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Tox-343

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

003813

Memorandum

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

To: Robert J. Taylor
Product Manager 25
Registration Division (TS-767C)

Thru: Christine F. Chaisson, Ph. D. C.F. Chaisson 5/8/84
Section Head, Review Section 4
Toxicology Branch
Hazard Evaluation Division (TS-769)

From: Roger Gardner
Toxicologist Roger Gardner 5-2-84
Toxicology Branch
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Subject: Use of Paclobutrazol on greenhouse ornamentals and grass seed crops. EPA Reg. No. 10182-IE and 10182-EUP-GU. Acc. nos. 251746 and 251747. Tox. Chem. No. 628C

Actions Requested

1. Registration of a 50 WP formulation for use on greenhouse ornamentals.

2. Experimental Use Permit for a 50 WP formulation on grass seed crops.

3. Review of the following studies:

   90-day feeding study in rats
   6-week study in dogs
   1-year oral toxicity study in dogs
   21-day dermal toxicity study in rabbits
   Teratogenicity study in rats
   Teratogenicity study in rabbits
   Mutagenicity study (mouse micronucleus test)
   Mutagenicity study (in vitro with mouse lymphoma cells)
   Metabolism study in rats

Recommendations and Conclusions

1. The battery of mutagenicity studies submitted to the Agency evaluate the potential of paclobutrazol to cause gene mutations or chromosomal damage. However, additional testing is needed to fulfill the Agency's recommended testing for the potential to cause DNA damage (see Pesticide Regulations: Proposed Data Requirements. FEDERAL REGISTER. Vol. 47, No. 247. November 24, 1982. pages 53193-4 and 53195-6).

1/446

2. Acute dermal studies used a single dose that was half that recommended for a limit test (see Section III below page 40), and the results do not provide a basis for classification of the technical material or the formulation into a Toxicity Category for labeling purposes. On that basis an acute dermal toxicity study is needed on the technical material as well as the 50 WP formulation.

3. Results of the rat teratogenicity study indicated a dose-related incidence of fetotoxicity (delayed ossification) without establishing a no-observed effect level (NOEL). Since these effects occurred at doses below that at which maternal toxicity was reported and since the teratogenicity study in rabbits had deficiencies (see Section II and the Appendix below). additional teratogenicity studies are needed.

4. The metabolites and tissue residues were not identified in the submitted study. Because of the complex structure of the paclobutrazol molecule the nature of its metabolites should be investigated (see Section II. 5., and Appendix below).

5. As indicated by points 1. through 4. above additional toxicity data are needed to support the requested registration and experimental use permit.

I. Background

Paclobutrazol is a plant growth regulator which is proposed for use on greenhouse ornamentals (EPA Reg. No. 10182-IE) and for experimental use on grass seed crops (EPA Reg. No. 10182-EUP-GU). Its chemical name is (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1yl) pentan-3-ol.

Confidential Statements of Formulation indicate that the two formulations (BONZI™ for the greenhouse use and PARLAY™ for the seed grass use) are wettable powders with the same composition. The inert ingredients include [redacted]. The stated percentage active ingredient in both formulations is 55.2.

II. Data Summary

The following summary includes studies which were submitted in support of EPA Reg. No. 10182-IE. Data Evaluation Records for those new studies are included in the Appendix.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

A. Technical grade material

1. Acute toxicity .

The results of acute toxicity studies on technical paclobutrazole are summarized as follows:

Route of administration	Species	Sex	LD_{50} or LC_{50}	Toxicity Category
Oral	Rat	Male	1.954 g/kg	III
		Female	1.336 g/kg	III
	Mouse	Male	490 mg/kg	II
		Female	1.219 g/kg	III
	Guinea pig	Male	542 mg/kg	III
		Female	400-640 mg/kg	III
	Rabbit	Male	835 mg/kg	III
		Female	937 mg/kg	III
Intraperitoneal	Rat	Male	160-250 mg/kg	-
		Female	90 mg/kg	-
Dermal	Rat	Both	>1 g/kg	-*
	Rabbit	Both	>1 g/kg	-*

*See Section III below and recommendation 2. above.

Signs of acute toxicity were observed to begin one to three hours after dosing or exposure of test animals. Deaths occurred within 2 to 4 days after treatment. Treated animals exhibited subdued behavior, unsteady gait, loss of righting reflex, piloerection, coma, hypothermia, respiratory stress, and urinary incontinance. Surviving animals appeared normal within 6 to 9 days after treatment.

The technical material causes mild skin irritation that persists in rabbits for 72 hours (Toxicity Category III). It also causes reversible corneal opacities with irritation that persists in treated eyes of rabbits for 72 hours (Toxicity Category II). Paclobutrazol is not a skin sensitizer in guinea pigs.

003813

2. Subchronic toxicity

In a 90-day feeding study with rats a level of 1250 ppm caused increased liver weights and doubled the aminopyrene-N-demethylase activity in the females. These effects were noted to a lesser extent in the females given diets containing 250 ppm, but without concommitant histological changes, the effects at the mid dose are unlikely to be toxicologically significant. Therefore, the no-observed-effect level (NOEL) for rats given paclobutrazol in the diet for 90 days is 250 ppm, and the lowest effect level (LEL) is 1250 ppm.

In a preliminary subchronic oral study in dogs (6 weeks in duration) the liver weights and serum alkaline phosphatase levels in animals given 75 or 225 mg/kg/day (one of each sex per dose group) suggests that the liver is the primary target organ for paclobutrazol toxicity. Results from observations made at 26 weeks in a 1-year oral toxicity study in dogs are consistent with the results of the preliminary study. However, these results provide preliminary evidence that NOEL of 15 mg/kg/day is likely. The lowest-effect level is likely to be 75 mg/kg/day (See Appendix for a detailed discussion of these studies.).

All dose levels (10, 100, or 1000 mg/kg/day) in a 21-day dermal toxicity study with rabbits caused irritation of the abraded skin of the test animals. Only the 100 and 1000 mg/kg/day doses irritated the intact skin sites of test animals. The irritation began to appear during the second week of dermal applications, and the degree of irritation increased with the dose. Histologically, treated skin exhibited hyperkeratosis, acanthosis, and inflammatory changes of the superficial dermis. The NOEL for intact skin with respect to these effects is 10 mg/kg/day, and the LEL is 100 mg/kg/day. The 1000 mg/kg/day dose (highest dose tested) caused severe irritation after two weeks of application to the skin of the treated rabbits.

3. Teratogenicity

In a rat study the NOEL for maternal toxicity with respect to decreased bodyweight gain during dosing (days 6-9 of gestation) was 40 mg/kg/day (lowest dose tested). The LEL was 100 mg/kg/day. The highest dose caused mortality (5/24 animals in the group) as well as grossly observable liver effects (pallor and enlargement).

Fetuses exhibited a dose-related increase in the incidence of delayed ossification at all doses, and the authors concluded that a NOEL for these effects was not established.

In a rabbit teratology study fertility of the animals was low. Only the low and mid dose groups contained the recommended minimum number of litters at the end of the study. Because of this limitation the study can only provide preliminary information in an assessment of the teratogenic potential of paclobutrazol. Within this context the NOEL for maternal toxicity (as indicated by decreased bodyweight gain during the dosing period) is likely to be 25 mg/kg/day and the LEL is likely to be 75 mg/kg/day. There were no effects on the fetuses of low and mid dose group does that could be attributed to the test substance (likely NOEL is 125 mg/kg/day which is the highest dose tested), but a more complete study is needed to confirm these results.

4. Mutagenicity

Paclobutrazole did not induce mutations in Salmonella test strains or in mouse lymphoma cells in vitro. The chemical also failed to cause an increase in the incidence of micronuclei in treated mice.

5. Metabolism

In a study with rats given a single 10 mg/kg dose of ^{14}C labelled paclobutrazol a sex difference with respect to the excretion pattern was found. The major route of excretion in males was the feces, while that in female rats was the urine. Most of the urinary excretion occurred within 24 hours after dosing, but fecal excretion was slower with most of that occurring over the 48-hour period following treatment. Both of these results are indicative of significant absorption from the digestive tract. Residue concentrations and autoradiography three or four days after dosing indicated that paclobutrazol and its metabolites do not accumulate in the body of treated rats. Approximately 60% of the administered dose was recovered from excreta of both male and female rats during the 24-hour period immediately after dosing. The latter two findings indicate that paclobutrazol and its metabolites are likely to be rapidly cleared by the rat.

The metabolites and tissue residues were not identified in the submitted study. Because of the complex structure of the paclobutrazol molecule the nature of its metabolites are needed to determine whether or not toxicologically significant residues are formed. Based on this consideration, there is a need for additional metabolism studies to identify residues and metabolites formed in treated animals.

