6-17-83

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

002994

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 40810-L. Belclene 319 (terbuthylazine, GS-13529). Reviews of studies and interim reports submitted.

Tox Chem No. 125B

TO: A.E. Castillo/Laird
Product Team #32
Registration Division (TS-767)

THRU: Ed Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

6/17/83

The following studies on terbuthylazine (and/or on the formulation Belclene 319, as indicated) are not on file and are required for registration of Belclene 319 as a swimming pool algicide:

1. Acute oral toxicity study (formulation).
2. Dermal irritation study (formulation).
3. Eye irritation study (formulation).
4. Skin sensitization studies (one each, on technical material and formulation).
5. Ninety-day dermal studies (one each, on technical material and formulation).
6. Teratogenicity studies (two mammalian species).
7. Mutagenicity studies (a battery of tests to satisfy Federal Register 47:227, November 24, 1982).
8. Life-time feeding study (rodent).
9. Oncogenicity studies (two rodents).

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Page _____ is not included in this copy.

Pages 2 through 5 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

X Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

$$\frac{(\frac{1}{6} + 0) + (\frac{1}{6} + 0) + (0 + 0) + (0 + 0)}{4} = \frac{1}{3}/4 = 0.1$$

(Not an irritant)

Conclusion: Minimum Data; Tox Category IV.

Due to the low irritation as reported, the test falls within NAS-recommendations.

Eye Irritation Test with GS-13529 Technical in Albino Rabbits. IBT No. 601-06531; April 21, 1975. Accession No. 247618

Tox Chem No. 125B

The test material was identified as GS-13529 Technical, Batch No. FL-15889. The animals were young Albino rabbits (New Zealand strain) of unstated sex. Undiluted test material (100 mg) was instilled into the conjunctival sac of the right eye of each of six rabbits. For three of the rabbits a 30-second exposure was followed by a one-minute eye-wash with distilled water. For the other three rabbits, after the same exposure, the eyes were not washed. Scoring of effects on the cornea, iris, and conjunctiva was by the Draize Method at 24, 48, and 72 hours, and at 7 days. The left eye served as control.

For washed eyes, all tabulated values were zero, yielding a total score of 0/110, not an irritant. For unwashed eyes all tabulated values at each period (for each of the three tissues) were also zero, with exception of the score for conjunctiva, which showed redness (grade 1) in one rabbit at 24 and 48 hours. Thus, the average overall score for unwashed eyes (three rabbits) was listed as 0.7/110 (not an irritant).

Conclusion: Minimum Data; Tox Category IV; satisfies NAS-recommendations.

It may be noted (memo, R. B. Jaeger, October 23, 1974) that the score for the 80% WP formulation of terbuthylazine (undiluted 100 mg, rabbits) is reported as 60/110 (severely irritating, corneal opacity reversible in 7 days) and that the score for a 7.0% (w/v, H_2O) suspension of this formulation is 11.6/110 (minimally irritating).

Two-Year Chronic Oral Toxicity Study with GS-13529 Technical in Albino Rats. IBT No. B8211, August 2, 1972. Accession No. 247619.

Tox Chem No. 125B

This study has been classified as invalid (by the Canadian Task Force for Re-assessment of Chemical Safety, April 4, 1979) due to missing data, apparent use of "extra" animals, and too little histopathology. The report staces that the only compound-related effects were reductions in body weight gains, noted in all test groups after the first three months and persisting throughout the study. Indeed, at the end of study the weight gain at high dose (400 ppm) for either sex was less than half that for controls. Overall weight gains at low dose (100 ppm) were depressed 24% and 36% for males and females, respectively. Food consumption, however, was not significantly altered at either the 12-month or 24-month period.

Hematological parameters were tabulated for high dose and controls. Total leukocyte count was depressed at 24 months, particularly in males. Lymphocyte count was slightly increased at termination (24 months). Among agranulocytes the neutrophil count was slightly decreased at termination, but the basophil count was zero in both tabulated groups throughout, and the eosinophil counts were increased at termination. Thus, there was no indication of agranulocytosis. Clinical chemistry and urinalysis data are unremarkable.

