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Tox-4352

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Use of Paclobutrazol on turf, ornamental plants, and trees (EPA Reg. Nos. 10182-EUP-GU, 10182-IE, 10182-TT, and 10182-EUP-GA). Tox. Chem. No. 628C.

TO: Robert J. Taylor
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Registration Division (TS-767C)

THRU: Jane Harris. Ph. D., Section Head Jane E. Harris 3-14-85
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

WDB 3/15/85

FROM: Roger Gardner, Toxicologist
Review Section 6
Toxicology Branch Roger Gardner 3-14-85
Hazard Evaluation Division (TS-769)

Actions Requested

1. Registration of 50WP formulations for use as an ornamental plant growth regulator (10182-IE, Record No. 131519) and as a tree growth retardant (10182-TT, Record No. 131518)

2. Experimental Use Permit for a 4 g/l formulation on container grown ornamentals (10182-EUP-GA, Record No. 142734)

3. Experimental Use Permit for a 50 WP formulation on grass seed crops (10182-EUP-GU, Record Nos. 131517, and 145722)

4. Review of the reports listed in Section IV (pages 12-14)

Recommendations and Conclusions

1. There are adequate data to support the Experimental Use Permits (10182-EUP-GU and -GA). However, the results of the rat teratogenicity study indicate that the label should require protective clothing if there is potential exposure for women of child bearing age.

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2. There was no information on the nature of the exposure associated with the ornamental plant and tree uses which would provide a basis for determination of data requirements (see point 8. below, for example). Therefore, comments on data needed in support of the two proposed uses on ornamentals and trees are deferred until information on the frequency of applications and the individuals is provided.

3. Acute toxicity data indicate that the a 4 g/l formulation should be classified into Toxicity Category IV for acute oral toxicity and Category III for acute dermal toxicity. The technical grade and 50% wettable powder formulations are classified into Toxicity Category III for acute dermal toxicity. The 4 g/l formulation is not a primary eye or skin irritant (Category IV), and it does not cause skin sensitization.

4. A one-year study in dogs established a NOEL of 15 mg/kg/day in the dog (Section II. B., page 6).

5. A NOEL of 10 mg/kg/day was established for fetotoxicity in rats (Sections II. C., page 6; and III. C., page 9).

6. No effects were seen in a dominant lethal study in mice or in an *in vivo* cytogenetics study in rats at doses up to 300 mg/kg (Sections II. D., page 6; and III. A. , page 8).

7. Rats and dogs readily absorb low and high oral doses of paclobutrazol, and excretion is rapid. The major route of excretion is the urine (Section II. E., page 7). Metabolites include free or conjugated diol and carboxylic acid forms of paclobutrazol with the halogenated phenyl and triazol groups remaining unchanged in rats (page 9).

8. Historical control data indicated that rabbits used in the teratogenicity study were possibly immature and that the artificial insemination technique had not been perfected at the time of the paclobutrazol study (Section III. C., page 11). In view of the NOEL for fetal effects noted in Point 5., above, the rabbit study should be repeated under the improved conditions indicated by the historical information. This requirement applies to uses for which significant exposure of human females of child bearing age can reasonably be expected (see §158.35).

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I. Background

A. Formulations and Uses

Paclobutrazol is a plant growth regulator with the chemical name (2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-(1,2,4-triazol-1-yl) pentan-3-ol. Its formulations, proposed uses and EPA Registration numbers are as follows:

Formulation=	Uses	EPA Reg. no.
PARLAY™ 50WP*	Grass seed yield enhancer	10182-EUP-GU
BONZI™ 50WP***	Ornamental plant growth regulator	10182-IE
CLIPPER™ 50WP	Ornamental tree growth regulator	10182-TT
BONZI™***	Plant growth regulator**	10182-EUP-GA

*The name of this formulation was changed from "PARLAY™ 50 WP Plant Growth Regulator" to "PARLAY™ Grass Seed Yield Enhancer" (see Record No.) and the data submitted for this registration also apply to the other 50WP formulations (see Record Nos. 13158 and 13159).
**Contains 0.4% active ingredient.
***These formulations are proposed for use in greenhouses. The BONZI™ Liquid (4 g/l) formulation is for container grown ornamentals, and BONZI™ 50WP is proposed for use on trees (by injection).

Confidential Statements of Formulation indicate that the three wettable powder formulations contain the [redacted]

Foliar application rates range from 0.60 to 4.8 mg active ingredient/ft^2, and soil drench application rates range from 0.12 to 3.8 mg/6 inch pot. Use dilutions recommended on the labels ranged from 31.3 to 250 ppm (118 to 944 mg/2 Qt for application to a 100 ft^2 area).

B. Previously Submitted Toxicity Data

1. Data Summary

The Toxicology Branch "One-liners" are included in Appendix I below.

The Toxicity Categories for acute toxicity are summarized as follows:

Type of Toxicity	Toxicity Categories: Technical grade	Wettable powder	4 g/l liquid
Acute oral	III	III	-*
Acute dermal	-*	III	-*
Acute inhalation	-**	II	-*
Primary skin irritation	III	IV	-*
Primary eye irritation	III	II	-*

*See Section II A., page 6.
**No data available.

The technical grade and 50% formulations did not cause skin sensitization in guinea pigs.

No-observed effect levels (NOEL) were established as follows:

Study	ppm	mg/kg/day
90-day feeding - rats	250	12.5
6-week oral - dogs*	---	15
26-week oral - dogs*	---	15

*Preliminary or interim reports (see Section II. B., page 6.

In a rat study the NOEL for maternal toxicity (decreased body weight gain during dosing) was 40 mg/kg/day (lowest dose tested). The LEL was 100 mg/kg/day. The 200 mg/kg/day (highest dose tested) caused mortality (5/24 animals in the group) as well as grossly observable liver effects (pallor and enlargement). A NOEL for fetal toxicity (delayed ossification) 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. Within those limitations the NOEL for maternal toxicity (as indicated by decreased body weight gain during the dosing period) was 25 mg/kg/day and the LEL was 75 mg/kg/day. There were no effects on the fetuses of low and mid dose group that could be attributed to the test substance (NOEL was 125 mg/kg/day, the highest dose tested).

Paclobutrazol 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.

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.

2. Data Requested

Letters from the Agency to the Registrant dated July 13 and 20, 1984 identified the following data gaps:

1. Additional mutagenicity testing is required (see FR Vol. 47, No. 227, November 24, 1982, pages 53193-53194 and 53195-53196).

2. Additional acute dermal testing for both the technical material and the 50 WP formulation is required. Existing data are not adequate to classify either the technical or the formulation into toxicity categories.

3. Additional teratogenicity studies are required. Existing rat data showed dose-related effects without establishing a no-observed effect level, and the rabbit study had deficiencies.

4. Additional metabolism studies are needed to identify residues and metabolites in treated animals.

II. New Data

Data Evaluation Records (DER) for the studies discussed in this section are included in Appendix II below.

A. Acute Toxicity

The acute toxicity results from submitted studies are summarized as follows (References 1-3):

Route	Formulation	Species	Sex	LD_{50} or I_{50}	Toxicity Category
Oral	4 g/l	Rat	Both	>5346 mg/kg	IV*
Dermal	4 g/l	Rat	Both	>2000 mg/kg	III*
Dermal	Technical	Rat	Both	>2000 mg/kg	III*
Dermal	50% WP	Rat	Both	>2000 mg/kg	III*

*Limit test, highest dose tested.

Two experiments (4) showed that the 4 g/l formulation did not cause eye or skin irritation in rabbits, and a third study (5) showed that the formulation is not a skin sensitizer in guinea pigs.

B. Subchronic Toxicity

Elevated serum alkaline phosphatase and triglyceride levels, enlarged hepatic cells, increased liver weights, and increased hepatic aminopyrine N-demethylase activity were observed in dogs given 75 or 300 mg/kg/day for one year. However, results with respect to liver weights in males and serum alkaline phosphatase levels in females were slight, and the 15 mg/kg/day dose was described by the investigators as approaching an absolute NOEL (6).

C. Teratogenicity

A NOEL for maternal toxicity in the rat was greater than 100 mg/kg/day (highest dose tested) (7). Dose-related fetal effects (renal dilatation, hydroureter, and minor skeletal defects or variations) were observed at 40 and 100 mg/kg/day dose levels, and a NOEL of 10 mg/kg/day was established for fetal effects (see Section III. C., page 9 for additional discussion).

D. Mutagenicity

Single oral doses of 30, 150, or 300 mg paclobutrazol per kg body weight in rats did not cause clastogenic effects in bone marrow cells (8). Paclobutrazol also caused no dominant lethal mutations in mice given doses from 25 to 300 mg/kg/day for 5 consecutive days (9).

E. Metabolism

A preliminary study in rats indicated that a 5 mg/kg dose was non-toxic and a 250 mg/kg dose was toxic on the basis of increased liver weights (10). Whole body autoradiographic studies with the 5 mg/kg dose indicated that significant ^{14}C were present in the liver, kidneys, and gastrointestinal tract (11). On the basis of these studies two single-dose studies were conducted with the 5 and 250 mg/kg doses of ^{14}C-paclobutrazol (12 and 13)

The two single-dose studies indicated that there were sex- and dose-related differences with respect to excretion of paclobutrazol and its metabolites. Recovery of radioactivity in excreta (expressed as a percentage of the dose) during the 72 hours following dosing are summarized as follows:

	5 mg/kg dose		250 mg/kg dose	
Sample	Males	Females	Males	Females
Urine	55.2	57.0	43.4	33.4
Feces	32.3	23.7	29.7	39.7
72 hr Total	87.5	80.7	73.1	73.1
7 day Total	96.5	98.1	89.1	100.1

In a repeated dose study with rats given the 5 mg/kg dose each day for up to 49 consecutive days (14), peak concentrations of ^{14}C-paclobutrazol and its metabolites were found in the liver and kidneys after 28 days. Blood levels gradually increased throughout the 49-day dosing period. The maximum levels for the liver, kidney, and plasma were reported to be 2.22-2.55, approximately 1, and 0.158 ppm, respectively. There was no accumulation of the parent or its metabolites in fat of treated rats. Radioactivity was cleared from the liver and kidneys in a biphasic manner with half lives of 1.36 to 1.5 days and 7 to 9 days for the first and second phases, respectively (14).

Bile duct cannulated rats given a single 5 mg/kg dose (15) excreted most of the ^{14}C-labelled parent and metabolites in the bile during the 48 hours immediately following dosing. Those results (expressed as percentage of the dose) are summarized as follows:

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Sample	Males	Females
Bile	74.40	33.79
Urine	19.08	15.85
Feces	1.65	0.46
72 hr Total	95.1	50.10
96 hr Total	----	94.91

The pentanol portion of the paclobutrazol molecule is oxicized in male and female rats to form free and conjugated diol and carboxylic acid metabolites (15). The proportion of carboxylic acid metabolites is greater in males than in females, but the female rats given the 5 mg/kg dose produce a higher proportion of the acid metabolites than those given the 250 mg/kg dose. In addition, unchanged paclobutrazol is found in rats given the higher dose (unmetabolized parent accounts for approximately 5% of the dose) (15).

The halogenated phenyl and triazole portions of the molecule remain unchanged in treated rats (15).