003613

B. Toxicity of formulations

The acute toxicity results for the powder formulation containing 50% active ingredient are summarized as follows:

Route of administration	Species	Sex	LD_{50} or LC_{50}	Toxicity Category
Oral	Rat	Both	>5000 mg/kg	IV
Inhalation	Rat	Male	>766 mg/m^3	II
		Female	359-766 mg/m^3	II
Dermal	Rabbit	Both	>1000 mg/kg	-*

*See Section III below and recommendation 2. above.

The signs of toxicity observed in these studies are similar to those described above for the technical material.

The 50% formulation is classified into Toxicity Category IV for skin irritation and Toxicity Category II for eye irritation.

III. Discussion

As mentioned in the previous section and in the Appendix both of the subchronic toxicity studies in dogs and the teratogenicity studies in rats and rabbits provide preliminary information in support of the proposed registrations. They do not provide an adequate basis (establishment of NOEL's) for an assessment of potential hazards which are suggested by their results (fetotoxicity in rats and liver toxicity in dogs). These considerations indicate that teratology studies and the final report on the one-year dog study are needed before a final decision on the requested registrations can be made.

It should be noted that the previous Toxicology Branch review (Gardner, 1983) is misleading with respect to acute dermal toxicity studies submitted earlier. In that review the Toxicity Categories assigned to the technical and 50% WP formulations with respect to acute dermal toxicity were based on single dose studies. Those studies used a dose that is half that recommended by the Agency for a limit test (see Pesticide Assessment Guidelines: Subdivision F, November, 1982, §81-2, page 40), and the results do not provide a basis for classification of the technical material or the formulation into a Toxicity Category for labeling purposes. On that basis an acute dermal toxicity study is needed on the technical material as well as the 50 WP formulation.

The battery of mutagenicity studies submitted to the Agency evaluate the potential of paclobutrazol to cause gene mutations or chromosomal damage. However, additional testing is needed to fulfill the Agency's recommended testing for the potential to cause DNA damage (see Pesticide Regulations: Proposed Data Requirements. FEDERAL REGISTER. Vol. 47, No. 247. November 24, 1982. pages 53193-4 and 53195-6).

Finally, the metabolites and tissue residues were not identified in the submitted study, and the conclusions from that study are appropriate for the triazol containing residues. As indicated by the structural formula the test substance is likely to be metabolized to presently unidentified metabolites which could have toxicological significance. Therefore, a complete evaluation of the metabolism of paclobutrazol can not be made on the basis of the submitted study alone.

IV. Reference

Gardner, R. Memorandum dated December 20, 1083. To: Robert J. Taylor, Registration Division. Subject: Paclobutrazol (PP333) Request for Data Waiver (Correspondence #35) and Data Review (EPA Acc. No. 248688) Reg. No. 10182-TT. Tox. Chem. No. 628C.

003813

APPENDIX

Data Evaluation Records

Litchfield, M. H., P. B. Banham, D. T. Chalmers, I. S. Chart, C. W. Gore, S. Lindsey, M. Robinson, M. D. Stonard, M. Thomas, and B. Woollen. July 16, 1983. Paclobutrazole (PP333): 90 day feeding study in rats. Unpublished report no. CTL/P/760 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251746.

Clapp, M. J. L., A. E. Kalinowski, I. S. Chart, C. W. Gore, and M. D. C. Scales. June 16, 1983. PP333: Six-week oral dosing study in dogs. Unpublished report no. CTL/P/767 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Clapp, M. J. L., A. E. Kalinowski, I. S. Chart, and C. W. Gore. July 18, 1983. Paclobutrazol: 1 year oral dosing study in dogs; Interim report after 26 weeks. Unpublished report no. CTL/P/812 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Kynoch, S. R., G. K. Lloyd, J. R. Mallard, A. E. Street, W. A. Gibson, and D. E. Prentice. March 17, 1980. The effect of repeated applications of PP333 to the skin of rabbits for twenty-one days. Unpublished report no. ICI/256/79822 prepared by Huntingdon Research Centre, Huntingdon, Cheshire, UK. Submitted by ICI Americas, Inc. EPA Acc. No. 251746.

Killick, M. E., G. H. Pigott, P. B. Banham, and M. R. Thomas. July 13, 1983. Paclobutrazol: Teratogenicity study in the rat. Unpublished report no. CTL/P/842 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Killick, M. E., M. H. Litchfield, P. B. Banham, and M. R. Thomas. July 14, 1983. Paclobutrazol: Teratogenicity study in the rabbit. Unpublished report no. CTL/P/861 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Phillips, C. E., C. R. Richardson, D. Hart, and E. Longstaff. August 4, 1983. An evaluation of Paclobutrazol in the mouse micronucleus test. Unpublished report no. CTL/P/848 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

McGregor, D. B., and C. G. Riach. March, 1983. PP333: Assessment of mutagenic potential in the mouse lymphoma mutation assay. Unpublished report no. 2529 prepared by Inveresk Research International. Submitted by ICI Americas. EPA Acc. No. 251747.

Jones, B. K., C. K. Choo, D. M. Williams, and A. R. Soames. August 9, 1983. Paclobutrazol: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat. Unpublished report no. CTL/P/870 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

003813

DATA EVALUATION RECORD

Citation: Litchfield, M. H., P. B. Banham, D. T. Chalmers, I. S. Chart, C. W. Gore, S. Lindsey, M. Robinson, M. D. Stonard, M. Thomas, and B. Woollen. July 16, 1983. Paclobutrazole (PP333): 90 day feeding study in rats. Unpublished report no. CTL/P/760 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251746.

Materials and Methods

Test substance: The test substance contained 91.9% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Male and female Wistar derived Alderley Park strain rats were used. The animals were approximately 6 weeks of age at the start of the study.

Experimental proceudre: Four groups each containing 20 male and 20 female rats were given diets containing 0, 50, 250, or 1250 ppm for 90 days.

Each rat was observed twice daily for the occurrence of clinical signs of toxicity, mortality, or behavioral changes. Bodyweight of each rat was obtained at weekly intervals throughout the experiment, and food consumption was estimated on a weekly basis for each cage of four rats during the experiment.

Blood samples were drawn from the tail vein of 10 animals in each group prior to the beginning and after 4 weeks of the study. At the end of the study blood was obtained by cardiac puncture. Hematological observations included hemoglobin, hematocrit, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential white cell counts, and platelet count. Kaolin-cephalin and prothrombin times were also determined.

Clinical chemistry observations of blood samples included urea, glucose, triglycerides, albumin and total protein, cholesterol, alkaline phosphatase, alanine transaminase, and aspartate transaminase. These tests followed the same schedule as that of the hematology.

The animals were placed individually into metabolism cages for 18 hours one week prior to the start of the experiment as

well as at 4 weeks after treatment started and during the last week of treatment. Urine was collected, and the following observations were made: volume, pH, specific gravity, glucose, ketones, and urobilinogen.

During the week prior to the start of the feeding period and during weeks 9 and 10 for females and week 11 for males 5 rats of each sex from each group were selected for urine collection. Collected urine was subsequently analyzed for the presence of the butyl acid metabolite of the test substance.

The eyes of each test rat in the control and 150 ppm dose groups were examined two weeks prior to the start of the study and at termination.

At the end of the 90-day feeding period the test animals were sacrificed and subjected to a post mortum examination. The liver, kidneys, heart spleen, gonads, adrenals, and brain were removed and weighed. Tissues representative of the digestive, respiratory, circulatory, endocrine, reproductive, lymphatic, nervous, hematopoietic, and excretory systems were prepared and examined histologically. The report stated that tissues from all animals from the control group and high dose group were examined, while only tissues from the liver, kidney, heart, adrenals, and any grossly abnormal tissues from the low and mid dose group animals were examined microscopically.

Samples of tissue from livers of 10 male and 10 female rats from each group were taken for determination of hepatic aminopyrene-N-demethylase activity.

Statistical analyses: The report stated that analysis of variance was used for bodyweight, food consumption, and food utilization results. The results of assays for aminopyrene-N-demethylase activity were also analyzed in this manner, but the data were transformed logarithmically prior to the analysis.

Organ weights were evaluated by analysis of variance and analysis of covariance on final bodyweights.

Biochemical results were evaluated by analysis of covariance on pre-test values for the 4 and 13 week samples. The authors stated that results from male and female animals were combined to determine common estimates of error variance and covariate regression. The report stated that the common

covariate regression and separate male and female pre-test means were used to adjust the means of biochemical test results obtained during the feeding period.