Inspection of organ weights and organ/body-weight ratios, after three-month sacrifice, shows no clear evidence (in the face of severe general weight loss) for organ-specific weight alterations.

Histopathology of animals (10 of each sex at control and high dose) sacrificed at three months showed mainly the respiratory conditions not unusual in the albino rat, occurring about equally in controls and treated animals.

The report states that gross pathologic findings among test animals at final sacrifice were not significantly different from findings in control animals.

At final sacrifice the liver-to-body weight ratio is significantly elevated (compared to controls) in both sexes at high dose. and in females at mid-dose. This elevation may indicate a compound-related effect at high dose (and at mid-dose in females) even in the face of decreased absolute liver weights, due to the very severe general weight loss (C. S. Weil, 1970).

On the other hand, the elevated kidney ratios in females at the two high doses provide less evidence for compound effect due to the fact that this kidney-ratio elevation has frequently occurred in food-deprived females (Robbins, 1968, in C. S. Weil, 1970). Likewise, both hypertrophy of the testes and increased heart-to-body weight ratio in the females may

result from a general metabolic effect rather than from a specific organ effect (Robbins, 1968).

Brain weights at all doses in both sexes (except low dose males) are significantly elevated, as are the brain-to-body weight ratios. The significantly elevated absolute brain weight is unusual and may be compound related, as suggested in the validation review (but not mentioned in the study).

The report states that general histopathologic examination showed no lesions attributable to test material. All observed lesions are stated as naturally occurring due to disease or aging. In regard to elevated brain weights, only one animal (female at high dose) was tabulated as presenting focal encephalitis (nosematosis).

Gross examination for tumor formation was conducted (according to the report) on all animals--those succumbing before termination and those terminally sacrificed. All tumors were submitted for histopathologic examination. The observed tumors are stated to be normal for a random population of rats, and not compound related.

Tabulation shows four malignant tumors, distributed as follows: controls, zero; low dose, adenocarcinoma (abdomen); mid dose, metastatic adenocarcinoma (thymus) and adenocarcinoma (mammary); high dose, adenocarcinoma (mammary).

(Previously recorded on one-liner sheet.)

One-year Status Report on the Ongoing Lifetime Carcinogenicity and Chronic Toxicity Study with GS 13529 Technical in Rats. Ciba-Geigy, Ltd. Proj. No. 785196. Aug. 7, 1981. Accession No. 247620

Tox Chem No. 125B

The test material was GS-13529 technical (98%), Lot No. 590305. Rats were of the strain Tif: RAIF (SPF)* from Ciba-Geigy, with initial mean group body weight of 95g (males) and 92g (females) at age 4 weeks. Animals were assigned to dose groups by IBM-generated random numbers. Animals were housed in groups of five in Macrolon cages with granulated soft wood bedding. The animal room was air conditioned with controlled temperature, humidity, and light cycle. Diet (Nafag No. 890 Tox) and tap water were ad lib. The feed was pelletized after mixing with test material. (Controls were fed pellets without test material.) Mean concentration of active ingredient in diet ranged 72-11[illegible]% of the added amount (by chemical analysis).

Dietary dose levels were 0, 30, 150, and 750 ppm administered to respective dosage groups, each containing 80 animals of each sex. Calculation from chemical analysis showed that mean dosages were 1.6, 8.7, and 51.2 mg/kg BW/day (males); and 1.7, 9.4, and 60.5 mg/kg BW/day (females).

Mortality and symptoms were observed daily, and body weight and food consumption were recorded weekly. Mean food conversion was calculated as

$$\frac{\text{weekly food consumption}}{\text{midweek body weight}} \times 1000 = \text{g feed/kg BW/day.}$$

Hematology and chemistry samples were collected from ten rats of each sex at weeks 17, 26, and 52. The protocol included 12 hematological parameters (plus differential count), 15 chemistries, and 11 urinalysis parameters.

