. nerve*
| | Esophagus*	| | Bone marrow*	| | Spinal cord (3 levels)*
| | Stomach*	|XX Lymph nodes* -axillary	|XX Pituitary*
| | Duodenum*	|XX Spleen* & mesenteric	| | Eyes (optic n.)*
| | Jejunum*	|XX Thymus*	Glandular
| | Ileum*	Urogenital	|XX Adrenals*
| | Cecum*	|XX Kidneys*	| | Lacrimal gland
| | Colon*	| | Urinary bladder*	| | Mammary gland*
| | Rectum*	|XX Testes*	| | Parathyroids*
|XX Liver*	| | Epididymides	| | Thyroids*
| | Gall bladder*	| | Prostate	Other
| | Pancreas*	| | Seminal vesicle	| | Bone*
Respiratory	|XX Ovaries	| | Skeletal muscle*
| | Trachea*	| | Uterus*	|X| Skin
| | Lung*		|X| All gross lesions and masses

A rectangular portion (2.5 cm X 7.5 cm) of treated skin, approximately 15 cm from the occiput, was removed from across the midline of the back. In addition, untreated sections of skin from the left thigh were examined.

Microscopic examinations were conducted on each animal at necropsy, including the high-dose animal succumbing before termination. Histopathologically examined tissues were as follows: axillary and mesenteric lymph nodes, kidney, liver, ovary, testis, spleen, thymus, skin (abdomen, back, and thigh), and any organs showing gross lesions.

Statistical analysis included a sequence of tests for outliers (D.M. Hawkins, 1980) and homogeneity of variances, followed by Dunnett's test. When normal distribution did not apply, data transformations or nonparametric tests were utilized. If sample sizes were adequate, data from microscopically investigated animals were analyzed (for each sex) by Fisher's exact test and for both sexes by computing convolved probabilities (w. Feller, 1950).

D. RESULTS

The Report states that the baseline data showed that all animals were healthy and suitable for use.

1. Mortality and Signs

One-high dose female was found dead on test day 29. Premonitory signs included few or no feces (from day 22),

muscle wasting (from day 17), lethargy, hypothermia, or cachexia. The same clinical signs were noted in all other high-dose females. Sporadic observations of few feces (in both sexes) were observed on days 5 to 18. One high-dose female showed red anal discharge and soft feces on days 5 to 8.

2. Dermal Effects

The Report states that 3/5 high-dose males and all high-dose females transiently exhibited slight erythema (Draize grade 1) on days 5 to 18.

3. Physical/Auditory and Eye Examinations.

In all three examination categories the results were unremarkable, according to the Report.

4. Body Weight and Food Consumption

In both sexes at high dose, after each week of the study, the body weight and body weight gain were significantly less than control ($p<0.05$ or 0.01 for males; $p< 0.01$ for females).

Food consumption at high dose is decreased from control values at all 4 weeks in both sexes. The decrease is very marked at week 1, and statistically significant ($p<0.01$) in females at weeks 2 and 3. The mid-dose in females also shows a significant decrease ($p<0.05$) at week 1. The pattern of decreased food consumption suggests a confounding with unpalatability at top dose.

5. Hematology and Clinical Chemistry

Statistically low values ($p<0.05$, 0.01) for eosinophils in males at low and mid-dose on day 27 are not accompanied by neutropenia. A dose-related decrease (not significant) also appears in the females at day 9, but not at day 27. In the absence of a wider agranulocytosis, no treatment effect is apparent.

Among clinical chemistry parameters, in males on day 27 the creatinine and glucose values are elevated ($p<0.05$, 0.01) at top dose, without dose relationship. The day-9 value in high-dose females is also elevated, but not statistically. Footnotes in the tables indicate that the day-9 values for females were analyzed with F-tests, while the day-27 values for males were analyzed with a two-tailed Dunnett t-test. No treatment effects are apparent, however.

6. Organ Weights

No treatment effect is present. Absolute thymus weights in low-dose males ($p<0.05$) and relative brain weights in high-dose females ($p<0.05$) are without dose relationship.

7. Gross and Histopathology

A separate Pathology Report shows by tabulation and statement that there were neither gross nor microscopic alterations attributable to Terbuthylazine. Among females (all doses combined) 6/14 (vs. zero in controls) exhibited lymphocytic inflammation in abdominal skin. This effect is not dose-related, and is possibly due to the occlusive dressing. The high-dose female found dead on day 29 showed red liver with endogenous pigment, smaller-than-normal spleen, and lymph nodes outsized or darkened. The study attributes this death to a compound effect.

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