The hematological results were not adjusted for pretest values because limited pre-test data were available. These results were evaluated by analysis of variance for male and female rats together.

The error mean square from analysis of variance was used in two-sided t tests to compare each dose group mean with the control group mean for each parameter.

Reported Results

The report stated that the dietary analyses showed a ± 10% fluctuation in the intended concentrations, and the test mixtures were homogeneous during the entire 90-day feeding period. The results of the analyses also indicated that the feed mixtures were stable for eight weeks according to the authors.

There were no mortalities or dose-related signs of toxicity noted by the authors during the study.

The authors noted statistically significantly decreased bodyweight gains in female rats from the high dose group as compared with those in the control group (t test, $p<0.05$).

These differences were noted during the 6th, 8th, and 10th through 13th weeks of the study. The investigators also noted slight decreases in bodyweight gain in the high dose males when compared with controls, but these differences were not statistically significant. Food consumption showed similar decreases as indicated by the authors. The report also noted that there was little indication of an effect of the test substance on food utilization as determined from the ratio of food consumption and bodyweight gain. At the end of the study group mean body weights were similar. These means (and standard deviations) are summarized as follows (bodyweights are in g):

Dose group	Males	Females
Control	493.8 (41.4)	269.3 (17.7)
Low	486.5 (45.2)	272.5 (16.6)
Mid	492.5 (41.3)	271.5 (20.6)
High	475.8 (30.6)	260.3 (16.0)

The authors noted small decreases in hemoglobin and associated values in the high dose group females. The reported group means for those parameters at the end of the feeding period are as follows:

Parameter	Test group	
	Control	High dose
Hemoglobin (g/dl)	15.09	14.67
Hematocrit (g/dl)	0.428	0.421
Red cell count (X 10^{12}/l)	7.88	7.81
Cell hemoglobin (pg)	18.76	18.42
Cell volume (fl)	55.4	54.9
Cell hemoglobin conc. (g/dl)	34.78	34.43*

*$p<0.05$, t test

The authors also noted that the prothrombin times were statistically significantly decreased in female rats in the high dose group from the values reported for control group femalse (17.00 and 14.54 sec. for the control and high dose groups, respectively at termination; $p<0.01$, t test). The 250 ppm group also exhibited a statistically significant decrease for this parameter (15.22 sec; $p<0.05$, t test). The prothrombin time for females in the low dose group was also decreased (15.66 sec.), but no statistical significance was reported. Kaolin-cephalin times were also significantly decreased by the highest dose (16.40 sec in the high dose females compared with 20.76 sec. in the control group at terminal sacrifice; $p<0.05$, t test).

The authors noted that the high dose group female rats had elevated serum cholesterol (45.7 mg/100ml compared with 40.3 mg/100 ml in the control animals at week 13; $p<0.05$, t test) and blood urea (58.5, 64.8, and 66.3 mg/100ml in the control, mid, and high dose groups, respectively at 13 weeks; $p<0.05$, t test). Male animals in the same group showed a decrease in group mean serum albumin (4.83 g/100ml compared with 5.06 g/100ml for the control group at 13 weeks; $p<0.05$, t test). Male rats from the high dose group also were reported to have a statistically significantly elevated ($p<0.05$, t test) alanine transaminase activity at week 13 of the feeding period (49.2 mU/ml compared with 42.6 in control rats) as well as at week 4 (49.1 and 68.8 mU/ml for control and high dose rats, respectively; $p<0.01$, t test).

The authors noted a slight decrease in urinary volume in treated males and an increase in protein output for the high

dose group male rats. The mean urinary volume reported for the control, low, mid, and high dose groups were 10.21, 6.77, 8.82, and 8.45 ml, respectively (measured during the 13th week). The differences between the high dose and control groups were statistically significant at $p<0.01$ (t test), while the differences between each of the other two groups were signigicant at $p<0.05$. The mean urine protein for the control group was reported to be 17.81 mg/TPV as compared with 21.18 for the high dose group males at 13 weeks ($p<0.05$, t test).

The mean urinary concentration (mg/l) of the butyl acid metabolite in test animals was reported as follows:

Dose level (ppm)	Males	Females
0	ND*	5.3**
50	2.0	2.2
250	13.1	11.7
1250	32.3	41.7

*Not detected
**Dosing error during weeks 9 through 11.

An increase in the hepatic aminopyrene-N-demethylase activity was reported in males of the high dose group and in females in the mid and high dose groups. The group mean activity (umol formaldehyde/h/g liver) for the control, mid, and high dose group females were reported to be 12.7, 16.8, and 29.1, respectively. The treatment group means were statistically significantly different from the control group mean ($p<0.01$; t test on transformed values). The respective group means for the control and high dose group males were reported to be 26.0 and 36.3 ($p<0.01$, t test using transformed values).

Organ weight data were statistically evaluated by analysis of variance and analysis of covariance with bodyweight as the covariate. Statistically significant differences were reported for group mean liver weights and liver weights adjusted for bodyweights for the mid and high dose group female rats and the high dose group male animals. Respective mean liver weights (g) for the control, mid, and high dose group females were reported to be 10.0, 10.7, and 11.6 g, respectively ($p<0.05$ for mid dose group mean and $p<0.01$ for the high dose group). The high dose group male rats had statistically significantly higher mean liver weight (19.1 g) when compared with the control group mean (18.0), but statistical significance was only achieved after adjusting for bodyweight differences.

The only histopathological findings which were associated with the administration of the test substance were reported in the liver. These changes were described as hydropic in nature. The reported incidences of minimum hydropic changes were reported to be 8, 5, 9, and 11 of 20 examined in the control, low, mid, and high dose group males, respectively. The respective incidences of these changes in females were 7, 2, 4, and 10 of 20 examined. Moderate hydropic changes were reported in 2 males from each of the control, low, and high dose groups, while 1 from the mid dose group showed moderate hydropic change. For female rats only one of the 20 examined from the high dose group exhibited the change. No other test animals had the lesion according to the report.

No other lesions were observed to occur in a dose-related manner according to the report.

Discussion and Conclusions

The results reported for bodyweight determinations do not suggest that a toxicologically significant effect was found. The bodyweights of the animals at the end of the study were not significantly different from group to group (<5% decreases in treated groups when compared to control group means).

Although there were statistically significant differences between group means for some hematological parameters discussed above, the authors noted that these differences were not toxicologically significant. However, they did not provide historical data from animals of the same age and strain to support their conclusion.

As the investigators indicated, there were increases in the liver weights of the high dose females along with increases in serum cholesterol levels and aminopyrene-N-demethylase activity. Based on those results the authors concluded that the test substance affected the liver at 1250 ppm. They apropriately described the effects in the 250 ppm group females as residual since the cholesterol and aminopyrene-N-demethylase levels were not statistically significantly elevated.

As noted above there were residues of the test substance detected in the urine of the control group females during the 9th week of the feeding period. In the discussion section of the report the authors stated:

> It is very unlikely that direct contamination by the metabolite during analysis was the cause since a stock of this compound was not available in the laboratory. It is probably not the result of a

> dietary make-up mistake since the batches of diet were shown to have the correct levels of PP333 and none was detected in the control diet (<2 ppm)..., it is unlikely that the diets were incorrectly fed to the female groups since the urinary outputs of PP333 metabolite from the treated groups was very similar to that from the male treated groups. There is no indication that the female controls have been adversely affected in any way with the values for the range of measured parameters showing normal figures.

The authors speculated further:

> The most probable explanation, although it could not be proven, was that a transposition of some kind occurred with the female urine samples after collection and before pooling.

The investigators concluded from these considerations that the study was not compromised. The data as reported support that conclusion.

An independent evaluation of the unadjusted individual animal data (t test) for urea and cholesterol measurements in the female rats indicated that the means reported in summary tables were adjusted as mentioned above (see Materials and Methods section). However, the report did not contain summary tables or worked examples of the analyses of variance and covariance procedures used in the evaluation of these data.

The results reported by tha authors support the conclusion that a level of 1250 ppm in the diet of female rats caused increased liver weights and doubled the aminopyrene-N-demethylase activity. These effects were noted to a lesser extent in the female rats given diets containing 250 ppm, and without concommitant histological changes the effects at the mid dose are unlikely to be toxicologically significant. Therefore, the no-observed-effect level (NOEL) for rats given paclobutrazole in the diet for 90 days is 250 ppm, and the lowest effect level (LEL) is 1250 ppm.

Core classification: Minimum

003813

DATA EVALUATION RECORD

Citation: Clapp, M. J. L., A. E. Kalinowski, I. S. Chart, C. W. Gore, and M. D. C. Scales. June 16, 1983. PP333: Six-week oral dosing study in dogs. Unpublished report no. CTL/P/767 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 91.9% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Sixteen to 18-week old male and female beagle dogs were used.