At 52 weeks, 10 animals per sex per dose group were subjected to detailed autopsy. Organ weights were obtained for liver, adrenals, brain, heart, kidneys, and gonads. These and 30 other organs and tissues (plus any tissue showing macroscopic change) were preserved in 10% neutral formalin. Liver was the only organ sectioned and stained for microsopic examination.

Results

Body weights in all groups were depressed from controls at all weeks (in dose-dependent fashion) from close to initiation to week 54. The differences from controls are tabulated as statistically significant (0.01) in all doses (both sexes) at all weeks, except the first 4 to 6 weeks at low dose. Mean food consumption in both sexes was reduced from control values, more strikingly in the males, and with dose-dependency and trend significance in both sexes. The upper two dose groups (both sexes) showed elevated food conversion values throughout the study.

Mortality data show no dose relation. Survival time, however, is tabulated as significantly increased in males (with dose dependence), and slightly prolonged in females. No clinical symptoms or signs were reported.

Hematological Parameters at 52 Weeks

(The following trends were not tabulated with reference to statistical significance.)

Males (p. 117)

Erythrocytes elevated at top two doses.
Hemoglobin elevated at top dose.
Hematocrit elevated at top two doses.
MCV lowered at top two doses.
Reticulocytes lowered at top two doses.
*Thrombocytes elevated at top dose.
*Leucocytes decreased at top two doses, elevated at low dose.
Segmented neutrophils decreased at top two doses.
*Lymphocytes increased at top two doses.
Prothrombin time increased at all doses.
Partial thromboplastin time elevated at top two doses.

Females (p. 128)

Erythrocytes decreased at top two doses.
MCV elevated at top dose.
*Thrombocytes elevated at top dose.
*Leucocytes decreased at top dose.
*Lymphocytes elevated at top dose.

At this point we do not remark upon the above hematological alterations. Comparison may be performed with ongoing or future studies. The asterisks indicate trends in both sexes.

Blood Chemistry at 52 Weeks

There was an increase (above physiological limits) in urea nitrogen at low and top dose in males and at mid and top dose in females.

Urinalysis at 52 Weeks

The report states that for a few values of urine volume and specific gravity there was a dose-related decrease in both sexes at the top two doses. The report interprets the effect as compound-related.

Organ-to-Body Weight Ratios

Decreases in absolute liver weight are concomitant with statistically increased liver-to-BW ratio at top dose in males. Also, for the kidney the decreasing values of absolute organ weight in males may partially reflect compound effect, as the kidney-to-BW ratio is statistically elevated at the top two doses. In females the statistically decreased heart weight is at top dose, as is the statistically elevated heart-to-BW ratio, and there is definite dose relation. Relative liver weights for females are statistically increased at the top two doses, with a decrease in absolute weight at the top

dose. The possibility cannot be excluded that the above trends in O/BW values indicate compound effect.

Increases in female relative kidney weights, however, may largely reflect the effect of dietary depletion (Robbins, 1968, in C.S. Weil, 1970). Likewise the male heart-to-body weight ratios, significantly increased at the top two doses (in combination with statistically decreased absolute weights) may partially result from poor feed conversion (Robbins).

Brain weights are somewhat elevated at all doses in males, with statistically elevated brain-to-BW ratios at the top two doses.

The scheduled interim sacrifice of 10 animals per sex per group, when added to the several moribund sacrifices (1, 1, 4, and 7 per dose group) resulted in histopathological and gross examination of 10 to 15 animals per sex per dose group. The summary tables show that for controls and the two low-dose groups one male in each group presented a single tumor. In the high dose one of each sex is reported as tumor bearing, again with only one tumor. Among treatment groups there are reported two benign testicular interstitial cell tumors, one mammary carcinoma, and one subcutaneous fibroma. There is no appearance of dose-relation in the tumor data.