Paclobutrazol is also rapidly absorbed and excreted in the dog (16). Peak levels of ^{14}C-paclobutrazol and its metabolites are observed in the plasma are observed an hour after dosing (4.453 and 4.106 ppm in males and females, respectively), and they decline below the limit of detection (<0.016 ppm) within 24 to 48 hours after dosing. There are no sex differences with respect to urinary excretion patterns, and most of the administered dose is recovered 24 hours after treatment (approximately 50%). There were sex differences with respect to fecal excretion rates (males excreted 25.2% and females excreted 37.1% during the first 24 hours after dosing) (16).

III. Discussion

The following discussion is oriented toward the points described in Section I. B. 2., above (page 5).

A. Mutagenicity

As shown in sections I. B. 1., and II. D. (pages 4 and 6) there are five assays available for consideration. Two of the tests (in mouse lymphoma cells and Salmonella typhimurium) indicated that there were no genetic mutations associated with paclobutrazol in those test systems. The remaining assays (dominant lethal assay in mice, micronucleus assay in mice, and an in vivo cytogenetics assay in rats) suggest that the chemical is not associated with chromosomal effects.

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B. Additional Acute Toxicity Studies

The requested studies were submitted and are summarized in Section II. A. (page 5) above and discussed in detail in Appendix II below.

C. Teratogenicity

An overview of the teratogenicity studies in rats was submitted with the second study (17). The overview noted the increased incidence of cleft palate found in the first rat teratogenicity study, and the discussion further noted that those effects occurred at maternally toxic doses. The second study (described in Appendix II below) showed no similarly increased incidence of cleft palate which indicates that the defect is not related to paclobutrazol except in the presence of maternal toxicity. The previous Toxicology Branch review of the first rat teratogenicity study including a discussion of the incidence of cleft palate is provided in Appendix III below (exerpted from Gardner R. Memorandum to Robert J. Taylor, Registration Division, dated May 18, 1984. Subject: Use of Paclobutrazol on greenhouse ornamentals and grass seed crops. EPA Reg. No. 10182-IE and 10182EUP-GU. Acc. nos. 251746 and 251747. Tox. Chem. No. 628C).

The report also described a second effect which was observed in the second rat study, but was not found in the first. That description was as follows:

> Defects of the kidney and ureter were of low incidence and showed no evidence of a reponse to treatment in the first study. By contrast the second study showed a higher incidence of the defects in all groups, including controls. This indicates a change in the background incidence and/or an increased sensitivity of detection as subsequent studies in this Laboratory have also shown a comparable or higher incidence of these defects. At doses of 40 or 100 mg/kg/day paclobutrazol the incidence was further elevated...

The report for the second rat study included historical control data to substantiate the conclusion that a change in the spontaneous rate of the kidney and ureter defects has occurred. Table 1 summarizes those results.

The extent of the limitations associated with the rabbit study (section I. B., page 4) can be considered with historical data provided in the Registrant's response to the Agency's July 13, 1984 letter mentioned above (page 5). Details of

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TABLE 1

Historical and concurrent control data on the incidence (%) of kidney and ureter defects in Alpk/AP strain rats

Dates	Unilateral	Bilateral	Combined
Kidney: pelvic dilatation			
Mar-Apr, 1982	2.1	0.0	2.1
Jul-Aug, 1982	2.6	1.0	3.6
Nov-Dec, 1982	3.6	0.7	4.3
Jan-Feb, 1983	1.0	0.3	1.3
Jun-Jul, 1983*	9.1*	2.3*	11.4*
40 mg/kg/day	20.9	5.3	26.2
100 mg/kg/day	16.1	5.2	21.3
Oct-Nov, 1983	6.4	1.1	7.5
Nov-Dec, 1983	2.9	1.6	4.6
Ureter: dilated			
Mar-Apr, 1982	0.4	0.0	0.4
Jul-Aug, 1982	0.0	0.0	0.0
Nov-Dec, 1982	0.0	0.0	0.0
Jan-Feb, 1983	1.0	0.0	1.0
Jun-Jul, 1983*	7.6*	1.1*	8.7*
40 mg/kg/day	13.6	3.3	16.9
100 mg/kg/day	19.1	6.9	26.0
Oct-Nov, 1983	20.3	6.0	26.3
Nov-Dec, 1983	20.3	3.6	23.9
Ureter: kinked			
Mar-Apr, 1982	0.0	0.0	0.0
Jul-Aug, 1982	0.0	0.0	0.0
Nov-Dec, 1982	0.0	0.0	0.0
Jan-Feb, 1983	0.0	0.0	0.0
Jun-Jul, 1983*	7.2*	0.8*	8.0*
40 mg/kg/day	18.9	1.0	19.9
100 mg/kg/day	16.4	2.6	19.0
Oct-Nov, 1983	21.0	2.5	23.5
Nov-Dec, 1983	14.7	1.3	16.0

*Paclobutrazol study

the fertility problem were described in the previous Toxicology Branch review (cited on page 9) as follows:

> 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.

In the response to the Agency letter the Registrant states:

> ICI's Central Toxicology Laboratory (CTL) changed from the Dutch to the New Zealand White rabbit in the middle of 1982 since there were difficulties with supply and the majority of laboratories use the New Zealand White Rabbit for teratogenic assessment...
>
> In the first two studies with the New Zealand White Rabbit (the paclobutrazol study and the RB0253 February 1983) the pregnancy rate was lower than expected due we believe to animals being sexually immature (heavy for their age) and the laboratory not having perfected the artificial insemination technique. Subsequently animals were obtained from the breeder at a known age (minimum 15 weeks) at a slightly higher body weight...

These circumstances and the results of the rat studies described in Sections I. B., II. C. (pages and), and Appendix II below indicate that the rabbit study should be repeated.

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D. Metabolism

As noted in section II. E., above (page) there are adequate metabolism studies.

E. Data Gaps

There are adequate data to support Experimental Use Permits (EPA Reg. Nos. 10182-EUP-GU and 10182-EUP-GA) according to §158.135.

In view of the NOEL for fetal effects in rats (Section II. C., page 6) and the circumstances of the rabbit teratogenicity study discussed on page 11 above, the rabbit study should be repeated. This requirement applies to uses for which significant exposure of human females of child bearing age can reasonably be expected (see §158.35).

IV. Bibliography

1. Rhodes, C., C. McKillop, R. Booth, and J. Southwood. August 17, 1984. Paclobutrazol: Acute oral toxicity and acute dermal toxicity studies on a 4 g/l formulation. Unpublished report no. CTL/P/1053 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

2. Prichard, V. K. November 16, 1984. Paclobutrazol: Acute dermal toxicity. Unpublished report no. CTL/P/1173 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 256655.

3. Barber, J. E. December 11, 1984. Paclobutrazol: Acute dermal toxicity. Unpublished report no. CTL/P/1176 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 256655.

4. Scott, R. C., C. Rhodes, J. Scott and J. Southwood. May 17, 1984. Paclobutrazol: Skin irritation and eye irritation studies on a 4 g/l formulation. Unpublished report no. CTL/P/1051 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

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5. Southwood, J. May 18, 1984. Paclobutrazol: Skin sensitisation on a 4g/l formulation. Unpublished report no. CTL/P/779 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

7. Clapp, M. J. L., A. E. Kalinowski, D. T. Chalmers, I. S. Chart, C. W. Gore, M. D. Stonard, and M. J. Godley. May 29, 1984. Paclobutrazol: 1 year oral dosing study in dogs. Unpublished report no. CTL/P/958 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

8. Killick, M. E., G. H. Pigott, P. B. Banham, and M. R. Thomas. June 1, 1984. Paclobutrazol: Second teratogenicity study in the rat. Unpublished report no. CTL/P/997 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

9. Richardson, C. R., C. A. Howard, E. Longstaff, M. G. Thomas, P. B. Banham, S. L. Beck, and M. J. Godley. May. 2, 1984. Paclobutrazol: A cytogenetic study in the rat. Unpublished report no. CTL/P/891 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

10. Whickramaratne, G. A., D. L. Kinsey, P. B. Banham, and M. G. Thomas. December 29, 1983. Paclobutrazol: Dominant lethal study in the mouse. Unpublished report no. CTL/P /922 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

11. Jones, B. K., D. M. Williams,and J. Galvin. May 31, 1984. Paclobutrazol: The effect of a single oral dose (5 mg/kg or 250 mg/kg) on liver weight in the rat. Unpublished report no. CTL/P/1065 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

12. Cresswell, D.G., J. Ward, and R. Hopkins. February, 1984. (^{14}C)-Paclobutrazol: Excretion and tissue retention of a single oral dose (250 mg/kg) in the rat. Unpublished report no. 3268-72/268 prepared by Hazleton Laboratories

Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

13. Cresswell, D.G., J. Vickers, and R. Hopkins. October, 1983. (^{14}C)-Paclobutrazol: Excretion and tissue retention of a single oral dose (5 mg/kg) in the rat. Unpublished report no. 3456-72/267 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

14. Jones, B. K., D. M. Williams, J. Galvin, and A. R. Soames. May 31, 1984. Paclobutrazol: Whole body autoradiography study in the rat following a single oral dose (250 mg/kg). Unpublished report no. CTL/P/1035 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

15. Greenslade, D., J. Vickers, and R. Hopkins. May, 1984. (^{14}C)-Paclobutrazol: Bioaccumulation of repeated oral doses (5 mg/kg) in the rat. Unpublished report no. 3743-72/269 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

16. Jones, B. K., R. M. Ladd, and J. Galvin. May 31, 1984. Paclobutrazol: Biotransformation in the rat. Unpublished report no. CTL/P/1036 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

17. Cresswell, D.G., J. Ward, and R. Hopkins. February, 1984. (^{14}C)-Paclobutrazol: Absorption, excretion, and tissue retention of a single oral dose (5 mg/kg) in the dog. Unpublished report no. 3494-72/270 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

18. Pigott, G. H. May 31, 1984. Paclobutrazol: An overview of teratogenicity studies in the rat. Unpublished report no. CTL/P/1058 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

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APPENDIX 1

Toxicology Branch "One-liners"
for previously reviewed studies

Tox Chem No. 628 C (PP333 Paclobutrazol) File Last Updated 8/14/84

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; Imperial Chemical Industries PLC, Central Tox. Lab.; Report no. CTL/P/842; July 13, 1983	Technical (92.4%)	251747	250 mg/kg/day (highest dose tested) caused mortality (5/24), liver enlargement, and pallor of the liver. 100 mg/kg/day caused slight decrease in bodyweight gain and food utilization efficiency. NOEL for the maternal effects = 40 mg/kg/day (lowest dose tested). All three test doses increased the incidence of delayed ossification in fetuses. No NOEL established for fetotoxicity. The 250 mg/kg/day dose also induced cleft palate in 3 fetuses from 2 litters. Levels tested by gavage in Wistar derived Alderley Park strain - 0, 40, 100, and 250 mg/kg		Supplementary 003813
Teratology - rabbit; Imperial Chemical Industries PLC, Central Tox. Lab.; Report no. CTL/P/ /861; July 14, 1983	Technical (92.4%)	251747	Teratogenic NOEL > 125 mg/kg (HDT) Maternal NOEL = 25 mg/kg/day Maternal LEL = 75 mg/kg/day (decreased body weight gain) Feto toxic NOEL > 125 mg/kg (HDT) Low fertility with only the mid and low dose groups having the minimal number of animals recommended Levels tested by gavage in New Zealand white strain - 0, 25, 75 and 125 mg/kg		Supplementary 003813
90-Day feeding - rat; Imperial Chemical Industries PLC, Central Tox. Lab; CTL/P/760; July 16, 1983	Technical (91.9%)	251746	At 1250 ppm liver weights were elevated in females along with serum cholesterol, hepatic aminopyrene-N-demethylase activity, and alanine transaminase levels. NOEL=250 ppm. 50 ppm was the lowest dose tested. No effects in males.		Minimum 003813