Experimental proceudre: Four groups each containing one male and one female were given daily doses of 0, 15, 75, or 225 mg test substance per kg bodyweight for six weeks. Doses were administered in capsules.

The report stated that the animals were given full clinical examinations prior to the start and at the end of the study. The examinations included cardiac and pulmonary ausculation and ophthalmoloscopic observations. Each dog was also observed twice daily for the occurrence of clinical signs of toxicity. Fecal consistency was also noted for one week prior to the beginning of the study and throughout the treatment period. Bodyweights of each dog were obtained at weekly intervals through the 4-week acclimatization period and the experiment, and food consumption was measured daily for one week prior to the study and throughout the experiment.

Blood samples were drawn from the jugular vein of each animal at weekly intervals beginning one week prior to the start and continuing through the six-week test period. These samples were taken prior to the daily feeding. Hematological observations included hemoglobin, hematocrit, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential white cell counts, and platelet count. Kaolin-cephalin and prothrombin times were also measured.

Clinical chemistry observations of blood samples included urea, glucose, triglycerides, albumin and total protein, cholesterol, electrolytes, alkaline phosphatase, alanine

transaminase, aspartate transaminase, and creatine kinase.

The animals were placed individually into metabolism cages for 18 hours one week prior to the start of the experiment as well as at 3 and 6 weeks after treatment was started. Urine was collected, and the following observations were made: volume, pH, specific gravity, glucose, ketones, bilirubin, urobilinogen, blood, and protein. Urine was also centrifuged, and the deposits were examined microscopically.

At the end of the six-week feeding period the test animals were sacrificed and subjected to a post mortum examination. The livers were removed and weighed. Tissues representative of the digestive, respiratory, circulatory, endocrine, reproductive, nervous, hematopoietic, and excretory systems were prepared and examined histologically.

Reported Results

There were no effects on mortality, clinical obsrvations, food consumption, hematology, urinalysis, and histopathology according to the report.

The authors noted that the liver from the mid-dose group male appeared enlarged, and the liver-to-bodyweitht ratios for the high dose dogs were approximately 25% greater than those noted in the control group animals.

Other isolated changes which were described by the authors included a 50% lower bodyweight gain in the high-dose group male than was noted in the control male during the six-week treatment period. This dog was also observed to gain less weight during the pre-test period. The only other dog which was observed to have decreased weight gain was the low-dose group female. No other animals from the other treatment groups exhibited significant decreases according to the report.

Serum alkaline phosphatase activity in the mid-dose group female and the two high-dose group animals were elevated above control levels (214, 279, and 309 mU/ml during the 6th week for control, mid, and high dose group females, respectively; 182 and 289 mU/ml for control and high dose males, respectively)

Discussion and Conclusions

The results of this study are preliminary in nature since only one animal of each sex was used in each group. The liver weights and the alkaline phosphatase levels reported for animals in the 75 and 225 mg/kg/day groups suggests that the liver is the primary target organ for PP333.

Core classification: Supplementary because of the small number of animals used in the study and the short duration of dosage administration (6 weeks).

003813

DATA EVALUATION RECORD

Citation: Clapp, M. J. L., A. E. Kalinowski, I. S. Chart, and C. W. Gore. July 18, 1983. Paclobutrazol: 1 year oral dosing study in dogs; Interim report after 26 weeks. Unpublished report no. CTL/P/812 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 91.9% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Eighteen to 24-week old male and female beagle dogs were used.

Experimental proceudre: Four groups each containing six males and six females were given daily doses of 0, 15, 75, or 300 mg test substance per kg bodyweight for 26 weeks. Doses were administered in capsules shortly before feeding each day.

The report stated that the animals were given full clinical examinations prior to the start and at weeks 13 and 26 of the study. The examinations included cardiac and pulmonary ausculation and ophthalmoloscopic observations. Each dog was also observed twice daily for the occurrence of clinical signs of toxicity and behavioral changes. Bodyweights of each dog were obtained at weekly intervals through the experiment, and food consumption was measured daily during the study.

Blood samples were drawn from the jugular vein of each animal starting one week prior to the first day of dosing and at weeks 4, 8, 12, 16, 20, and 26 of the study. These samples were taken prior to the daily feeding. Hematological observations included hemoglobin, hematocrit, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential white cell counts, and platelet count.

Clinical chemistry observations of blood samples included urea, glucose, triglycerides, albumin and total protein, cholesterol, electrolytes, alkaline phosphatase, alanine

transaminase, aspartate transaminase, and creatine kinase.

The animals were placed individually into metabolism cages for 18 hours one week prior to the start of the experiment as well as at 8, 17. and 26 weeks after treatment was started. Urine was collected, and the following observations were made: volume, pH, specific gravity, glucose, ketones, bilirubin, urobilinogen, blood, and protein. Urine was also centrifuged, and the deposits were examined microscopically.

Reported Results

The report noted that one of the control group males was inadvertantly given the low dose, and a low dose male was given the control capsules for a period of 16 days (weeks 8 to 10 of the study).

No mortalities were reported. There were also no effects on hematological or urinalysis parameters.

The authors noted that the high-dose group male and female animals had reduced bodyweight gains during the study. Tabulated group mean bodyweights for the treatment group were not more than 3% less than the control group mean bodyweights during the 26-week feeding period.

Food consumption was reported to be unaffected by administration of the test substance.

The investigators observed dose-related increases in serum alkaline phosphatase in male and female dogs from the mid and high dose groups. These values (mU/ml) at week 26 of the study are summarized as follows:

Dose group	Males	Females
Control	99	95
Mid	153	191
High	450	674

The differences in the treated group means and the control means were statistically significant ($p<0.05$, two-tailed Student's t test).

Triglyceride levels were also described as increased in the high-dose group animals throughout the treatment period. In males the control and high-dose group means were 25 and 45 mg %, respectively at the 26-week observation ($p<0.01$; t test).

For female dogs the respective means for the control and high-dose groups were 33 and 49 mg % (no statistically significant difference was noted).

Other group means which were reported to be statistically significantly different from controls included albumin, total protein, and calcium levels. Those means which were tabulated in the report from the 26-week observations are summarized as follows:

	Males		Females	
Observation	Control	Treated	Control	Treated
Albumin*	3.5	3.1	3.7	3.4
Total protein*	5.7	5.1	5.7	5.5
Calcium**	10.8	10.7	11.0	10.7

*g %
**mg %

Discussion and Conclusions

The report was described as an interim report and has no histological or other post mortum observations to accompany the clinical chemistry data presented. However, the results of this study and the preliminary 6-week study discussed elsewhere (DER on report no. CTL/9/767) suggest that a no-observed-effect level (NOEL) of 15 mg/kg/day is likely. The lowest-effect level is likely to be 75 mg/kg/day.

Core classification: Supplementary because the study is an interim report of a long term experiment.

003813

DATA EVALUATION RECORD

Citation: Kynoch, S. R., G. K. Lloyd, J. R. Mallard, A. E. Street, W. A. Gibson, and D. E. Prentice. March 17, 1980. The effect of repeated applications of PP333 to the skin of rabbits for twenty-one days. Unpublished report no. ICI/256/79822 prepared by Huntingdon Research Centre, Huntingdon, Cheshire, UK. Submitted by ICI Americas, Inc. EPA Acc. No. 251746.

Materials and Methods

Test substance: (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1yl) pentan-3-ol (paclobutrazole). Batch no. P15, reference no. ERH 283.78JH.

Test species: Male and female New Zealand white rabbits weighing approximately 2.1 to 3.1 kg were used. The animals were 11 to 14 weeks of age.

Experimental procedure: Twenty-four hours prior to the first application of the test substance hair was clipped from the mid dorsal region of each rabbit. About 10% of each animal's surface area was clipped. Ten male and 10 female rabbits were assigned to each of four test groups, and the clipped treatment sites on 5 animals of each sex in each group were abraded. The treatment sites on the remaining animals in each group were left intact. Abrasions were to penetrate the stratum corneum without causing bleeding according to the report. Hair clipping was repeated as needed during the 21-day treatment period, and abrasion was done on a weekly basis during the test.

The test substance was suspended in 1% aqueous carboxymethyl cellulose and applied to the prepared skin sites at rates of 0, 10, 100, or 1000 mg per kg bodyweight. After each application the treated areas were covered with an occlusive dressing for six hours. At the end of that time the bandages were removed, and each test site was washed with warm tap water and blotted dry.

This procedure was followed once each day 5 days each week for 3 consecutive weeks.

During the study the animals were observed daily for the appearance of toxic signs, mortality, and behavioral changes.