The report does not present summary tabulation of histopathological findings other than tumors. All other microscopical changes are stated as attributable to naturally occurring conditions in rats. To the reviewer there appeared to be no dose-relation to any of the individually tabulated findings.

Reviewer's Comments

This study does not exhibit a NOEL for reasons as follows:

1. Decreased body-weight gain (both sexes, all doses).

2. Increased BUN at low dose in males, and in both sexes at the other two doses.

Increased organ-to-body weight ratios indicate the possibility of compound effects as follows:

Liver: top dose in males, top two doses in females.
Kidney: top two doses in males.

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Heart: top dose in females.
Brain: top two doses in males (with somewhat elevated absolute weights at all doses).

The reported data on tumors and other histopathological findings appear unremarkable.

Summary tables for clinical studies (and tables for individuals) are too small for accurate examination. Tables showing statistically significant values, although readable, do not display trends and do not allow for examination of correlations.

The registrant discontinued this lifetime study at the end of the first year.

One-year Status Report on the Ongoing Lifetime Carcinogenicity and Chronic Toxicity Study with GS-13529 Technical in Rats. Ciba-Geigy Proj. No. 791229, Oct. 8, 1981. Accession No. 247620. Tox Chem No. 125B.

The test material, Tektamer 38 (GS-13529), technical, was from Lot 590305, 98% purity. The SPF rats are identified as the F_3-hybrid of RII 1/Tif x RII 2/Tif. Body weights ranged 72 to 75 g at approximately three weeks of age. The animals were housed in groups of five in Macrolon cages type 4, with granulated softwood bedding. Controlled conditions included temperature (22°C), relative humidity (55%), ventilation (16 changes/hour), and photoperiod (10 hours/day). Test material was homogeneously mixed with feed (Nafag No. 890 Tox) and then pelletized. Analysis showed that the active ingredient in the feed ranged 70 to 108% of the added amount. Tap water and feed were available ad lib.

Observations included mortality and symptoms (daily) and body weight and food consumption (weekly). Mean food conversion was calculated as

$$\frac{\text{weekly feed consumption (g)}}{\text{midweek body weight (g)}} \times \frac{1000}{7} = \text{g feed/kg BW/day}$$

The protocol involved dietary dosing to 80 males and 80 females per dose group at concentrations of 0, 6, and 30 mg/kg feed. Chemical analysis determined average dosages at 0.43 and 1.94 mg/kg BW/day for males, and 0.43 and 1.92 mg/kg BW/day for females.

Separate groups of 10 animals/sex/group were utilized for hematological evaluation and for biochem/urinalysis. (The same 20 animals per sex/group were scheduled for 24-month sacrifice.) Groups of 50 animals/sex/dose group were maintained for oncogenic evaluation. Interim sacrifice at month 12 was performed on 10 animals/sex/group.

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Blood samples were collected at weeks 26 and 52 with sampling between 8:00 and 9:00 AM. Food was withheld for 20 hours prior to blood chemistry sampling. Blood was removed from the orbital sinus without anesthesia. Food and water were withheld during urine collection. Ten hematological parameters were measured, in addition to leucocyte counts. Clinical chemistry evaluation comprised 14 parameters, and urinalyses included 11 parameters.

Autopsy was performed at 52 weeks on 10 animals/sex/dose group, bled under ether anesthesia. Organ weights were obtained for liver, adrenals, brain, heart, kidneys, and gonads. These and 33 other tissues (plus any showing macroscopic change) were preserved in 10% neutral formalin. The slides were stained with H and E.

Statistical analysis utilized non-parametric methods, including the Jonckheere trend test.

Results

Body weight for each sex at low dose was very slightly (but not significantly) depressed from controls starting at about week 20. Weight depression was quite definite (and significant at high dose in both sexes from about week 10. At various measurements during the first 20 weeks the food consumption at high dose (both sexes) was significantly depressed. The data appear to show that the low dose food consumption trended in the same direction as the high dose during the same period of the study. Food conversion data showed no trends.