Tox Chem No. 628C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
6-Week oral dosing-dog; Imperial Chemical Industries PLC, Central Tox. Lab.; CTL/P/767; June 16, 1983	Technical (91.9%)	251747	At doses of 75 and 225 mg/kg dogs had increased liver weights and serum alkaline phosphatase levels. NOEL = 15 mg/kg/day (lowest dose tested). Only one male and one female dog was tested at each dose.		Supplementary 003813
21-Day dermal - rabbits; Imperial Chemical Industries, Central Tox. Lab.; Report no. ICI/256/79822; March 17, 1980	Technical	251746	Applications of 10, 100, or 1000 mg/kg/day caused irritation of abraded skin. Intact skin was irritated by the 100 and 1000 mg/kg/day doses. Irritation appeared during the 2nd week, and dose-related effects included hyperkeratosis, acanthosis, inflammation of the superficial dermis. 1000 mg/kg/day caused severe irritation. NOEL (intact skin) = 10 mg/kg/day. LEL (intact skin) = 100 mg/kg/day.		
1-Year oral dosing - dog; Imperial Chemical Industries PLC, Central Tox. Lab.; Report no. CTL/P/812; July 18, 1983	Technical (91.9%)	251747	INTERI REPORT (26 weeks) The serum alkaline phosphatase levels in dogs receiving 75 or 300 mg/kg/day were elevated. Triglyceride levels were also increased by the highest dose tested (300 mg/kg/day). No effects were observed in dogs getting the lowest test dose (15 mg/kg/day).		Supplementary 003813

Tox Chem No. 628C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism - rat; Imperial Chemical Industries PLC, Central Tox. Lab.; Report no. CTL/P/870; August 9, 1983	99% a. i.	251747	During the four days following oral administration of a single 10 mg/kg dose 39.18 and 52.6% of the dose was recovered in the urine of male and female rats, respectively. Fecal recoveries were 53.49% in males and 37% in females. Approximately 60% of the administered dose was accounted for in feces and urine of both male and female rats during the first 24 hours after dosing. Residue concentrations in tissues 3 or 4 days after dosing did not indicate accumulation, and urinary excretion along with slow fecal excretion indicated significant gastrointestinal absorption of the dose.		003813
Mutagenic (point mutation assay) - mouse - lymphoma cells; Inveresk Research International; Report no. 2529; March, 1983.	Technical (92.4%)	251747	Mutation frequencies observed at concentrations of 1, 3.3, 10, 33, 60, 80, 100, 120, or 140 ug/ml in L5178Y cells in vitro were not increased above those observed for vehicle control cultures.		Adequate by "Gene Tox" 003813
Mutagenic (micronucleus test) - mouse; Imperial Chemical Industries PLC, Central Tox. Lab.;Report no. CTL/P/848; 8/4/83	Technical (92.4%)	251747	Doses of 87.5 or 140 mg/kg administered intraperitoneally did not increase the incidence of micronuclei in treated mice. The doses were equivalent to 50 or 80% of the acute i. p. LD_{50}.		Adequate by "Gene Tox" 003813
Mutagenic - ames test in salmonella; ICI Central Tox. Lab.; #CTL/P/722; 9/9/82	Technical (92.4% pure)	248688	Negative at doses up to 5000ug/plate in strains: TA 1535,TA 1537,TA 1538, TA 98, TA 100, with and without metabolic activation. Cytotoxicity and precipitation at 5000 ug/plate. 5 positive controls used.		Acceptable 003457

Tox Chem No. 628C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Dermal sensitization - guinea pig; ICI Central Tox. Lab.; #CTL/P/741; 9/24/82	Technical (92.4% pure)	248688	Not a skin sensitizer Dunkin Hartly albino guinea pigs used. (Males only).		Minimum 003457
Acute oral LD_{50} - rat; ICI Central Tox. Lab.; #CTL/P/748; 10/82	Technical (97% pure)	248688	LD_{50} = 1954 (1147-4985) mg/kg (M) LD_{50} = 1336 (837-1969) mg/kg (F) Toxicity: Appeared within 1 hr.; unsteady gait, loss of righting reflex, piloerection, coma, hypothermia, respiratory stress, urinary incontinence, survivors normal 9 days post treatment. Alderley Park SPF rats used.	III III	Minimum 003457
Acute oral LD_{50} - mice; ICI Central Tox. Lab.; #CTL/P/748; 10/82	Technical (97% pure)	248688	LD_{50} = 490 (394-642) mg/kg (M) LD_{50} = 1219 mg/kg(F)(no range given) Toxicity: Appeared within 1 hr.; same as rats; survivors normal 6 days post treatment. Alderley Park SPF mice used.	II III	Minimum 003457
Acute oral LD_{50} - guinea pig; ICI Central Tox. Lab.; #CTL/P/748; 10/82	Technical (97% pure)	248688	LD_{50} = 542 (432-717) mg/kg (M) LD_{50} = between 400 and 640 mg/kg (F) Toxicity: Appeared within 3 hrs.; subdued behavior, unsteady gait, survivors normal 3 days post treatment. Dunkin Hartley albino guinea pigs used.	III III	Minimum 003457
Acute oral LD_{50} -rabbit; ICI Central Tox. Lab; CTL/P/748; 10/82	Technical (97% pure)	248688	LD_{50} = 835 mg/kg (M) LD_{50} = 937 mg/kg (F) Toxicity: Appeared within 1 hr.; subdued behavior, unsteady gait, survivors normal 12 days post treatment. New Zealand white rabbits used.	III III	Minimum 003457

Tox Chem No. 628C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute dermal LD_{50} - rabbit; ICI Central Tox. Lab; #CTL/P/748; 9/82	Technical (97% pure)	248688	LD_{50} > 1000 mg/kg (M) (only dose tested). LD_{50} > 1000 mg/kg (F) (24 hr. exposure). New Zealand white rabbits used.	II II	Minimum 003457
Acute intraperitoneal LD_{50} - rat; ICI Central Tox. Lab.; #CTL/P/748; 9/82	Technical (97% pure)	248688	LD_{50} between 160 and 250 mg/kg (M) LD_{50} = 99 mg/kg (F) Toxicity: Appeared within 1 hr.; same as rats receiving oral dose; survivors normal 6 days post treatment. Alderley Park SPF albino rats used.		Minimum 003457
Primary dermal irrit.- rabbit; ICI Central Tox. Lab.; #CTL/P/741; 9/24/82	Technical (97.0% pure)	248688	Caused mild skin irritation that persisted for 72 hours. PIS: mean scores At 24 hrs. = 1.3/8 (intact) = 1.5/8 (abraded) At 72 hrs. = 1.0/8 (intact) = 1.0/8 (abraded) Dose tested: 500 mg/kg(in olive oil) 24 hr. exposure.(Albino New Zealand strain).	III	Minimum 003457
Primary eye irritation- rabbit; ICI Central Tox. Lab.; #CTL/P/741; 9/24/82	Technical (97.0% pure)	248688	Reversible diffuse corneal opacities with conjunctival irritation that persists for 72 hours. PIS = mean scores 17/110(unwashed)24hr. post treatment 14/110(washed) 48hr. post treatment. Dose tested: 100 mg of powdered test substance. Albino New Zealand strain	II	Minimum 003457
Acute dermal LD_{50} - rat; ICI Central Tox. Lab; CTL/P/748; 10/82	Technical (97.0% pure)	248688	LD_{50} > 1000 mg/kg (M) LD_{50} > 1000 mg/kg (F) (only dose tested). 24 hr. exposure	II II	Minimum 003457

Tox Chem No. 628C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOFL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irritation - rat; ICI Central Tox. Lab; CPL/P/741; 9/24/82	Technical (97.0% pure)	248688	Slight irritant: 3/5 (M) showed erythema. 2/5 (F) showed desquamation and scabbing. Effects did not persist. Dosage: suspension in PEG (12.5% W/V). 5 applications of 250 mg/kg during alternate 24 hr. periods. SPF Albino Alderley Park rats used.	IV	Minimum 003457
Dermal Sensitization - guinea pig; ICI Central Tox. Lab.; #CTL/P/742; 6/82	50% a.i. wettable powder	248688	Not a skin sensitizer Dunkin Hartley guinea pigs.		Minimum 003457
Acute oral LD_{50} - rat; ICI Central Tox. Lab.; #CTL/P/742; 6/82	50% a.i. wettable powder	248688	LD_{50} > 5000 mg/kg(only dose tested); (1/5 [M] and 1/5 [F] died). Alderley Park SPF rats. Toxic signs: appeared 48 hrs. post treatment, included; decreased activity, piloerection, cromodacryorrhea, upward curvature of spine, and pinched in sides. Persisted through 14 day observation period.	IV	Minimum 003457
Acute dermal LD_{50} - rabbit; ICI Central Tox. Lab.; #CTL/P/742; 6/82	50% a.i. wettable powder	248688	LD_{50} > 1000 mg/kg (only dose tested) LD_{50} > 1000 mg/kg (F) 24 hr. exposure. (1/5 [F] died)-New Zealand white albino rabbits.	II	Minimum 003457
Primary dermal irrit.- rabbit; ICI Central Tox. Lab.; #CTL/P/742; 6/82	50% a.i. wettable powder	248688	Mild irritation persisted for 72 hrs (24 hr. exposure). Dose tested: 500 mg/kg Intact skin: Well defined erythema and moderate edema. Abraded skin: no erythema to well defined erythema and slight to severe edema.	III	Minimum 003457

Tox Chem No. 628C

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation-rabbit; ICI Central Tox. Lab.; #CTL/P/742; 6/82	50% a.i. wettable powder	248688	Reversible diffuse corneal opacities with irritation. Opacities persisted for up to 4 days. Dose tested: 100 mg powdered test substance. PIS: 25/80 at 24 hr. New Zealand white albino rabbits.	II	Minimum 003457
Acute inhalation LC_{50}-rat; ICI Central Tox. Lab.; #CTL/P/759; 10/4/82	50% a.i. wettable powder	248688	LC_{50} > 766 mg/m^3 (M) (no deaths) LC_{50} = 359-766 mg/m^3 (F) (doses causing 0 to 100% mortality) Alderley Park Wistar rats.	II	Minimum 003457
Acute oral LD_{50} - rat; WARF Inst.; #60064414; 10/19/77	Granular (0.40% a.i.) Scotts Turf Builder 27-3-3		LD_{50} > 20 g/kg (males) (only dose tested). No signs of toxicity or mortality. Sprague Dawley rats.	IV	Minimum 003457
Acute dermal LD_{50} - rabbit; WARF Inst.; #60064414; 10/19/77	Granular (0.40% a.i.) Scotts Turf Builder 27-3-3		LD_{50} > 8 gm/kg. (Male) 24 hr. exposure. (Only dose tested). New Zealand white rabbits.	IV	Minimum 003457
Primary eye irritation - rabbit; WARF Inst.; #60064414; 10/19/77	Granular (0.40% a.i.) Scotts Turf Builder 27-3-3		Causes no irritation Dose tested: 0.1 g amount of the formulation. New Zealand white rabbits.	IV	Minimum 003457
Primary dermal irrit.-rabbit; WARF Inst.; #60064414; 10/19/77	Granular (0.40% a.i.) Scotts Turf Builder 27-3-3		No irritation was found. 24 hr. exposure. (5 g amount of the formulation). New Zealand white rabbits.	IV	Minimum 003457

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APPENDIX II

Data Evaluation Records for
studies listed in Section IV

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: 4 g/L liquid, 0.39% active ingredient (Reference nos. D2110/40, JF9457).