Treated skin was observed daily for irritation and scored according to the following criteria:

Observation	Erythema and eschar formation	Edema formation
None	0	0
Slight	1[a]	1[a]
Well-defined	2	2[c]
Moderate	3	3[d]
Severe	4[b]	4[e]

[a]barely perceptible
[b]severe edema to aschar with injuries in depth
[c]raised area
[d]raised approximately 1 mm
[e]raised more than 1 mm and extended beyond the treated area

The rabbits were weighed prior to the first application of the test substance and at weekly intervals thereafter. Food consumption was determined at weekly intervals also.

Blood samples were drawn from the median artery of the ear of each rabbit prior to dosing and during the third week of the study. Hematological observations and determinations included packed cell volume, hemoglobin, cell counts (red blood cells, total and differential white cells, and platelets), mean corpuscular hemoglobin concentration, and mean cell volume. Blood chemistry observations included urea, total protein, albumin, electrolytes, creatinine, cholesterol, glucose, alkaline phosphatase, and glutamic-pyruvic transaminase.

Survivors were sacrificed 21 days after the study began. Those animals as well as rabbits which were found dead or sacrificed in extremis during the experiment were necropsied. After gross examination the adrenals, brain, heart, kidneys, liver, lungs, gonads, and spleen were removed and weighed. In addition to these organs sample tissues from the cecum, cervix, duodenum and ileum, eyes, pancreas, lymph nodes, mammary glands, bone marrow, esophagus, pituitary, prostate, salivary glands, skeletal muscle, treated and untreated skin, stomach, thymus, thyroids and parathyroids, trachea, urinary bladder, uterus, and any grossly observed lesion were taken from each animal and prepared for microscopic examination.

Reported Results

The authors noted that the control rabbits exhibited slight erythema and edema occassionally during the test period. By the second week of the study the investigators noted slight to well-defined erythema with or without slight edema at abraded and intact sites on rabbits receiving the 10 mg/kg application. A similar response was noted in the 100 mg/kg group although the authors did not report well-defined erythema as frequently. Also the report stated that the responses in the 100 mg/kg group tended to ameliorate during the third week of treatment.

The skin reactions noted in the 1000 mg/kg/day group were described as well-defined to moderate dermal irritation which was persistent. During the third week the skin reactions on some animals intensified and were classified by the investigators as severe. Treatment sites of 15 of 20 of the rabbits hardened during the third week, and cracking, hemmorrhaging, scabbing, and sloughing were noted.

Mortalities were reported to occur in a manner which was not dose related, and the cause of these deaths could not be determined. In the control group two of the five males with abraded skin died, while one of five males with intact skin and two of five males with abraded skin in the 100 mg/kg/day group died during the study. One female with abraded and one with intact skin from the 100 mg/kg/day group were reported to have died during the experiment also. No other deaths were noted.

According to reported observations, the control and treated groups were similar with respect to hematology, blood chemistry, bodyweight, food consumption, and organ weights.

There were no dose-related macroscopic or microscopic abnormalities with the exception of those noted in treated skin. Histopathological findings in skin were confined to treated areas according to the report. These lesions were described as acanthosis and hyperkeratosis of the epidermis with associated scab formation and inflammatory cells and edema. The authors also noted ulceration in the skin of several rabbits given the highest dose. The control group males and females and the 10 mg/kg/day group female rabbits were reported to have normal skin sections on microscopic examination.

Although there were no summary tables for the incidence of

frequently observed microscopic abnormalities in the test animals, the report noted histopathology in the respiratory tract, liver, kidneys, and cardiovascular system.

In the lungs the majority of rabbits in control and treated groups the authors noted peribronchiolar and perivascular aggregations of lymphoid cells. Focal areas of vascular congestion with or without hemmorrhaging in the parenchyma was noted in a "proportion" of animals in all groups including the controls.

The kidneys from a "small number" of rabbits in the control and treated groups were reported to exhibited dilated cortical tubules lined with basophilic or degenerate tubular epithelium. These lesions were often accompanied by interstitial infiltration of lymphocytes and mineralization of the tubular lumens according to the report.

The authors also noted degenerative medial changes in the aorta of a "small number" of animals in control and treated groups.

None of these changes were associated by the investigators with the dermal application of the test substance.

Discussion and Conclusions

There were adequate data presented in the report to support the conclusions of the investigators. All dose levels (10, 100, or 1000 mg/kg/day) caused irritation of the abraded skin of test animals. Only the 100 and 1000 mg/kg/day doses irritated the intact skin sites of test animals. The irritation began to appear during the second week of dermal applications, and the degree of irritation increased with the dose. Histologically, treated skin exhibited hyperkeratosis, acanthosis, and inflammatory changes of the superficial dermis. The no-observed-effect level (NOEL) for intact skin with respect to these effects is 10 mg/kg/day, and the lowest-effect level (LEL) is 100 mg/kg/day. The 1000 mg/kg/day dose (highest dose tested) caused severe irritation after two weeks of application to the skin of rabbits.

Core classification: Minimum

DATA EVALUATION RECORD

Citation: Killick, M. E., G. H. Pigott, P. B. Banham, and M. R. Thomas. July 13, 1983. Paclobutrazol: Teratogenicity study in the rat. Unpublished report no. CTL/P/842 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 92.4% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Female Wistar derived Alderley Park strain rats were used. Each female was mated overnight with a male and the following morning vaginal smears were examined for the presence of spermatozoa. The day spermatozoa were found was designated Day 0 of gestation. Test animals weighed between 222 and 280 g and were 12 weeks old when selected for the study.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 15 of gestation. Doses of 0, 40, 100, or 250 mg test substance per kg body weight were given to groups of 24 mated dams.

Each dam was observed daily for occurrence of toxic signs and mortality. Bodyweight determinations were made on days 0, 6-15, and day 21 of gestation. Food consumption was estimated for three day periods throughout gestation according to the report.

The rats were sacrificed on day 21 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and two-thirds of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and abnormalities were noted.

Early embryonic deaths were described as implantation sites with decidual or placental tissue only, while late deaths showed embryonal or fetal tissue with placenta at implantation sites according to the report.

The degree of ossification in the manus and pes was assessed according to the following scale:

1 = good---metacarpals/metatarsals and first, second, and third phalanges fully ossified.

2 = metacarpals/metatarsals and first and third phalanges fully ossified, some of second row not ossified.

3 = metacarpals/metatarsals fully ossified; all first and third row present, the majority fully ossified; most of second row not ossified, occassionally phalanx may be partially ossified.

4 = one metacarpal or metatarsal may be partially ossified, while the remainder of these bones may be fully ossified; second row of phalanges not ossified, most of first and third rows ossified.

5 = poor---one metacarpal or metatarsal partially ossified or not ossified at all, the remainder of these bones may be fully ossified; second row of phalanges not ossified, occassionally phalanges of the first and third rows partially ossified, and the rest are not ossified.

Major abnormalities were characterized as rare or possibly lethal, and minor abnormalities were defined as those commonly observed. The report stated that variations in the degree of ossification were considered as minor defects when observed to occur more frequently than similar observations in control or background data. Extra thoracic ribs were considered to be minor variants.

Statistical procedures are discussed below as apropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

Reported Results

The report stated that one rat died and four others were sacrificed in extremis. All of these animals were from the high dose group, and they died after 2 to 5 doses. The only clinical sign which was related to treatment according to the authors was staining of the genital and ventral areas. There were 4, 3, or 6 of 24 with the staining in the control, low, and mid dose groups, respectively, while 10 of the 19 survivors in the high dose group exhibited the effect.

Maternal bodyweight gain during the treatment period (days 6-15 of gestation) showed a dose-related decrease (not statistically significant). During that period the control, low, mid and high dose groups gained an average of 54.5, 53.2, 50.6, and 49.2 g, respectively. The only statistically significant difference between treated and control group means was reported for the high dose group dams during days 6-9 of gestation (3.9 g compared with 11.3 g for the control group; $p<0.01$, Student's t test). The authors also noted a slight decrease in bodyweight gain (8.8 g) during the same period for the mid dose group, but they noted no statistical significance.

Group mean food consumption for the high-dose group was also statistically significantly less than the control group. The control group animals consumed an average of 23.4 g of food per observation period during dosing compared with 20.7 g for the high dose group ($p<0.01$, Student's t test). During days 6-9 and 9-12 of gestation the mean food consumption values for the high dose group were 15.2 and 20.6 g, respectively. The respective control group values for the two times were reported to be 20.4 and 23.4 g.