Mortality is noted for two animals each, in the low and high dose groups, and one among controls. The report states that no symptoms or signs were observed.

Hematology data at 52 weeks indicated the possibility of mild compound-related effects. The alterations itemized below differ significantly from controls at a significance level of approximately $p < 0.1$ (or more significant) by one-sided t-test. Effects indicated by asterisk occurred with the same trend in Ciba-Geigy Project No. 785196 (One-Year Status Report on GS-13529 in Rats).

Males

Hemoglobin decreased at top dose.
Reticulocytes increased at low dose and somewhat at high dose.
*Leucocytes increased at low dose.
*Neutrophils and basophils somewhat reduced at top dose.
Nucleated RBC-normoblasts increased (over three fold) at low dose.

Females

*Erythrocytes decreased at top dose.
Leucocytes elevated at high dose.
*Mean corpuscular volume elevated at high dose.
Neutrophils elevated at high dose.
Lymphocytes depressed at both doses.
Nucleated RCB-normoblasts significantly elevated in some individuals at high dose.

We note the elevations of immature RCB's (reticulocytes and normoblasts) particularly in males at low dose. These effects are compatible with decreased erythrocytes (females, top dose), elevated MCV (females, top dose), and decreased hemoglobin (males, top dose). There is also a suggestion of agranulocytosis in males at top dose.

Clinical chemistry parameters at 52 weeks show the following alterations, reported at a significance level of approximately $p < 0.1$ (or more significant, by one-sided t-test). The trends in BUN (both sexes) were reported in Ciba-Geigy Project No. 785196 (One-Year Status Report on GS-13529 in Rats).

Males

Increased sodium at high dose.
Increased potassium at high dose (dose related).
Slight decrease (not significant) in alkaline phosphatase at high dose (compatible with indicators of anemia).

Increased urea nitrogen at both doses.

Females

Increased potassium at high dose (dose related).
Increased GOT in a few individuals at low dose (slight increase at high dose).
Increased LDH at both doses in several individuals (means are dose related).
Increased glucose at low dose.
Increased urea nitrogen at both doses (dose related).
Increased cholesterol at both doses.

The elevated BUN (both sexes) is compatible with the anuria reported in Project No. 785196.

Urine pH was significantly elevated above controls at high dose in males.

Among organ weights and O/BW ratios there appear increases ($p < 0.1$, one-sided t-test) in adrenal ratios at both doses in males; the absolute weights are also elevated at both doses.

Primary tumor summary indicates that among the high dose there was one nephroblastoma (male), one subcutaneous tissue fibroma (female), and one mammary carcinoma (female). Controls presented one pituitary adenoma and one malignant thymoma, both in females.

Tabulation of microscopical findings shows that 6/22 at high dose (sexes combined) showed inflammatory cell infiltration in the lung vs. zero in controls. In the heart and liver there also was a tendency for elevated inflammatory cell infiltration at high dose. Lymphocytic infiltration in the liver at low dose was three-fold larger than control incidence. The pituitaries presented three developmental cysts at low dose (sexes combined) and two at high dose vs. zero in controls.

Reviewer's Comments

We note the elevation of immature erythrocytes in males at low dose. At top dose we have pointed to decreases in erythrocytes and hemoglobin, and elevated mean corpuscular volume. All of these hematological parameters are compatible. There is also a suggestion of agranulocytosis in males at top dose.

At both doses in both sexes there is an increased blood urea nitrogen, compatible with the anuria reported in Ciba-Geigy Project No. 785196.

Adrenal weights and their O/BW ratios are somewhat elevated in males at both doses, a possible indicator of stress.

Primary tumor data appear unremarkable.

Microscopical tabulation showed inflammatory-cell infiltration at high dose, reported as exceeding control incidence in three organs. Developmental cysts of the pituitary occurred at both doses (3/22, 2/22: low, high) but were absent in controls.