3. STUDY/ACTION TYPE: Acute oral and dermal - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Rhodes, C., C. McKillop, R. Booth, and J. Southwood. August 17, 1984. Paclobutrazol: Acute oral toxicity and acute dermal toxicity studies on a 4 g/l formulation. Unpublished report no. CTL/P/1053 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 254865.

5. REVIEWED BY:

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Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
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Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSIONS: There were no mortalities in this study, and the acute oral LD_{50} is >5346 mg/kg. The results are sufficient to place the 4g/l formulation into Toxicity Category IV with respect to acute oral toxicity.

The acute dermal LD_{50} is >2 ml/kg, and the results indicate that the formulation should be classified into Toxicity Category III for acute dermal toxicity.

Core classification: Minimum for both studies

8. MATERIALS AND METHODS

Test species: Five to seven-week old male and female Alderley Park specific pathogen free rats were used. The males weighed from 200 to 315 g, and the females weighed from 150 to 212 g.

a. Acute oral study: One group containing 5 male and 5 female rats was given a single dose of 5346 g test material per kg body weight by oral gavage. Test animals were weighed on the day prior to dosing, the day of treatment, and on days 3, 8, and 15 after dosing. Observations for the occurrence of toxic signs and mortality were made between 30 and 90 minutes, 4 and 7 hours, and daily throughout the 15-day observation period following dosing.

At the end of the observation period surviving animals were sacrificed and subjected to gross necropsy.

b. Acute dermal study: A 100 X 50 mm area of the dorso-lumbar region of rats in a group containing 5 males and 5 females was clipped free of hair, and 2 ml undiluted test material per kg body weight was applied to the prepared skin. The test sites were then occluded with gauze and adhesive tape, and after 24 hours the occulusive dressings were removed. The treated skin was washed to remove excess test substance.

Test animals were weighed, observed for appearance of toxic signs, and examined at necropsy according to the same schedule as that described for the acute oral study in paragraph 8. b., above.

9. REPORTED RESULTS

No mortalities were observed in either study according to the report.

The only changes noted in the acute oral study were decreases in body weight resulting from fasting of the animals prior to dosing.

The report stated that signs of toxicity were observed in 9 of the 10 animals in the dermal study on the second day after dosing and in the remaining animal on day 7 following treatment. The most frequently observed signs were diarrhea and urinary incontinence. No signs of skin irritation were observed by the investigators, and the test animals were reported to have decreased body weights early in the observation period. The group mean body weights on the day of dosing were 288 and 202 g for males and females, respectively,

9. REPORTED RESULTS: (continued)

and the respective weights on day 3 after dosing were 282 and 199 g. By the eighth day the group mean body weights were increased in comparison to those reported for day 1 (297 g for males and 219 g for females).

10. DISCUSSION:

The report stated that preliminary studies were conducted to determine the doses to be tested, but those experiments and their results were not included. The report also contained individual animal data for all observations, and large doses were used. There is enough information to support the conclusions of the authors, and there is no need for further comment about the studies.

001352

[redacted]

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Technical grade 92.4% active ingredient (Reference nos. P29 D2517/62).

3. STUDY/ACTION TYPE: Acute dermal - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Prichard, V. K. November 16, 1984. Paclobutrazol: Acute dermal toxicity. Unpublished report no. CTL/P/1173 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 256655.

5. REVIEWED BY:

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Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSIONS: The acute dermal LD_{50} is >2 g/kg, and the results indicate that the formulation should be classified into Toxicity Category III for acute dermal toxicity in rats.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Eight to ten-week old male and female Alderley Park specific pathogen free rats were used. The males weighed from 281 to 349 g, and the females weighed from 213 to 228 g.

Experimental procedure: A 100 X 50 mm area of the dorso-lumbar region of rats in a group containing 5 males and 5 females was clipped free of hair, and 2 ml undiluted test material per kg body weight was applied to the prepared skin. The test sites were then occluded with gauze and adhesive tape, and after 24 hours the occulusive dressings were removed. The treated skin was washed to remove excess test substance.

Test animals were weighed on the day of treatment and on days 3, 8, and 15 after dosing. Observations for the occurrence of toxic signs and mortality were made between 30 and 90 minutes, 4 and 7 hours, and daily throughout the 15-day observation period following dosing.

At the end of the observation period surviving animals were sacrificed and subjected to gross necropsy.

9. REPORTED RESULTS

According to the report there were no mortalities in treated animals during the study. The most frequently observed signs of toxicity were urinary incontinence and upward curvature of the spine. These signs were reported to appear on the day of dosing and persisted until the fifth day after dosing. The investigators also noted skin irritation which they described as desquamation of test sites with small scattered scabs.

The report stated that some rats lost weight initially, but all animals with the exception of one female gained weight by the 8th day after dosing. By the end of the observation period all animals gained weight.

The investigators stated that no gross lesions were observed at necropsy.

10. DISCUSSION

There were adequate data presented to support the conclusion that the acute dermal LD_{50} is >2 g/kg, and the results indicate that the formulation should be classified into Toxicity Category III for acute dermal toxicity in rats.

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: 50WP Formulation (49.9% active ingredient (Reference nos. GFU 029).

3. STUDY/ACTION TYPE: Acute dermal - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Barber, J. E. December 11, 1984. Paclobutrazol: Acute dermal toxicity. Unpublished report no. CTL/P/1176 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 256655.

5. REVIEWED BY:

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6. APPROVED BY:

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7. CONCLUSIONS: The acute dermal LD_{50} is >2 g/kg, and the results indicate that the formulation should be classified into Toxicity Category III for acute dermal toxicity in rats.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Five to seven-week old male and female Alderley Park specific pathogen free rats were used. The males weighed from 230 to 280 g, and the females weighed from 220 to 260 g.

Experimental procedure: A 100 X 50 mm area of the dorso-lumbar region of rats in a group containing 5 males and 5 females was clipped free of hair, and 2 ml undiluted test material per kg body weight was applied to the prepared skin. The test sites were then occluded with gauze and adhesive tape, and after 24 hours the occulusive dressings were removed. The treated skin was washed to remove excess test substance.

Test animals were weighed on the day of treatment and on days 3, 8, and 15 after dosing. Observations for the occurrence of toxic signs and mortality were made between 30 and 90 minutes, 4 and 7 hours, and daily throughout the 15-day observation period following dosing.

At the end of the observation period surviving animals were sacrificed and subjected to gross necropsy.

9. REPORTED RESULTS

According to the report there were no mortalities in treated animals during the study. The most frequently observed signs of toxicity were urinary incontinence and stains around the nose. These signs were reported to persist until the fourth day after dosing. The investigators also noted slight skin irritation in one male and two females, and a third female showed moderate irritation. The irritation was described by the authors as as desquamation of test sites.

The report stated that four male and two female rats lost weight initially, but all animals gained weight by the 8th day after dosing. By the end of the observation period all animals gained weight.

The investigators stated that no gross lesions were observed at necropsy.

10. DISCUSSION

There were adequate data presented to support the conclusion that the acute dermal LD_{50} is >2 g/kg, and the results indicate that the formulation should be classified into Toxicity Category III for acute dermal toxicity in rats.

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: 4 g/L liquid, 0.39% active ingredient (Reference nos. D2110/40, JF9457).

3. STUDY/ACTION TYPE: Skin and eye irritation - rabbits; (EUP for new chemical)

4. STUDY IDENTIFICATION: Scott, R. C., C. Rhodes, J. Scott and J. Southwood. May 17, 1984. Paclobutrazol: Skin irritation and eye irritation studies on a 4 g/l formulation. Unpublished report no. CTL/P/1051 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 254865.

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6. APPROVED BY:

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7. CONCLUSIONS: The 4 g/l formulation of Paclobutrazol is a non-irritant to the skin and eyes of rabbits. The results indicate that the formulation should be classified into Toxicity Category IV for skin and eye irritation.

Core classification: Minimum for both studies

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8. MATERIALS AND METHODS (continued)

Test species: New Zealand White female rabbits (Hacking and Churchill, Abbots Ripton Road, Wyton, Nr Huntingdon, Cambridgeshire, UK for the eye irritation and Mellon Rabbits, Chadderton Heights, Nr Oldham, Greater Manchester, UK for the skin irritation study) were used. The rabbits weighed from 2341 to 3927 g and were between 11 and 17 weeks of age.

a. Skin irritation study: On the day before treatment the hair was clipped from the backs of rabbits to expose the skin for application of the test substance. Only rabbits without skin defects were selected for the study, and 6 rabbits were used. No abraded skin sites were tested.

Four-tenths ml undiluted test substance or a 1:16 dilution (described as spray concentration) was applied to 25 x 25 mm skin sites (one per flank), and gauze pads were then placed over each of the test sites on each animal. The trunks of each rabbit were wrapped with plastic. The plastic and gauze pads were removed 4 hours after they were applied, and the skin was scored for signs of irritation. Test sites were also evaluated 44 and 68 hours after treatment.

Erythema and eschar formation as well as edema were scored on a 5-point scale (0-4) with a maximum possible score of 8 for any site. Scoring was done according to the following classifications:

Erythema and eschar		Edema	
No erythema	0	No edema	0
Slight erythema	1	Very slight edema	1
Well-defined erythema	2	Slight edema	2
Moderate to severe erythema	3	Moderate edema	3
Severe erythema to slight eschar formation	4	Severe edema	4

The report stated that mean erythema and mean edema scores were calculated for the right and left flanks separately. They were based on the 20, 44, and 68hour observations for all six rabbits. If the maximum mean erythema or edema score was less than two, the investigators classified the test material a non-irritant. Maximum mean scores greater than two were indicative of an irritant.

8. MATERIALS AND METHODS (continued)

b. Eye irritation study: Nine rabbits, previously found without signs of eye defects or irritation, were used in the experiment. One-tenth ml of undiluted test substance was instilled into one eye of each rabbit, and the eyelids were gently held together for one or two seconds.

Immediately after instillation of the test substance, the eyes were assessed for pain reaction according to the following scale:

Score	Animal reaction	Descriptive rating
1	No response	No initial pain.
2	A few blinks only; normal within 1 to 2 minutes	Practically no initial pain.
3	Rabbit blinks, tries to open eye, but reflexes close it.	Slight initial pain.
4	Rabbit holds eye shut and puts pressure on lids; may rub eye with paw.	Moderate initial pain.
5	Rabbit holds eye shut vigorously; may squeal.	Severe initial pain.
6	Rabbit holds eye shut vigorously; may squeal, claw at eye, and try to escape.	Very severe initial pain.

Twenty to thirty seconds after instillation the treated eyes of three rabbits were washed for one minute with lukewarm water. The treated eyes of the six remaining rabbits were left unwashed.