The ratio between bodyweight gain and food consumption (g bodyweight gain per 100 g food consumed) was significantly decreased in the high dose group below that reported for the control group dams during days 6-9 of gestation. The reported group means were 17.8 and 5.3 ($p<0.01$, Student's t test).

At necropsy the investigators noted pallor, lobulation, and enlargement of the livers in 10 of the 19 survivors in the high-dose group dams as well as the 5 which died during the study. Pallor of the kidney was also noted in the high dose group animals. No other group was reported to have dose related gross pathology.

The reported group mean corpora lutea per dam ranged from 13.5 in the mid dose group to 14.7 in the control group. Group mean implantations per dam ranged from 12.8 in the low and mid dose groups to 13.7 in the control group (high-dose group averaged 13.0), and the group mean number of live fetuses per litter ranged from 11.8 in the mid-dose group to 12.7 in the control group (the mean for the high dose group was 12.4). None of these three parameters exhibited a relationship to dose.

Group mean gravid uterine weights for the control, low, mid and high-dose groups were reported to be 86.5, 86.0, 83.1,

and 87.2 g, respectively. The respective mean fetal weights were 5.1, 5.2, 5.3, and 5.1 g for the control, low, mid, and high dose groups.

The overall incidence of fetuses with defects in each group was reported as follows:

	Dose groups			
Observation	Control	Low	Mid	High
	External/visceral			
No. examined*	305	297	283	234
With external defects (%)	15 (5)	16 (5)	12 (4)	12 (5)
	Skeletal			
No. examined**	204	198	190	153
With defects (%)	84 (41)	110 (56)	117 (61)	111 (73)

*All fetuses were examined externally. Also includes those examined for visceral abnormalities (one-third of the fetuses.

**Two-thirds of the fetuses were examined for skeletal defects.

The authors noted that there were 3, 2, 1, and 3 fetuses in the control, low, mid, and high dose groups with major defects. One fetus from the low dose group was reported to have cleft palate along with three from the high dose group. Two of the latter group were litter mates, and the third exhibited exencephaly according to the report. The other major defects noted included hydrocephaly and multiple defects of the vertebrae, sternebrae and ribs in effected fetuses.

The report stated that a dose-related increase in the incidence of skeletal defects was observed in fetuses from treated dams. The defect which contributed most to the increase was classified as a minor defect and involved partial ossification of the 7th cervical vertebra's transverse processes. Incidence data for this and other skeletal observations which were reported to be dose-related are summerized as follows:

Observation	Dose groups: Control	Low	Mid	High
No. examined	204	198	190	153
Cervical defect (%)	13 (6)	32 (16)	49 (26)	47 (31)
Extra rib (uni lateral) (%)	22 (11)	36 (18)	101 (53)	104 (68)
Extra rib (bi- and unilateral) (%)	54 (26)	54 (27)	135 (71)	126 (82)

Partial ossification was also noted in the mid and high dose group fetuses in the odontal bone as well as in the occipital bone of high dose group fetuses. Control, mid, and high dose groups had 9.3, 18.9, and 23.5% of the fetuses with the first effect, while the high dose group and controls had respective incidences of 11.1 and 2.5% for the latter effect.

Discussion and Conclusions

The data presented in the report are adequate to support the conclusions of the investigators. They concluded that the no-observed-effect level (NOEL) for maternal toxicity with respect to decreased bodyweight gain during dosing (days 6-9 of gestation) is 40 mg/kg/day (lowest dose tested). The lowest-effect dose (LEL) is 100 mg/kg/day. The highest dose caused mortality (5/24 animals in the group) as well as grossly observable liver effects (pallor and enlargement).

Fetuses exhibited a dose-related increase in the incidence of delayed ossification at all doses, and the authors concluded that a NOEL for these effects was not established. They also presented a discussion of the incidence of cleft palate observed in the study. They stated:

> Cleft palate is rare as a spontaneous abnormality in the Alderley Park rat with a historic incidence of 1 in approximately 1500 fetuses in recent studies...in this Laboratory...The observed incidence of cleft palate in this study at 250 mg/kg/day paclobutrazol may be of biological significance...When the results of the preliminary study are taken into account (Dosages of 80 mg/kg/day caused cleft palate in 1 of 110 fetuses.) the possibility of a treatment related effect cannot be ignored.

These effects occurred at maternally toxic doses.

The dose related increase in the number of fetuses with skeletal abnormalities is associated with the increases in delayed ossification as shown in the tabulated summaries of incidence data above. However, the uncertainty with regard to the occurrence of cleft palate in fetuses from treated dams in this and a preliminary study suggest that paclobutrazol may have a teratogenic potential at maternally toxic doses.

Core classification: Supplementary since there is no NOEL for fetal effects.

DATA EVALUATION RECORD

Citation: Killick, M. E., M. H. Litchfield, P. B. Banham, and M. R. Thomas. July 14, 1983. Paclobutrazol: Teratogenicity study in the rabbit. Unpublished report no. CTL/P/861 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 92.4% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Female New Zealand white rabbits weighing between 2.7 and 4.0 kg were given an intravenous injection of chorionic gonadotropin 3 weeks prior to mating. These rabbits were then mated with proven males or artificially inseminated, and one hour after mating they were given chorionic gonadotropin again to insure ovulation. Day 0 of gestation was designated the day of mating.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 18 of gestation. Doses of 0, 25, 75, or 125 mg test substance per kg body weight were given to groups of 18 mated does. Dosages were adjusted according to bodyweights obtained on gestation days 6 through 18.

Each doe was observed daily for occurrence of toxic signs and mortality. In addition to bodyweight determinations mentioned above the animals were weighed on days 0 and 29. Food consumption was estimated for three day periods throughout gestation according to the report.

The rabbits were sacrificed on day 29 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each doe were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and dissected, and abnormalities were noted. The numbers of early and late resorptions as well as abortions were also recorded.

Early embryonic deaths were classified as implantation sites with decidual or placental tissue only, while late deaths showed embryonal or fetal tissue with placenta at implantation sites according to the report.

The degree of ossification in the manus and pes was assessed according to the following scale:

1 = good---metacarpals/metatarsals and first, second, and third phalanges fully ossified.

2 = metacarpals/metatarsals and first and third phalanges fully ossified, some of second row not ossified.

3 = metacarpals/metatarsals fully ossified; all first and third row present, the majority fully ossified; most of second row not ossified, occassionally phalanx may be partially ossified.

4 = one metacarpal or metatarsal may be partially ossified, while the remainder of these bones may be fully ossified; second row of phalanges not ossified, most of first and third rows ossified.

5 = poor---one metacarpal or metatarsal partially ossified or not ossified at all, the remainder of these bones may be fully ossified; second row of phalanges not ossified, occassionally phalanges of the first and third rows partially ossified, and the rest are not ossified.

Major abnormalities were characterized as rare or possibly lethal, and minor abnormalities were defined as those commonly observed. The report stated that variations in the degree of ossification were considered as minor defects when observed to occur more frequently than similar observations in control or background data. Extra thoracic ribs were considered to be minor variants.

Statistical procedures are discussed below as apropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

Reported Results

Of the 18 female rabbits naturally mated or artificially inseminated in each test group 10, 12, 15, or 9 were reported to be pregnant in the control, low, mid, and high dose groups. Two animals from the mid dose group aborted and were sacrificed prior to the end of the experiment, and one animal from the control group was killed in extremis. Two from the high

dose group and one from the control group were found dead. The report stated that no compound-related changes could be found during macroscopic examination of these animals.

There were 9, 12, 13, and 7 rabbits with live fetuses at the end of the experiment in the control, low, mid, and high dose groups, respectively. One of the pregnant animals in the high-dose group was reported to carry no live fetuses at the end of the study.

The investigators noted no clinical or macroscopic changes related to treatment with the test substance. During the first three days of dosing (days 6-9 of gestation) the group mean bodyweight gain for the high dose group (-0.09 kg) was statistically significantly less than that for the control animals (0.01 kg) at $p<0.05$ (two-tailed t test). At all other times there were no statistically significant differences between the treated groups and controls with respect to mean bodyweight gain. However, the authors noted that the animals in the mid-dose group also exhibited slightly lower weight gains during the dosing period than did those in the control group.

Decreased food consumption was also reported for the high dose group animals. The authors pointed out that the decreases occurred before dosing began (days 0-3 of gestation) and during dosing (days 6-9 and 12-15 of gestation). The pre-treatment means were the only ones which were reported to be statistically significantly different (539 g for controls and 402 for the high-dose group; $p<0.05$, two-sided t test).