The registrant should be advised that tables showing means and dispersions for clinical data are entirely inadequate with regard to readability. Proper legibility is not possible even with a magnifying glass! Tables summarizing statistically significant values do not display trends and do not allow for examination of correlations.

Tox Branch will make further comment on this two-year study after reviewing the final report. At this stage in the study the protocol appears adequate for a combined chronic/oncogenicity study. This study provides data for dose levels of 0.4 and 1.9 mg/kg/day. We accept data from the one-year rat interim report (Ciba-Geigy No. 735196) as providing one-year data for the higher doses 9 and 55 mg/kg/day.

Three-Generation Reproduction Study with GS-13529 Technical in Albino Rats. Feb. 16, 1972. IBT No. B8272 (valid). Access. No. 247619. Tox Chem No. 125B.

The test material is described as GS-13529 Technical. The test animals were weanling Charles River Albino rats. The F_0 generation consisted of three dosage groups and a control group, each consisting of 8 males and 16 females. From the second litters of each dose group (F_{1b} and F_{2b}), 8 males and 16 females were selected at weaning as parents for the succeeding generation(s). The study terminated at the weaning of the F_{3b} litters. Animals of each generation (from weaning to sacrifice after second litters were weaned) consumed diets (Special Mix Mouse Chow, Ralston Purina) containing essentially two dosage levels: 50 and 100 ppm.

A second dose group of 100 ppm was carried through the treatment of the F_0 animals only. (The report does not specify the age at which treatment of the F_0 generation commenced). During the second week the dose for one of the 100 ppm groups for the F_0 was raised to 200 ppm, but was returned to 100 ppm because of poor acceptance by males. These (virtual) duplicate dosage groups at 100 ppm apply only to the F_0 animals (and to the F_1 while nursing).

Mating was initiated at age 100 days. Males were rotated with females within their dietary group at ten-day intervals to enhance conception. After weaning of the first litters, parental females were rested for 10 days prior to second mating to produce the second litters (F_{1b}, F_{2b}, F_{3b}). Females not impregnated during the first mating were not rebred.

From the second litters of each group, 8 males and 16 females were selected at weaning (on the basis of health and body weight) as parents for each succeeding generation. After weaning of the second litters, all surviving parental males and 8 females from each group were autopsied. In addition, parental animals succumbing during test (and weanlings of the F_{3b} litters) were subjected to gross pathologic examination, which included 31 tissues. Final body weights were recorded along with weights of liver, kidneys, spleen, gonads, heart, and brain. Both absolute and relative organ weights were subjected to ANOVA and t-tests.

Histopathologic examination was stated to have been performed upon 38 tissues from 5 males and 5 females of the control and high dose parental groups from each generation, and upon weanlings from the F_{3b} litters.

Results

Final parental body weight for both doses and both sexes and in all generations (F_0, F_1, and F_2) appeared slightly lower than controls, except for F_1 males at low dose and F_0 females at low dose (statistical parameters are not provided). This weight depression at approximately 30 weeks (at doses of 50 and 100 ppm) can be compared with "slight weight depression" occurring in both sexes (Ciba-Geigy Project No. 791229) at 6 ppm by week 20.

Mortality data and gross autopsies fail to show effect of the test material. There were no untoward signs reported among parental animals.

Significantly reduced relative (O/BW) and absolute liver weights in some F_2 animals may be due to poor diet acceptance. Food consumption data are not available, but poor diet acceptance was mentioned for males. The relative heart and ovary weights in F_1 females are statistically elevated at both doses. It is not clear that these changes represent treatment effects.

In both the F_1 and F_2 generations, however, the relative brain weights are statistically elevated in females at both doses. This effect on the brain compares with the statistically elevated relative brain weights at the top two doses in males, as reported in the "One-year Status Report...," Ciba-Geigy No. 785196. Reports of generally elevated absolute and relative brain weights at all doses and in both sexes (except low-dose males) are found in the two-year rat feeding study (IBT No. B8211).