All eyes of test animals were examined 24, 48, and 72 hours after treatment. Examination of one rabbit was also conducted 96 hours after instillation of the test substance according to the report. Corneas were examined for the presence and extent of opacities. The condition of the iris was also noted, and the conjunctivae were evaluated for chemosis,

8. MATERIALS AND METHODS (continued)

redness, necrosis, and discharge. These observations were scored according to the following scales:

Corneal opacity

Degree of density

1 - scattered or diffuse area, details of iris visible
2 - easily discernible transluscent areas, details of iris slightly obscured
3 - opalescent areas, no details of iris visible, size of pupil barely discernible
4 - opaque, iris invisible

Area of cornea involved

1 - one-quarter (or less but not zero)
2 - greater than one-quarter to less than one-half
3 - greater than one-half to less than three-quarters
4 - greater than three-quarters

score = score for degree x score for extent x 5
maximum = 80

Iris

1 - folds above normal, congestion, swelling, circumcorneal injection (any one or a combination of these), iris still reacting to light (sluggish reaction is positive)
2 - no reaction to light, hemorrhage, gross destruction (any one or all of these)

score = score for iris x 5
maximum score = 10

Conjunctivae

Redness

1 - vessels definitely injected above normal
2 - more diffuse, deeper crimson red, individual vessels not discernible
3 - diffuse beefy red

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8. MATERIALS AND METHODS (continued)

Chemosis

1 - any swelling above normal (including nictitation membrane
2 - obvious swelling with parital eversion of the lids
3 - swelling of lids about half closed
4 - swelling of lids about half to completely closed

Discharge

1 - any amount different from normal (does not include small amount in inner canthus of normal animals)
2 - discharge with moistening of the lids and hairs just adjacent to the lids
3 - discharge with moistening of the lids and considerable area around the eye

Score = sum of values for redness, chemosis, and discharge multiplied by 2. Maximum = 20

Interpretation of the scores was made according to the appended tables (Appendix 1) which are reproduced from the report.

9. REPORTED RESULTS:

a. Skin irritation: The reported mean irritation scores for edema was 0, but the investigators noted that one rabbit exhibited transient erythema one hour after test sites were washed. The mean edema score was also 0 for the undiluted formulation. The mean erythema score for the 1:16 dilution was 0, and the mean edema score was 0.056. One rabbit had slight edema 20 hours after treatment with the diluted formulation.

b. Eye irritation: The investigators reported that the treated rabbits had practically no initial pain reaction to a moderate initial pain reaction (see scale on page 3 above). Two of the six rabbits with nonirrigated eyes had conjunctivitis with slight redness, mild chemosis, and slight discharge one to two hours after treatment, and those rabbits recovered within 24 hours. The authors observed no other signs of irritation during the experiment. Mean scores for eye irritation are shown in Appendix 2 below.

10. DISCUSSION

The authors presented enough information to support their conclusions that the 4 g/l formulation of Paclobutrazole is not irritating to the skin or eyes of rabbits.

APPENDIX 1

Interpretation of eye irritation scores

PACLOBUTRAZOL: SKIN IRRITATION AND EYE IRRITATION STUDIES ON A 4g/l FORMULATION

APPENDIX - 5

INTERPRETATION OF THE SCORES IN THE EYE IRRITATION STUDY (KAY AND CALANDRA 1962)

Maximum Mean Total Score During First 4 Days	Persistence of Score	Descriptive Rating (and class)
0 to 0.5	Mean total score 1 day = 0 Mean total score at 1 day greater than 0	Non-irritating (1) Practically non-irritating (2)
0.5 to 2.5	Mean total score 1 day = 0 Mean total score at 1 day greater than 0	Non-irritating (1) Practically non-irritating (2)
2.5 to 15	Mean total score 2 days = 0 Mean total score at 2 days greater than 0	Slight irritant (3) Mild irritant (4)
15 to 25	Mean total score 3 days = 0 Mean total score at 3 days greater than 0	Mild irritant (4) Moderate irritant (5)

PACLOBUTRAZOL: SKIN IRRITATION AND EYE IRRITATION STUDIES ON A 4g/l FORMULATION

APPENDIX 5 - continued

INTERPRETATION OF THE SCORES IN THE EYE IRRITATION STUDY (KAY AND CALANDRA 1962)

Maximum Mean Total Score During First 4 Days	Persistence of Score		Descriptive Rating (and class)	
25 to 50	Mean total score at 7 days 20 or less	More than half of the individual total scores at 7 days 10 or less	Moderate irritant	(5)
		More than half of the individual total scores at 7 days greater than 10 but no individual total score at 7 days greater than 30	Moderate irritant	(5)
		More than half of the individual total scores at 7 days greater than 10 and any individual total score at 7 days greater than 30	Severe irritant	(6)
	Mean total score at 7 days greater than 20		Severe irritant	(6)

PACLOBUTRAZOL: SKIN IRRITATION AND EYE IRRITATION STUDIES ON A 4g/l FORMULATION

APPENDIX 5 - continued

INTERPRETATION OF THE SCORES IN THE EYE IRRITATION STUDY (KAY AND CALANDRA 1962)

Maximum Mean Total Score During First 4 Days	Persistence of Score		Descriptive Rating (and class)
50 to 80	Mean total score at 7 days 40 or less	More than half of the individual total scores at 7 days 30 or less	Severe irritant (6)
		More than half of the individual total scores at 7 days greater than 30 but no individual total score at 7 days greater than 60	Severe irritant (6)
		More than half of the individual total scores at 7 days greater than 30 and any individual total score at 7 days greater than 60	Very severe irritant (7)
	Mean total score at 7 days greater than 40		Very severe irritant (7)

PACLOBUTRAZOL: SKIN IRRITATION AND EYE IRRITATION STUDIES ON A 4g/l FORMULATION

APPENDIX 5 - continued

INTERPRETATION OF THE SCORES IN THE EYE IRRITATION STUDY (KAY AND CALANDRA 1962)

Maximum Mean Total Score During First 4 Days	Persistence of Score		Descriptive Rating (and class)
80 to 100		More than half of the individual total scores at 7 days 60 or less	Very severe irritant (7)
	Mean total score at 7 days 80 or less	More than half of the individual total scores at 7 days greater than 60 but no individual total score at 7 days greater than 100	Very severe irritant (7)
		More than half of the individual total scores at 7 days greater than 60 and any individual total score at 7 days greater than 100	Extremely severe irritant (8)
	Mean total score at 7 days greater than 80		Extremely severe irritant (8)
100 to 110	Mean total score at 7 days 80 or less		Very severe irritant (7)
	Mean total score at 7 days greater than 80		Extremely severe irritant (8)

APPENDIX 2

Reported mean eye irritation scores for treated rabbits

PACLOBUTRAZOL: SKIN IRRITATION AND EYE IRRITATION STUDIES ON A 4g/l FORMULATION

004352

TABLE 1

EYE IRRITATION: MEAN SCORES FOR RABBIT EYES WITHOUT IRRIGATION

Time After Instillation	Mean Scores			
	Cornea (max 80)	Iris (max 10)	Conjunctiva (max 20)	Mean Total Score (max 110)
1-2 Hr	0.0	0.0	1.0	1.0
1 day	0.0	0.0	0.0	0.0
2 days	0.0	0.0	0.0	0.0
3 days	0.0	0.0	0.0	0.0

Means based on 6 animals and rounded to one decimal place.
Individual animal data are given in Appendix 6, Table 5.

PACLOBUTRAZOL: SKIN IRRITATION AND EYE IRRITATION STUDIES ON A 4g/l FORMULATION

004352

TABLE 2

EYE IRRITATION: MEAN SCORES FOR RABBIT EYES WITH IRRIGATION

Time After Instillation	Mean Scores			
	Cornea (max 80)	Iris (max 10)	Conjunctiva (max 20)	Mean Total Score (max 110)
1-2 Hr	0.0	0.0	4.0	4.0
1 day	0.0	0.0	0.7	0.7
2 days	0.0	0.0	0.0	0.0
3 days	0.0	0.0	0.0	0.0

Means based on 3 animals and rounded to one decimal place.
Individual animal data are given in Appendix 6, Table 6.

004332

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: 4 g/L liquid, 0.39% active ingredient (Reference nos. D2110/40, JF9457).

3. STUDY/ACTION TYPE: Skin sensitization - guinea pigs; (EUP for new chemical)

4. STUDY IDENTIFICATION: Southwood, J. May 18, 1984. Paclobutrazol: Skin sensitisation on a 4g/l formulation. Unpublished report no. CTL/P/779 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratories, Alderley Park Macclesfield Cheshire, UK. Submitted by ICI Americas. EPA Acc. No. 254865.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSIONS: The 4 g/l formulation does not cause skin sensitization in guinea pigs when tested by the Maximization method.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Four to seven week old female Dunkin-Hartley strain guinea pigs were used. They weighed from 336 to 414 g.

Experimental procedure: The Maximization procedure was used. The authors stated that dosages used in the main study were selected according to the results of a "sighting" study. Two or three doses were tested in each of two groups made up of 2 animals per group. The dosing procedure for the preliminary study was described as follows:

i) Intradermal injection (induction): dilutions of the formulation in deionised water were tested to determine the highest concentration, up to 5% (w/v), that could be well tolerated locally and systemically;

ii) Topical application (induction): dilutions of the formulation in deionised water were tested to determine the highest concentration which did not produce excessive inflammation;

iii) Topical application (challenge): dilutions of the formulation in deionised water were tested to determine the highest concentration which did not produce excessive inflammation or irritation in animals that had been injected with Freund's complete adjuvant...at least fourteen days previously.

Main experiment: The hair was clipped from the scapular region of 30 test animals. One group containing 10 guinea pigs was designated the control group, and a second group of 20 made up the treated group. Each treatment group animal was given a series of three intradermal injections at prepared skin sites on either side of the midline. The first injection was Freund's complete adjuvant and water (1:1); the second was 5% (w/v) formulation in water; and the third contained the 5% dilution of the formulation and Freund's complete adjuvant.

One week later the test sites were clipped again, and the undiluted formulation was applied to the skin. An occlusive dressing was put over the application site for 48 hours. The control group was treated similarly without application of the test substance.

Two weeks after the first topical applications the hair on the flanks of test animals was clipped, and a second topical application was made. The test substance was applied to

8. MATERIALS AND METHODS (continued)

filter paper undiluted or diluted 1:16. The filter paper was attached to occlusive dressings so that the test substance was kept in contact with the clipped skin sites (one dilution on each side of the midline of each animal). After 24 hours, the dressings were removed and the test sites were scored (see Appendix 1 for scoring system description).

9. REPORTED RESULTS

The formulation did not cause any skin sensitization reactions under the test conditions (see Appendix 2 below).

10. DISCUSSION

There was adequate data to indicate that the 4 g/l formulation is not a skin sensitizer in guinea pigs when tested by the Maximization procedure.

APPENDIX 1

Scoring System for Skin Reactions

Scale:- 0 - no reaction
1 - scattered mild diffuse redness
2 - moderate diffuse redness
3 - intense redness and swelling

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To classify the sensitisation response, the percentage of the control animals that responded was subtracted from the percentage of test animals that responded and the net response was compared with the following scheme:

% net response	description
0	Not a sensitiser
>0- 8	Weak sensitiser
9-28	Mild sensitiser
29-64	Moderate sensitiser
65-80	Strong sensitiser
81-100	Extreme sensitiser

At the end of the study all the animals were killed.

~~3. RESULTS (CTL Study No GG2927)~~

Nine out of ten control animals were used for the challenge as one of the animals died during the experiment.

Following challenge with the undiluted formulation and the 1:16 dilution, none of the animals, test or control, showed any signs of erythema. Individual animal data are given in Appendix 2, Table 1.

In conclusion, therefore, the paclobutrazol formulation and its spray strength dilution (1:16) are not sensitisers to guinea pig skin.