Litter data (group means, per doe) are summarized from the report as follows:

		Dose groups		
Observation	Control	Low	Mid	High
Corpora lutea*	9.7	9.9	9.8	9.0
Implantations*	8.0	8.3	8.6	7.3
Viable fetuses*	7.0	7.4	7.5	6.5
Early deaths**	6.9	9.0	10.7	8.6
Late deaths**	5.6	2.0	2.7	1.7
Mean fetal weight***	38.6	38.8	39.5	38.9

*Group mean per doe
**Mean per cent

According to the report there were 63, 89, 97, and 52 fetuses

examined in the control, low, mid, and high-dose groups, respectively. The most frequently noted variation was slight renal pelvic dilatation which was observed in 9 of the low dose group fetuses. No other group contained fetuses with the variation with the exception of one from the mid dose group with moderate renal pelvic dilatation. All other visceral abnormalities were observed in one fetus from each group. The authors noted that one fetus from a high-dose group doe had several major abnormalities including exencephaly, a cardiac anomaly (not described in detail), clubbed foot, shortened forelimb, and multiple vertebral defects in the thoracic region. The report further stated that one fetus from the high-dose group had encephalocoele, and two others had multiple vertebral defects in the thoracic region.

One fetus in the mid-dose group was reported to have a cardiac anomaly, and another was found to have fused sacral vertebrae. The low-dose group contained one fetus with severe flexion of a forepaw and a second fetus with vertebral defects.

The other frequently reported skeletal defects included partially ossified hyoid and partially ossified transverse processes of the 4th lumbar vertebra. The incidence of these anomalies is summarized as follows:

Dose group	Hyoid	Lumbar Vertebra
Control	2/63	0/63
Low	8/89	5/89
Mid	7/97	8/97
High	0/52	0/52

A skeletal variant which was reported to occur at relatively high overall incidence was 27 pre-sacral vertebrae which involved 49.2, 18.0, 36.1, and 44.0% of the fetuses in the control, low, mid, and high dose groups. Extra ribs were also noted in a high percentage of the fetuses from each group (approximately 60% of the fetuses from each group).

Delayed ossification of the sternebrae was reported in approximately 20% of the fetuses in the control, low, and mid dose groups, while approximately 30% of the fetuses in the high dose group exhibited this variation.

The overall incidence of fetuses with external and visceral defects (including major and minor defects) was reported to be 1.6, 15.7, 8.2, and 7.7% in the control, low, mid, and high dose groups, respectively. The respective percentage

incidence of fetuses with major or minor skeletal defects was reported to be 27.0, 37.1, 46.4, and 30.8 for the control, low, mid, and high dose groups. The percentage of fetuses with skeletal variants was approximately 80% in the control, mid, and high dose groups while the low dose group had approximately 70% of the fetuses with skeletal variants.

The assessment of ossification in the pes and manus did not show a dose related effect on the scores according to tabulated group means.

Discussion and Conclusions

The authors indicated in the report that fertility of the rabbits was low. The report describes the use of both natural mating and artificial insemination as well as the use of more than the minimum number of rabbits recommended for each test group to compensate for that difficulty. Only the low and mid dose groups contained the minimum number of litters at terminal sacrifice of the test according to the report. These problems limit the sensitivity of the study with respect to detection of a potential teratogen, but they do not completely exclude the possibility of detecting a potentially teratogenic or fetotoxic test material. The extent of the limitations on sensitivity cannot be determined without additional and more detailed historical data from the laboratory in which the test was conducted.

Because of these limitations the study should only be used as supplementary information in an assessment of the teratogenic potential of paclobutrazol.

Under the limited test conditions the no-observed-effect level (NOEL) for maternal toxicity (decreased bodyweight gain during the dosing period) is likely to be 25 mg/kg/day and the lowest effect level (LEL) is likely to be 75 mg/kg/day. There were no effects on the fetuses of low and mid dose group does that could be attributed to the test substance (likely NOEL is 125 mg/kg/day), but an additional study should be conducted to confirm those results.

Core classification: Supplementary. See limitations which are discussed above.

003813

DATA EVALUATION RECORD

Citation: Phillips, C. E., C. R. Richardson, D. Hart, and E. Longstaff. August 4, 1983. An evaluation of Paclobutrazol in the mouse micronucleus test. Unpublished report no. CTL/P/848 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 92.4% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol.

Test species: Six to 8 week old male and female C57/BL/6J mice were used.

Preliminary study: A preliminary acute intraperitoneal toxicity study was conducted with male mice to determine dosage levels to be used in the main study. Single i. p. injections of 100, 250, 500, 750 or 1000 mg test substance per kg bodyweight were administered in corn oil to groups of 4 or 5 male mice. The animals were observed for mortality for 7 days following dosing.

Main study: Groups containing 15 male and 15 female mice were given corn oil, a 60 mg/kg dose of cyclophosphamide in physiological saline, and 140 or 87.5 mg test substance per kg bodyweight by intraperitoneal injection. Five animals of each sex from each group were sacrificed 24, 48, or 72 hours after the single i. p. injections of test substance. The femur of each mouse was removed, and marrow was taken with a fine paint brush moistened with 6% albumin. Three streaks of the bone marrow suspension were made on clean dry glass slides, and the slides were dried and stained with methylene blue. Five-hundred polychromatic erythrocytes were examined in each streak (3 per slide). Cells containing micronuclei were noted along with any evidence of cytotoxicity.

Treated groups were compared with the negative control group by a one-tailed Student's t test for statistical significance of differences in group means for cell counts.

Reported Results

There was a statistically significant increase in the incidence of polychromatic erythrocytes with micronuclei in the positive control group at 24, 48, and 72 hours after dosing as well as a statistically significant increase in those cells from mice given the 140 mg/kg dose. The corn oil control and cyclophosphamide dose group responses were reported as follows:

Control Group	24 hr	48 hr	72 hr
Negative	2.8	3.4	4.8
Positive	23.6	20.6	9.8

The reported mean incidence of polychromatic erythrocytes containing micronuclei for the 140 mg/kg mice was 6.2. Individual animal data reported for mice given the corn oil vehicle alone indicated a range of 0 to 12 cells with micronuclei per 1000 examined. In the high dose group the range was reported to be 2 to 12 in the males and 4 to 6 in the females.

Discussion and Conclusions

In the discussion of results the authors of the report note the unusual nature of the variation they observed in the negative control group. They stated that normal control ranges are from 0 to 6 cells containing micronuclei per 1000 examined. On that basis, they conclude that the statistically significant increased incidence of cells with micronuclei in the high dose group is not likely to be biologically significant.

The variability of the control results limits the sensitivity of the assay, but results with cyclophosphamide show that the conditions of the test are adequate to detect a potent clastogen.

Based on these considerations the conclusions of the authors with respect to Paclobutrazol are apropriate.

It is unlikely that paclobutrazol increases the incidence of polychromatic erythrocytes with micronuclei in mice treated with doses equivalent to 50 or 80% of the acute intraperitoneal LD_{50}.

003813

DATA EVALUATION RECORD

Citation: McGregor, D. B., and C. G. Riach. March, 1983. PP333: Assessment of mutagenic potential in the mouse lymphoma mutation assay. Unpublished report no. 2529 prepared by Inveresk Research International. Submitted by ICI Americas. EPA Acc. No. 251747.

Materials and Methods

Test substance: PP333 (Y00001/001/011) was used. No purity was given. The reference mutagens included ethyl methanesulfonate (EMS) and 2-acetylaminofluorene (2-AAF), and the inducer substance was Aroclor 1254.

Test species: Mouse lymphoma (L5178Y cells) heterozygous at the thymidine kinase (TK) locus were used.

Media, cultures, and S-9 mixture: For growth and maintenance of cultures Fischer's medium supplemented with penicilin, streptomycin, sodium bicarbonate, sodium pyruvate, pluronic acid, glutamine, and donor horse serum was used . This medium was designated $F_{10}P$ in the report. Cloning medium contained additional serum and sodium pyruvate.

Cultures were incubated at 37° C in an atmosphere of 5% CO_2 and 95% air. Cell density of cultures was calculated using a hemocytometer, and cultures were diluted with the growth medium to a density of 3 X 10^5 cells/ml.

Cultures were "cleansed" of $TK^{-/-}$ cells which spontaneously arise from mutations of heterozygous ($TK^{+/-}$) cells. This procedure used two stock solutions in the base medium (culture medium without serum). One solution which was designated THG in the report contained thymidine, hypoxanthine, and glycine, and the other which was designated THGM contained methotrexate in addition to the three other ingredients. Approximately 5 days prior to initiation of the experiment THGM was added to a culture which was then incubated for 24 hours. At that time a sample containing 3 milion cells was centrifuged, and the pellet of cells was resuspended in culture medium with THG and incubated for three more days.