Absolute spleen weights were significantly reduced for high-dose females (F_1) and for high-dose males (F_2).

Histopathologic tabulations for the three generations showed (relative to controls) a higher incidence of inflammation and hyperemia among the F_0 generation at high dose (the only dose tabulated).

Parental reproductive performance was graded for the following parameters: mating index, incidence of pregnancy, fertility index, incidence of parturition, and mean gestation time. For the F_{1a} animals (at all three tabulated doses) the mating index and the incidence of pregnancy (see Validation Study,

p. 388) were significantly lower than controls. Also, in the F_{1a} the fertility index at the two higher doses was significantly lower than controls.

A compound effect with genetic component may possibly be producing the effect on mating index. Evidence for the genetic component is the depressed values in the F_{3a} animals (the "generation skip") for the mating index at both doses (and also for the fertility index at high dose).

All viable progeny, according to the report, appeared outwardly normal and showed normal growth increase through lactation. At weaning, all pups were judged free of gross external abnormalities.

Progeny data (per litter) included the following: number delivered, number stillborn, number cannibalized, number born viable, numbers of pups at lactation days 1 and 5, number of pups retained at lactation day 5, and number of weanlings (male, female). The F_{2a} animals (low dose only) showed depressed values (means per litter) for pups at lactation days 1 and 5, for pups retained at lactation day 5, and for number of weanlings per litter, in both sexes. Survival indices for F_{2a} animals at low dose were depressed at the 24-hour and 5-day periods. Weanling body weights of progeny showed no effects.

Conclusion

Deficit in mating index in the F_{1a} and F_{3a} animals at both 50 and 100 ppm. Minimum Data.

Depressed lactation and survival indices for the F_{2a} animals were not dose related. The elevated brain weights at both doses in females (F_1 and F_2) support the reports of elevated brain weights in Ciba-Geigy No. 785196 and IBT No. B8211 (two-year feeding study).

The report gives no explicit statement on teratologic response.

Teratogenic Study with GS-13529 Technical in Albino Rats. IBT No. B904, May 11, 1972. Accession No. 247619. Tox Chem. No. 125B.

The test material was GS-13529 Technical (98.7%), Batch No. FL-15889, ARS No. 1940/71. Test animals were Charles River Albino rats, shipped to the laboratory on Day 1 of gestation (day zero was the day of insemination).

Dosage was at 25 or 50 mg/kg/day, respectively, to groups of 21 females. The gavage dosing was at one percent (a.i.)

in corn oil, daily from the 6th thru the 15th day of gestation. The contol group of 21 females was treated on the same schedule with water (rather than corn oil). Concomitant controls were not used. Historical control data was from animals under test 17 months earlier. The animals were permitted food and water ad lib. Mean group body weights were recorded at day 6 of gestation (initial dosing), day 9, day 12, day 15 (final dosing), and day 20 (sacrifice). Records of mortality and signs were maintained.

Sacrifice was by CO_2 asphyxiation on gestation day 20. Tabulations are presented for live and dead fetuses, fetal weights (after blotting), implantation sites, resorption sites, and corpora lutea. External examination emphasized the detection of 18 external abnormalities, including exencephaly, cleft lip, size of limbs, spina bifida, and scoliosis. Approximately half the fetuses of each sex per litter were examined for skeletal abnormalities, and the other half for internal development. Skeletal development was examined by Hurley's method of Alizarin staining, and internal development was evaluated by the sectioning technique of Wilson and Warkany.

Results

For the treated parental females the differences in weight gain (during the dosing period) appear not significant, compared to controls. At high dose there is a 7.7 percent decrease in weight gain, relative to controls. The report states there were no deaths nor unusual signs among either test or control females. The autopsy findings for dams indicate that the parameters studied (corpora lutea, implantation sites, resorption sites, and viable fetuses) did not differ significantly from controls.