JS/JMP/SJB/WD/JMP (438A)
10/05/84

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APPENDIX 2

Reported Scores

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PACLOBUTRAZOL: SKIN SENSITISATION STUDY
ON A 4g/l FORMULATION

004352

TABLE 1

GUINEA PIG SKIN SENSITISATION (MAGNUSSON AND KLIGMAN MAXIMISATION METHOD):
ERYTHEMA SCORES

Induction: a) Intradermal injection (conc and vehicle):

(i) 50% (v/v) Freund's adjuvant and deionised water
(ii) 5% (w/v) test substance in deionised water
(iii) 5% (w/v) test substance in Freund's complete adjuvant and deionised water

b) Topical application (conc and vehicle): Undiluted formulation

Challenge: (conc and vehicle):
Undiluted formulation: LHS
Spray strength dilution (1:16): RHS

Study No: GG2927

TEST ANIMALS

Animal Number	Erythema Scores (LHS)		Animal Number	Erythema Scores (RHS)	
	24 hr	48 hr		24 hr	48 hr
Induction plus challenge					
89	0	0	89	0	0
90	0	0	90	0	0
91	0	0	91	0	0
92	0	0	92	0	0
93	0	0	93	0	0
94	0	0	94	0	0
95	0	0	95	0	0
96	0	0	96	0	0
97	0	0	97	0	0
98	0	0	98	0	0
99	0	0	99	0	0
100	0	0	100	0	0
101	0	0	101	0	0
102	0	0	102	0	0
103	0	0	103	0	0
104	0	0	104	0	0
105	0	0	105	0	0
106	0	0	106	0	0
107	0	0	107	0	0
108	0	0	108	0	0

The scale used for scoring erythema is given on page 6.
LHS = left hand side RHS = right hand side

PACLOBUTRAZOL: SKIN SENSITISATION STUDY
ON A 4g/l FORMULATION

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TABLE 1 - continued

GUINEA PIG SKIN SENSITISATION (MAGNUSSON AND KLIGMAN MAXIMISATION METHOD):
ERYTHEMA SCORES

Induction: a) Intradermal injection (conc and vehicle):

(i) 50% (v/v) Freund's adjuvant and deionised water
(ii) 5% (w/v) test substance in deionised water
(iii) 5% (w/v) test substance in Freund's complete adjuvant and deionised water

b) Topical application (conc and vehicle): Undiluted formulation

Challenge: (conc and vehicle):
Undiluted formulation: LHS
Spray strength dilution (1:16): RHS

Study No: GG2927

CONTROL ANIMALS

Animal Number	Erythema Scores (LHS)		Animal Number	Erythema Scores (RHS)	
	24 hr	48 hr		24 hr	48 hr
Challenge only					
109	0	0	109	0	0
110	-	-	110	-	-
111	0	0	110	0	0
112	0	0	112	0	0
113	0	0	113	0	0
114	0	0	114	0	0
115	0	0	115	0	0
116	0	0	116	0	0
117	0	0	117	0	0
118	0	0	118	0	0

The scale used for scoring erythema is given on page 6.
LHS = left hand side
RHS = right hand side
- = animal died before challenge application

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (92.4%) was used (see Item 1.)

3. STUDY/ACTION TYPE: One-year feeding study - dogs; (EUP for new chemical)

4. STUDY IDENTIFICATION: Clapp, M. J. L., A. E. Kalinowski, D. T. Chalmers, I. S. Chart, C. W. Gore, M. D. Stonard, and M. J. Godley. May 29, 1984. Paclobutrazol: 1 year oral dosing study in dogs. Unpublished report no. CTL/P/958 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254865.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Geneticist
Organization: Review Section 6
Toxicology Branch
Signature: Jane C Harris
Date: 3/13/85

7. CONCLUSION Elevated serum alkaline phosphatase and triglyceride levels, the enlarged hepatic cells, increased liver weights, and increased hepatic aminopyrine N-demethylase activity indicate that the two highest doses (75 and 300 mg/kg/day) have definite effects. However, no toxicologically significant effects were observed at the 15 mg/kg/day dose level. The NOEL is 15 mg/kg/day, and the LEL is 75 mg/kg/day in beagle dogs.

Core classification: Minimum

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8. MATERIALS AND METHODS

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 body weight for one year. 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, 26, 39, and 52 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. Body weights 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, 26, 39, and 52 of the study. These samples were taken prior to the daily feeding. Hematological observations in blood 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 include urea, glucose, triglycerides, albumin, 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. 26; and 50 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 week 53 all dogs were sacrificed and gross necropsies were performed. The gonads, liver, pituitary, brain, spleen, thymus, lungs, heart, adrenals, thyroid/parathyroid, and kidney were removed and weighed.

Tissue samples from the weighed organs as well as the aorta, large and small intestines, coccum, cervix, epididimydes,

8. MATERIALS AND METHODS (continued)

eyes, gall bladder, ileum, lymph nodes, mammary glands, esophagus, sciatic nerve, pancreas, prostate, rectum, rib, skin, salivary glands, spinal cord, stomach, trachea, urinary bladder, uterus, muscle, and gross lesions were processed for histological examination.

Samples of liver from 4 dogs of each sex in each group were collected for analysis of hepatic aminopyrine N-demethylase (APDM) activity. The method was described by citation only (Mazel, 1971).

Statistical methods are described in Appendix 1 below.

9. 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 body weight gains during the study. Tabulated group mean body weights for the high dose group males were 6% less than the control group mean body weights at the end of the 52-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 52 of the study are summarized as follows:

Dose group	Males	Females
Control	79	83
Mid	137	182
High	537	598

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

9. REPORTED RESULTS (continued)

Alkaline phosphatase activity was also elevated in the low dose group females, but the investigators attributed the increase to two of the six dogs in that group (see DISCUSSION below).

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 23.7 and 41.2 mg/100 ml, respectively at the 52-week observation ($p<0.01$; t test). For female dogs the respective means for the control and high-dose groups were 28.7 and 51.7 mg/100 ml ($p<0.01$; two-tailed t test).

Other group means which were reported to be statistically significantly different from controls included albumin, total protein, and calcium levels in high-dose group animals. The means which were reported for the 52-week observations are summarized as follows:

	Males		Females	
Observation	Control	Treated	Control	Treated
Albumin*	3.60	3.18	3.70	3.50
Total protein*	5.92	5.55	5.98	5.80
Calcium**	10.90	10.60	11.10	10.00

*g %
**mg %

The only compound related effects on organ weights were noted for liver weights in males and females, the kidneys of males, and the adrenals of females all in the high-dose group. The group mean organ weights for these organs are summarized in Table 1. No organ-to-body weight ratios were presented.

Appendix 2 contains the reported histopathology summary. The most frequently observed lesions occurred in the liver, and hepatocellular enlargement was the most frequent observation. The authors noted that 2 of the 6 female dogs in the mid-dose group had focal ballooning hepatocytes along with 3 of the 6 in the high-dose group.

The group mean hepatic aminopyrine N-demethylase activities (umol formaldehyde/h/g liver) were reported as follows:

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9. REPORTED RESULTS (continued)

Table 1

Selected group mean organ weights (g)

Dose group	Liver		Kidneys	Adrenals
	Males	Females	Males	Females
Control	362	395	60.0	1.67
Low	409*	382	63.4	1.64
Mid	450**	434	63.1	1.79
High	502**	511**	65.3***	1.99**

*Statistically significantly different from controls at $p<0.05$, two-tailed t test.
**Statistically significantly different from controls at $p<0.01$, two-tailed t test.
***Adjusted group means statistically significantly different from control group, $p<0.01$.

Table 2

Group mean aminopyrine N-demethylase activity (umol formaldehyde/h/g liver)

Dose group	Males	Females
Control	12.6	12.1
Low	14.6*	13.6
Mid	19.4*	17.8*
High	30.1*	24.0*

*Statistically significantly different from the control group mean.

10. DISCUSSION

Elevated serum alkaline phosphatase and triglyceride levels, the enlarged hepatic cells, increased liver weights, and increased hepatic aminopyrine N-demethylase activity results indicate that the two highest doses (75 and 300 mg/kg/day) have definite effects. However, results with respect to liver weights and serum alkaline phosphatase activity are not as clearly affected as they are at two higher dose levels. For example, the authors suggested that the elevated SAP levels in the low dose females could be attributed to two

10. DISCUSSION (continued)

females in that group. A comparison of results (mU/ml) for the group and for each of the two animals is as follows:

Time of measurement	Control group mean*	Treated group mean*	Animal # 21	Animal # 23
Pre-test	154	176	178	233
52-weeks	83	110	157	114

*Unadjusted means (see Appendix 1 below)

At 52 weeks the individual values for each female in the low dose group were 82, 97, 157, 123, 114, and 83 mU/ml. The reported individual values for the control group females at 52 weeks were 74, 109, 92, 76, 79, and 66 mU/ml.

As shown by pre-test group means, the low-dose group females showed generally higher SAP levels from the beginning of the study. These differences, which persisted throughout the experiment, the absence of elevations in liver weight (see Table 1 above) and the absence of liver histopathology (see Appendix 2 pages CTL/P/958-59 and -60) suggest that the elevated SAP in the low-dose-group females is not necessarily related to the test substance.

Results from an independent calculation of the liver-to-body weight ratios from reported means (liver weights divided by group mean body weights for pre-sacrifice multiplied by 100) is summarized as follows:

Dose group	Males	Females
Control	2.59	3.05
Low	2.84	2.89
Mid	3.27	3.29
High	3.86	4.05

The liver-to-body weight ratios for male dogs shows an increasing trend in all treated groups which reflects the increases in absolute organ weight (see Table 1). For purposes of establishing a no-observed-effect level (NOEL), the low-dose group should be reconsidered.

Mean body weight for the low dose group dogs was slightly increased above controls (14.42 kg compared with 13.97 kg for controls, a 3.22% increase). The increase in absolute

10. DISCUSSION (continued)

liver weight in low-dose males was approximately 12% which may not be toxicologically significant by itself. As noted above, there were no other effects in the low-dose-group males.

In view of the SAP results in low-dose females and the liver weight results in the low-dose males, the authors conclusion that the 15 mg/kg/day dose could not be described as an absolute NOEL is supported. However, the absence of histopathological changes in the low dose group animals, the small increase (10%) in absolute liver weight in males, and the absence of consistent effects on clinical chemistry observations suggests that the 15 mg/kg/day dose is a NOEL in dogs.

Paclobutrazol scientific review

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___ Description of product 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

___ Information about a pending registration action

_X_ 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.

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (92.4%) was used (see Item 1.)

3. STUDY/ACTION TYPE: Cytogenetics - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Richardson, C. R., C. A. Howard, E. Longstaff, M. G. Thomas, P. B. Banham, S. L. Beck, and M. J. Godley. May, 2, 1984. Paclobutrazol: A cytogenetic study in the rat. Unpublished report no. CTL/P/891 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Geneticist
Organization: Review Section 6
Toxicology Branch
Signature: Jane C Harris
Date: 3/13/85

7. CONCLUSION: Single oral doses of 30, 150, or 300 mg paclobutrazol per kg body weight in rats did not cause clastogenic effects in bone marrow cells.

8. MATERIALS AND METHODS

Test species: Six to eight-week old male and female Alderly Park strain specific pathogen free rats were used. They weighed from 193 to 254 g.

Positive control substance: Cyclophosphamide was used as the reference mutagen in this study.

Experimental procedures: Doses were selected on the basis of a preliminary study (reproduced from the original report in Appendix 1 of this review).

Three groups containing 24 male and 24 female rats were given single oral doses of 30, 150, or 300 mg paclobutrazol per kg body weight. The test substance was administered in corn oil by gavage. One group of 36 males and 36 females was given corn oil without the test substance, and a second group of the same size was given 30 mg cyclophosphamide per kg in physiological saline.