For preparation of the S-9 mixture used in the metabolic activation experiments (see below) male Fischer 344 rats were used. The animals were given a single 500 mg/kg i. p. injection of Aroclor 1254, and the rats were sacrificed 5 days later. The livers were removed and homogenized, and the

homogenate was centrifuged at 9000 X g for 10 minutes. Supernatant was decanted and stored in liquid nitrogen. Before use in the mutation assays samples of the supernatant were evaluated for total protein concentration, cytochrome P450/P_1-450 concentration, benzo(a)pyrene hydroxylase activity, and N-demethylase activity. The S-9 mix also included phosphate buffer (pH 7.4), NADP, glucose-6-phosphate, $MgCl_2$, and KCl.

Toxicity test: Culture samples containing 3 X 10^6 cells/ml were incubated with the test substance in saturated solutions or 4 and 10-fold dilutions of the saturated solution. Each of the three test solutions was tested in the presence or absence of the S-9 mixture. These cultures were incubated for 3 hours, and then harvested by centrifugation. The harvested cells were resuspended in growth medium and incubated for the next 3 days. Daily cell counts were made.

Main experiment: Based on the results of the toxicity studies doses of 1.0, 3.3, 10, 33, and 100 ug/ml medium were used in the first experiment and 60, 80, 100, 120, and 140 ug/ml were used in the second. Concentrations of EMS used (for experiments without the S-9 mix) were 200 and 400 ug/ml, while those for 2-AAF (used in studies with the S-9 mix) were 50 and 100 ug/ml. The solutions of these substances were prepared in methanol which was also added to control cultures.

After 3-hour incubation periods with the test substance and reference mutagens with or without the S-9 mix, the cells were harvested in the same manner as described above. The centrifuged cultures were resuspended in culture medium. A sample of each suspension was plated on soft agar to determine day 0 survival.

After sampling of cultures for the survival assays, the cultures were incubated for 3 days. On the third day following exposure of the cells to test substances, samples were again taken and assayed for survival. Then the cultures were grown in cloning medium containing trifluorothymidine (designated TFT medium) for selection of mutant cells. These cultures were then incubated on TFT plates for 8 to 10 days. At the end of the incubation period the colonies on each plate were counted. There were 3 plates per dose level.

According to the report the number of colonies observed in the Day 3 survival assay and the number of TFT resistant colonies found on the selective medium were used to calculate the mutation frequency. The authors estimated that 1.5 X 10^6

cells were plated in each dish. Frequencies were expressed as the number of mutants per 100,000 surviving cells.

The criteria used to indicate a positive response were a doubling in a treatment group of the mutation frequency over that observed in the vehicle control group as well as a dose-related increase shown in at least two consecutive doses. The authors also stated that an observable increase in the absolute mutation frequencies was desirable to indicate a significant effect of the chemical on mutation frequency.

Reported Results

The toxicity assay showed that all cells exposed to the 1025 ug/ml concentration with and without the S-9 mixture were killed. Survival relative to vehicle controls at lower doses are summarized from the report as follows:

Concentration (ug/ml)	Survival (% vehicle control) With S-9	Without S-9
0.1	55	124
1.0	91	134
10.3	74	91
102.5	3	6

Mutaticn frequencies are summarized in the table below.

Discussion and Conclusions

There were adequate data presented in the report to support the conclusions of the authors that the test substance did not increase the frequency of mutations at the TK locus in L5178Y cells under the test conditions.

SUMMARY OF REPORTED RESULTS

Compound	Concentration (ug/ml)	Mutation frequency (per 10^5 survivors)* With S-9	Without S-9
Methanol	---	7	4
		7	3
2-AAF	50	19	-
		24	-
	100	200	-
		30	-
EMS	200	-	17
		-	12
	400	-	27
		-	29
Paclobutrazol	1.0	5	2
	3.3	6	3
	10.0	5	3
	33.3	4	4
	100.0	5	4
	60	5	4
	80	7	5
	100	7	5
	120	6	4
	140	8	6

*Means of results from 3 culture dishes per dose.

DATA EVALUATION RECORD

Citation: Jones, B. K., C. K. Choo, D. M. Williams, and A. R. Soames. August 9, 1983. Paclobutrazol: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat. Unpublished report no. CTL/P/870 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

Materials and Methods

Test substance: The test substance contained 99% (w/w) (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol. Radiolabelled paclobutrazol (^{14}C in the triazol ring) was also used. The specific activity of the labelled test substance was reported to be 1.76 GBq/mM.

Test species: Eight to 11 week old male and female Wistar rats were used.

Analytical procedures: Plasma, urine, fecal extracts, and cage washing samples were added to scintillant for direct liquid scintilation counting. Samples of adipose tissue, gonads, kidneys, liver, feces, and residues from fecal extracts were weighed and homogenized. The resulting homogenate was then oxidized and the radioactivity was determined from the resulting $^{14}CO_2$.

Expired air was drawn through hexane, methanol, and sodium hydroxide traps, and the radioactivity in each trap was measured in liquid scintillant.

Feces were extracted with methanol, and the extracts were also assayed by liquid scintillation directly.

Experimental procedures: The study was conducted in two parts. The first part consisted of autoradiography of one treated rat of each sex, and the second involved treatment of three rats of each sex. All animals received a single dose by gavage of 10 mg test substance per kg bodyweight. The specific activity of the dose administered in the autoradiographic experiment was reported to be 10 MBq/kg, while that for the dose administered in the second experiment was reported to be 2 MBq/kg.

Urine and feces were collected at 24 hour intervals after dosing and assayed (see above) to determine the amount of radioactivity present. Expired air was sampled over the 48 hours following dosing at 24 hour intervals, and assay of radioactivity in the traps (see above) was conducted.

Both animals in the autoradiography experiment were sacrificed 72 hours after dosing, while those rats in the second experiment were sacrificed 4 days after dosing.

Each cage was washed with an aliquot of methanol after removal of the animal, and the washings were assayed for radioactivity.

For autoradiography, the sacrificed animals were frozen and longitudinal sagital sections were prepared and exposed to x-ray film for 6 weeks. Based on results of developed autoradiographs tissues to be assayed in the second experiment were selected.

The tissues mentioned in the description of analytical procedures above were taken at sacrifice of the animals in the second experiment.

Reported Results

Group mean percentages of dose recovered in the urine and feces of the animals in the second study during the four days after dosing were reported as follows:

Sample	Males	Females
Urine	39.18	52.60
Feces	53.49	37.00
Cage washings	0.76	0.71
Tissues	0.05	0.09
Total	93.48	90.40

According to tabulated results in the report, the majority of the urinary excretion occurs during the first 24 hours following dosing. In male rats an average of 34.71% of the administered dose was recovered, while in female rats an average of 43.96% was recovered.

The majority of the dose excreted via the feces was recovered during the first 48 hours after dosing. Tabulated results indicated that males excreted 27.31% and 21.93% of the dose during the fisrt and second 24-hour sampling periods,

respectively. The respective recoveries for female rats were 15.34 and 14.74%.

At the end of the first 24 hours after dosing the average amounts recovered from urine and feces combined were approximately 60% in male and female rats.

The authors noted that the most intense areas of radioactivity in the whole body autoradiographs were in the gastrointestinal tract. The small intestine was reported to contain the highest intensity followed by the large intestine and the stomach. The report further stated that the liver contained radioactivity at an intensity similar to that observed in the stomach with an even distribution throughout the organ.

Residues found in the liver accounted for approximately 0.1% of the administered dose, while those in the kidneys, gonads, and whole blood each accounted for approximately 0.005% of the dose according to the report. The levels found in adipose tissue (renal fat samples) were approximately 8 times less than that found in the liver (based on ug equivalents of the test substance per g tissue). In female rats the level found in adipose tissue was 18 times less than that found in the liver. Blood levels were reported to be <0.01 ug equivalents/g.

Radioactivity was not found in expired air or in the solvent traps indicating that significant exhalation of metabolites is unlikely.

Discussion and Conclusions

The reported results are adequate to support the conclusions of the investigators. There was a sex difference with respect to the excretion pattern for paclobutrazole in that the major route of excretion in males appeared to be the feces, while that in female rats is the urine. Most of the urinary excretion occurs within 24 hours after dosing, but fecal excretion is slower with most of that occurring over the 48 hour period following treatment. Both of these results are indicative of significant absorption from the digestive tract.

It should be noted that the metabolites and tissue residues were not identified chemically, and the above mentioned conclusions are considered appropriate for the triazol containing residues. As indicated by the structural formula which was provided in the report, the test substance is structurally complex and is likely to be metabolized to unidentified residues which may have toxicological significance. Therefore, a complete evaluation of the metabolism of paclobutrazol is still needed.