For fetal skeletal development the data are not reported completely enough to permit adequate evaluation of either teratogenicity or fetotoxicity. The Ciba-Geigy Validation Report states (p. 425) that 25% of the control fetuses examined for the study were not tabulated. Supernumerary ribs were tabulated as totally absent in the controls examined, while both treatment groups showed a 9% incidence. For each treatment group and control group the value of total abnormal fetuses appears to include 3 to 5 major malformations, but these are neither described nor tabulated as such.

The tabulations for fetal internal development show that the incidences of outsized atria (small, large) were 9.8, 20.2, and 19.3% in the control, low dose, and high dose, respectively.

Neither fetal body weights nor sex ratio differed significantly from control values, according to the report.

Conclusion Invalid study.

The incidences of large and small atria are entirely out of line with the knowledge and data available to TB regarding this anomaly in any rat strain. We suspect that the problem may reside in an artifact of the examination technique. This apparent artifact together with the incomplete data for fetal development causes us to discredit the remainder of the data; and we classify the study as invalid.

Salmonella/Mammalian - Microsome Mutagenicity Test with GS-13529. Ciba-Geigy Rept. No. PH 2.632, July 20, 1977. Accession No. 247619.

Tox Chem No. 125B.

The test material was stated as Preparation GS-13529, tested with and without microsomal activation at the following concentrations: 10, 50, 100, 500, and 1000 ug/0.1 ml. Acetone was the solvent, which was used for negative controls.

The bacteria were the histidine-auxotrophic TA 98, TA 100, TA 1535, and TA 1537 strains of S. typhimurium. Preparation of activation mixture (rat liver microsomes and co-factors) was stated by reference to three articles by Ames et al.

Positive controls for strains without activation were as follows:

Strain	Positive Control
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (5 ug/0.1 ml DMSO)
TA 1537	9-aminoacridine hydrochloride monohydrate (100 ug/0.1 ml DMSO)
TA 98	daunoblastin (5 ug/0.1 ml phosphate buffer)
TA 100	4-nitroquinoline-n-oxide (0.25 ug/0.1 ml phosphate buffer)

Positive control for activated experiments was cyclophosphamide (250 ug/0.1 ml phosphate buffer) tested with strain TA 100.

For trials of test substance, three Petri dishes were prepared per dose for each strain. For trials on the positive controls two Petri dishes were used per dose per strain.

Each Petri dish contained approximately 25 ml of minimum agar containing Vogel-Bonner Medium E and glucose. To each plate was added 0.1 ml of the test solution (or vehicle) and 0.1 ml of culture (in nutrient both), all in 2.0 ml soft agar. The soft agar contained 0.6% NaCl, 0.5 mM histidine, and 0.5 mM biotin.

For activated trials, the plates also contained 0.5 ml of S9 fraction (from liver of rats induced with Aroclor 1254) plus co-factors.

Results

At doses above 50 ug/0.1 ml for strains TA 1535 and TA 1537 (without activation) the number of colonies of histidine-prototrophic mutants was much lower than controls. This result was stated as an inhibitory effect of the test material on bacterial growth.

For both activated and non-activated trials at all doses and for all strains (with the foregoing exception) the mean numbers of colonies of back-mutants were entirely comparable to solvent control values. All positive controls showed extremely large increases in revertant counts. At the top dose (1000 ug/0.1 ml) the test material precipitated in the soft agar.

Conclusion

Not mutagenic by Ames test at doses up to 500 ug/0.1 ml (activated) and up to 50 ug/0.1 ml (non-activated). Adequate protocol.

TOX:VAN OMER:DCR-17990:6/1/83:FILE-TOX-23
REVISED:06/03/83:DCR-11392:TOX-23
DCR-11393:VanOrmer,D.G.:jad:TOX21:6/7/83
REVISED:06/13/83:DCR-11396
REVISED-6/16/83:DCR-11129:efs: ALL WORK ON NEW DISKET [TOX - 25]