Eight animals of each sex from each of the three groups given paclobutrazol were sacrificed 12, 24, and 48 hours after dosing. Twelve rats of each sex from each of the two control groups were also sacrificed at the same times. Two hours before the animals were sacrificed each was given an intraperitoneal injection of 30 mg colchicine per kg body weight to arrest dividing cells in c-metaphase.

The report stated that bone marrow cells were then harvested from both femurs by aspiration with Hank's Balanced Salt solution. Cells were treated with a hypotonic KCl solution (0.075 M) and fixed with glacial acetic acid:methanol (1:3). Slides were made from these preparations and air dried. They were stained with Giemsa stain and mounted in DPX for microscopic observation.

The reported noted that 50 cells from each animal were examined, and only those with 40 or more centromeres were considered. Chromosomal abnormalities were classified as chromatid gaps, chromosome gaps, breaks, fragments, minutes, or others (Robertsonian translocations were given as an example).

Cytotoxicity was determined by the mitotic index for each sample.

8. MATERIALS AND METHODS (continued)

Statistical analyses: Descriptions of procedures used in this study are reproduced from the original report and included as Appendix 2 of this review.

9. REPORTED RESULTS

The report of the main study did describe toxic signs in the animals in the main study. Discussion of toxic effects was confined to the reported preliminary study (see Appendix 1).

The authors noted that the incidence of cells with less than 40 or more than 42 chromosomes was generally low with the cells from cyclophosphamide treated animals exhibiting the highest incidence. They stated that the range was from 0 to 6.9%.

Only one animal was reported to have less than 20 cells examined. That animal was a male from the 24 hr sacrifice in the negative control group.

A statistically significant increase in the group mean percentage of cells with chromosomal abnormalities was reported for the high dose group males at the 12-hour sacrifice. These results are summarized as follows:

Males	Abnormality	Control	High dose group
All animals	Inc. gaps	1.00	3.00*
	Exc. gaps	0.67	1.75*
All animals: only cells with 40, 41, or 42 chromosomes	Inc. gaps	1.00	3.07*
	Exc. gaps	0.67	1.81*

Females	Abnormality	Control	High dose group
All animals	Inc. gaps	1.00	1.50
	Exc. gaps	0.67	1.00
All animals: only cells with 40, 41, or 42 chromosomes	Inc. gaps	1.00	1.52
	Exc. gaps	0.67	1.01

*Statistically significant at the 5% level using one-tailed Student's t test on transformed data (see Appendix 2).

9. REPORTED RESULTS (continued)

The increased incidence of minutes was the stated reason for the change observed in the high dose males (see individual animal data reproduced in Appendix 3).

There were no compound related effects on the incidence of chromosomal abnormalities noted in the high dose groups at the 24 and 48-hour sacrifices (see Appendix 4 for summary results reproduced from the report).

The positive control group was evaluated at the 24-hour sacrifice, and the results were summarized in the report as follows:

Males and Females	Abnormality	Negative control	Positive control
All animals	Inc. gaps	0.60	12.70**
	Exc. gaps	0.30	10.70**
All animals: only cells with 40, 41, or 42 chromosomes	Inc. gaps	0.61	10.07**
	Exc. gaps	0.31	7.86**
All animals for which at least 20 cells were examined: cells with 40, 41, or 42 chromosomes	Inc. gaps	0.64	10.07**
	Exc. gaps	0.32	7.86**

**Statistically significant at the 1% level using one-tailed Student's t test on transformed data (see Appendix 2).

The report stated that there was no compound related effect on mitotic indices in the samples examined. The reported group means for the 3 sacrifice times are summarized in Appendix 4 below.

10. DISCUSSION

There were adequate data to support the authors conclusion that the increased percentage of cells with chromosomal abnormalities noted at 12 hours is not biologically significant. The reported group mean percentage for the high dose group was within the range observed for the negative control group at 12, 24, and 48 hours after dosing. A comparison of the results is shown in Table 1 below.

Table 1

Summary of group mean percentages for negative control male rats 12, 24, and 48 hours after dosing

	Abnormality	12-hour control	24-hour control	48-hour control	High dose group at 12 hours
All animals	Inc. gaps	1.00	0.60	2.01	3.00
	Exc. gaps	0.67	0.30	1.46	1.75
All animals: only cells with 40, 41, or 42 chromosomes	Inc. gaps	1.00	0.61	2.43	3.07
	Exc. gaps	0.67	0.31	1.47	1.81

APPENDIX 1

Preliminary study to determine doses to be tested in the single-dose rat cytogenetics study with paclobutrazol

Paclobutrazol scientific review

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___ Description of the product manufacturing process

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071352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (99%) was used (see Item 1.)

3. STUDY/ACTION TYPE: Liver effects study - dogs; (EUP for new chemical)

4. STUDY IDENTIFICATION: Jones, B. K., D. M. Williams,and J. Galvin. May 31, 1984. Paclobutrazol: The effect of a single oral dose (5 mg/kg or 250 mg/kg) on liver weight in the rat. Unpublished report no. CTL/P/1065 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Geneticist
Organization: Review Section 6
Toxicology Branch
Signature: Jane C Harris
Date: 3/13/85

7. CONCLUSION

There were adequate data reported to support the authors' conclusion that:

> ..., the results presented justify the use of single oral doses of 5 and 250mg paclobutrazol/kg as "no effect" and "effect" levels respectively for metabolism studies.

004352

8. MATERIALS AND METHODS

Test species: Eight to eleven-week old male and female Alpk/AP strain rats were used.

Experimental procedure: Groups of five male and five female rats were given single oral doses of 0, 5, or 250 mg test substance per kg body weight. Doses were administered by gavage in polyethylene glycol 600. An additional group was undosed.

Seventy-two hours after dosing the animals were weighed and sacrificed. The livers were then dissected out and weighed. Liver-to-body weight ratios were calculated.

Data analysis: Liver-to-body weight ratios for each treatment group were compared with the vehicle- and undosed-control groups by the Student's t test.

9. REPORTED RESULTS

The reported results are shown in Appendix 1 below, and the results of statistical analysis are included in Appendix 2.

The investigators noted that the liver-to-body weight ratios for the 250 mg/kg dose group were significantly increased above both vehicle and undose control groups for male rats and significantly increased above vehicle controls for the female rats in that dose group. There were no significant differences between the control groups and the group given the 5 mg/kg dose or the 250 mg/kg group females compared with the undosed control group.

10. DISCUSSION

There were adequate data reported to support the authors' conclusion that:

> ..., the results presented justify the use of single oral doses of 5 and 250mg paclobutrazol/kg as "no effect" and "effect" levels respectively for metabolism studies.

APPENDIX 1

Reported Results

Paclobutrazol scientific review

Page _____ is not included in this copy.

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The material not included contains the following type of information:

___ Identity of product inert ingredients

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004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (92.4%) was used (see Item 1.)

3. STUDY/ACTION TYPE: Teratogenicity - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Killick, M. E., G. H. Pigott, P. B. Banham, and M. R. Thomas. June 1, 1984. Paclobutrazol: Second teratogenicity study in the rat. Unpublished report no. CTL/P/997 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSIONS:

A no-observed-effect level (NOEL) for maternal toxicity in this experiment is greater than 100 mg/kg/day (highest dose tested). Dose-related fetal effects (renal dilatation, hydroureter, and minor skeletal defects or variations) were observed at 40 and 100 mg/kg/day dose levels, and a NOEL of 10 mg/kg/day was established for fetal effects.

Core classification: Minimum

8. MATERIALS AND METHODS

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 1 of gestation. Test animals weighed between 262 and 300 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 7 through 16 of gestation. Doses of 0, 2.5, 10, 40, or 100 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. Body weight determinations were made on days 1, 4, 7-16, 19 and 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.

8. MATERIALS AND METHODS (continued)

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.

9. REPORTED RESULTS

The report stated that there were no treatment-related effects on dams with respect to the occurrence of toxic signs, mortality, body weight, or macroscopic observations at necropsy.

The reported group mean corpora lutea per dam ranged from 14.4 in the 10, 40, and 100 mg/kg/day groups to 14.8 in the 2.5 mg/kg/day dose group (the control group mean was 14.6). Group mean implantations per dam ranged from 12.9 in the control group to 14.0 in the 2.5 mg/kg/day dosed group, and the group mean number of live fetuses per litter ranged from 12.0 in the control group to 13.3 in the lowest-dosed group. None of these three parameters exhibited a relationship to dose.

Group mean gravid uterine weights for the 0, 2.5, 10, 40, and 100 mg/kg/day dosed groups were reported to be 76.4, 85.3, 82.3, 87.9, 85.4, respectively. The respective mean

9. REPORTED RESULTS (continued)

fetal weights were 4.7, 4.9, 4.9, 4.8, and 4.9 g.

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

		Dose (mg/kg/day)			
Observation	Control	2.5	10	40	100
	External/visceral				
No. examined*	264	318	301	302	305
With external defects (%)	60 (22.7)	35 (23.6)	73 (24.3)	138 (45.7)	147 (48.2)
	Skeletal				
No. examined**	176	213	199	200	202
With defects (%)	61 (34.7)	80 (37.6)	75 (37.7)	89 (44.1)	106 (52.5)

*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, 0, and 9 fetuses in the control, 2.5, 10, 40, and 100 mg/kg/day dose groups with major defects, respectively. Eight of the 9 fetuses in the highest dosed group were reported to have hydroureter. Two litters contained one fetus each with the defect, and two additional litters contained 3 each with the defect. The authors stated that all cases were associated with some renal pelvic dilatation, and 3 litter mates from dam number 116 were reported to have distended bladders also. The reported incidence of urogenital defects is presented in Appendix 1 below.

The reported minor skeletal defects were characteristic of delayed ossification, and the authors stated:

> The only individual defect to show a substantial treatment-related increase was the incidence of

9. REPORTED RESULTS (continued)

partial ossification of the transverse processes of the seventh cervical verterbra...There were other minor indications of increased or decreased ossification seen in these dose groups (40 and 100 mg/kg/day groups) but none attained statistical significance.

The only skeletal variation reported to be significantly increased by the two highest doses was the incidence of 14th rib. The incidence of these defects is presented in Appendix 2.

The number of litters with one or more fetuses with external/visceral or skeletal defects is summarized as follows:

	Dose (mg/kg/day)				
Observation	Control	2.5	10	40	100
	External/visceral				
No. examined	22	24	24	24	24
With external defects	17	20	21	23	23
	Skeletal				
With defects	22	21	22	24	24

No other observations in the study showed compound related effects.

10. DISCUSSION

The authors noted that a no-observed-effect level (NOEL) for maternal toxicity in this experiment is greater than 100 mg/kg/day (highest dose tested). Dose-related fetal effects (renal dilatation, hydroureter, and minor skeletal defects or variations) were observed at 40 and 100 mg/kg/day dose levels, and a NOEL of 10 mg/kg/day was established on the basis of the results described above and historical control data (see Appendix 3).

Adequate data were presented in the report to support the authors' conclusions. The investigators also provided a discussion of published literature to which substantiates their interpretation of the results (see Appendix 4).

APPENDIX 1

Incidence of Urogenital Defects

Paclobutrazol scientific review

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004332

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (92.4%) was used (see Item 1.)

3. STUDY/ACTION TYPE: Dominant lethal-mice; (EUP for new chemical)

4. STUDY IDENTIFICATION: Whickramaratne, G. A., D. L. Kinsey, P. B. Banham, and M. G. Thomas. December 29, 1983. Paclobutrazol: Dominant lethal study in the mouse. Unpublished report no. CTL/P/922 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254864.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Geneticist
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSION: Paclobutrazol does not cause dominant lethal mutations in mice given doses from 25 to 300 mg/kg/day for 5 consecutive days.

8. MATERIALS AND METHODS

Test species: Seven to eight-week old male and female Charles River CD-1 strain mice were used.

Positive control substance: Cyclophosphamide was used as the reference mutagen in this study.

Experimental procedures: Determination of fertility. Prior to the main experiment mice were acclimated to the laboratory for 10 days. A group of 240 males were then mated with females to determine their fertility. Each male was cohabitted with two females for a mating period of 7 days. According to the report, mating was assumed to occur soon after cohabitation was started, and the females were not checked for the presence of vaginal plugs or the presence of sperm in vaginal smears. Sixteen days after the beginning of the mating period the females were sacrificed and uteri were examined. Numbers of live implantation sites as well as early embryonic and late fetal deaths were recorded for each female. Based on the results of these tests, males were selected for the main experiment according to specified criteria (see Appendix 1 below).

Main experiment. Four groups of 20 males were given 5 consecutive daily doses of 0, 25, 100, or 300 mg test substance per kg body weight. The test substance was suspended in corn oil and administered by gavage. On the 5th day of dosing a group of 20 males was given an intraperitoneal injection of 200 mg cyclophosphamide per kg body weight. Doses of the test substance were selected on the basis of results from a preliminary study which is reproduced in Appendix 2 below.

On the third day after the last dose was administered, 15 males were selected from each group of 20 for the matings that followed (see Appendix 3 for selection criteria). The mating procedure and examination of females were similar to those used in the fertility study described above. Males were 13 to 14 weeks of age when mated with 10-week old females. After each 7-day mating period the two original females with each male were replaced by two other previously unmated females. Replacements were made weekly for 8 consecutive weeks after dosing of the males.

Treated animals were weighed prior to dosing and daily during the dosing period. They were also observed daily for condition, occurrence of clinical signs, and changes in behavior throughout the study.

8. MATERIALS AND METHODS (continued)

Data Analysis: Procedures are described as reported in Appendix 4 below.

9. REPORTED RESULTS

Appendix 5 contains summary tables from the original report which include results appropriate to the discussion that follows.

The authors noted that one male in the 300 mg/kg/day group died on the fourth day of the dosing period. Clinical signs which were noted (see Table 3 in Appendix 5) included tremors, urinary incontinence, and piloerection in some animals. No treatment-related effect was noted on body weight (see Table 4 in Appendix 5) or fertility of the males (see Table 5 in Appendix 5).

The report stated that results from one female in the 25 mg/kg/day dose group were excluded from the study because an escaped male from another dose group was found in the cage with that female.

There were no compound-related effects on the number of live implants, early deaths or late deaths (see Tables 6 through 13 in Appendix 5).

The authors noted statistically significantly reduced pregnancy rates in the mid dose group at weeks 4 and 8 and in the low dose group at week 8 (see Appendix 6 for individual animal data). Since no reductions in pregnancy rate were seen in the high dose group when compared with that for the negative control group, and since a dose-related decrease in pregnancy rate was not observed, the authors concluded that there is no evidence that a compound related effect on pregnancy occurred.

Positive control results indicated that dominant lethal effects occurred during weeks 1, 2, and 3, and reduced pregnancy rate was noted in week 7.

10. DISCUSSION

There is adequate information presented in the report to suggest that paclobutrazol does not cause dominant lethal mutations in mice given doses from 25 to 300 mg/kg/day for 5 consecutive days.

APPENDIX 1

Selection Criteria for Male Mice to Be Used in the Main Experiment

PACLOBUTRAZOL: DOMINANT LETHAL STUDY IN THE MOUSE

004352

APPENDIX 5

SELECTION AND RANDOMISATION OF MALES

Dominant Lethal Animals

A pre-experiment fertility test was carried out on an excess number of individually ear-marked males housing 1 male:2 females. The results of these matings were used to select the required number of males for the main study, the process being in two stages:-

1) Selection of males for dosing

Fertile males with a low background dominant lethal frequency were required. Excess males were used to allow for any which might have become sick or ill during dosing since a top dose level producing overt toxicity is usual in dominant lethal studies in this Laboratory. The selection process was performed by the ARTEMIS computer system using the male fertility selection option of the dominant lethal suite.

The parameters used to specify the selection of males were as follows:-

(a) Select males paired with two females.

(b) Reject males producing the following criteria in the females:-

(i) only one pregnant female with more than three dead implantations.
(ii) both females pregnant with a total of more than four dead implantations.
(iii) both females are pregnant and either female has more than two dead implantations.
(iv) one or both females pregnant and with a total of less than eight live implantations.

APPENDIX 5 - continued

SELECTION AND RANDOMISATION OF MALES

(c) The remaining males were allocated by selecting their number (using the 'picking out of a hat' technique) and distributing one to each experimental group in turn. Preference was given first to males which had mated successfully with both females (ie both females pregnant).

(d) Should there be insufficient numbers of animals available it may be necessary to include some males producing criteria (i)-(iv), using first those least affected under criteria (ii)-(iv). These animals should be distributed evenly between groups.

APPENDIX 2

Report of the Preliminary Study Conducted for Determination of Doses to Be Used in the Main Experiment

APPENDIX 2

Results of Statistical Analysis

PACLOBUTRAZOL: THE EFFECT OF A SINGLE ORAL DOSE (5mg/kg OR 250mg/kg) ON LIVER WEIGHT IN THE RAT

004352

TABLE 3

STATISTICAL COMPARISON OF LIVER WEIGHT/BODYWEIGHT RATIOS BETWEEN GROUPS OF RATS ADMINISTERED A SINGLE ORAL DOSE OF PACLOBUTRAZOL (5 OR 250 mg/kg) AND GROUPS OF CONTROL ANIMALS

Liver weight/bodyweight ratios for groups of test and control animals were compared statistically using Student's t-test. An example of the statistical analysis is given in Appendix 4.

Statistical comparison between rats dosed with paclobutrazol and control rats dosed with polyethylene glycol			
Group	mg paclobutrazol/kg	t	Significance
Males (1-5)	250	5.74	significant **
Females (6-10)	250	2.57	significant *
Males (11-15)	5	0.63	not significant
Females (16-20)	5	0.04	not significant

Statistical comparison between rats dosed with paclobutrazol and undosed control rats			
Group	mg paclobutrazol/kg	t	Significance
Males (1-5)	250	4.11	significant **
Females (6-10)	250	4.60	significant **
Males (11-15)	5	0.81	not significant
Females (16-20)	5	2.08	not significant

** Statistically significantly different from the control group mean at the 1% level (t-test, two sided).

* Statistically significantly different from the control group mean at the 5% level (t-test, two sided).

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Radiolabelled paclobutrazole was used along with unlabelled test substance (99% purity) [(2RS, 3RS)1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 (^{14}C)-triazole-1-yl)-pentan-3-ol; specific activity = 1.68 GBq/mM). Labelled paclobutrazol was mixed with the unlabelled compound for use in the experiment.

3. STUDY/ACTION TYPE: Metabolism - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Jones, B. K., D. M. Williams, J. Galvin, and A. R. Soames. May 31, 1984. Paclobutrazol: Whole body autoradiography study in the rat following a single oral dose (250 mg/kg). Unpublished report no. CTL/P/1035 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 254864.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane P. Harris
Date: 3/13/85

7. CONCLUSIONS: The disposition of ^{14}C label after a single oral dose of 250 mg/kg is limited to the gastrointestinal contents and, to a lesser extent, the liver and kidney.

8. MATERIALS AND METHODS

Test species: Eight to eleven-week old male and female Alpk/AP strain rats were used.

Experimental procedure: One male and one female were given a single oral doses of 5 mg test substance per kg body weight. The doses were administered by gavage in polyethylene glycol 600. Samples of urine, feces, and expired air were collected for analysis 24 and 48 hours after dosing.

Forty-eight hours after dosing both animals were sacrificed, and the bodies were frozen. Longitudinal sagital sections were made through areas containing major organs, and the sections were autoradiographed on x-ray film.

Analytical methods: Fecal samples were homogenized in methanol. Measured amounts of homogenates were air dried and ground to a fine powder for combustion. The combustion products were absorbed in 2-methoxyethylamine which was subsequently mixed with liquid scintillant (8 g 2,5-diphenyl-oxazole + 800 mg of p-bis-(O-methylstyryl)-benzene per liter of toluene; Fisolfour) for counting.

Cage washings, urine, and methanol extracts of fecal samples were added directly to liquid scintillant (Fisoflour) for counting.

Expired $^{14}CO_2$ was absorbed in sodium hydroxide, and samples of the caustic solution were counted in Fisoflour liquid scintillant.

9. REPORTED RESULTS

Reported mean percentages of the administered dose recovered in urine, feces, and expired air are summarized as follows:

Time of Sample	Urine		Feces		Expired air	
	Male	Female	Male	Female	Male	Female
24 h	20.95	10.21	6.40	1.15	0.021	0.020
48 h	8.83	26.06	22.28	6.10	0.015	0.017
	29.78	36.27	28.67	7.25	0.036	0.037

The cage washings at 48 hours after dosing contained 1.68 and 1.85% of the administered radioactivity for the male and female rats, respectively.

9. REPORTED RESULTS (continued)

The investigators noted that the pattern of distribution for the radiolabel in the autoradiographs was similar in the male and female. They further stated that the radioactivity appeared more intense in the female. The majority of the activity was reported to be in the alimentary tract. The intensity of label in the small and large intestines of the male were similar, and the stomach showed a lesser intensity. In the female, the relative intensities of label were described as similar in the stomach and small intestine and less in the large intestine. No autoradiographs were included in the report.

The report also stated:

> Radioactivity was detected in only two organs, the liver and the kidneys, with levels considerably lower than seen in the gastrointestinal contents. The liver was uniformly labelled, with a greater intensity in the female. In the kidney more labelling was seen in the renal pelvis than in either the cortex or the medulla. A few foci were seen in perirenal fat in both sexes.

10. DISCUSSION

As the authors concluded, the disposition of the test substance appears to be minimal in that it was confined to the gastrointestinal contents after oral administration of a 250 mg/kg dose. There was some radioactivity found in organs involved in the excretion of the test substance (liver and kidney).

Since there were no autoradiographs included in support of the authors conclusions, this study should be considered with others in which tissue levels have been reported. c ✓

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol
(±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Radiolabelled paclobutrazol was used [(2RS, 3RS)1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 (^{14}C)-triazole -1yl)-pentan-3-ol; specific activity = 158.6 uCi/mg). Labelled paclobutrazol was mixed with the unlabelled compound for use in the experiment.

3. STUDY/ACTION TYPE: Metabolism - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Cresswell, D.G., J. Vickers, and R. Hopkins. October, 1983. (^{14}C)-Paclobutrazol: Excretion and tissue retention of a single oral dose (5 mg/kg) in the rat. Unpublished report no. 3456-72/267 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254865.

5. REVIEWED BY:

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6. APPROVED BY:

Name: Jane Harris, Ph. D.
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Date: 3/13/85

7. CONCLUSIONS:

There are adequate data presented in the report to support the conclusions of the investigators.

Most of the 5 mg/kg dose was excreted within 72 hours after treatment (70-80%), and there was no significant retention in tissues.

8. MATERIALS AND METHODS:

Test species: Eight to twelve-week old male and female Sprague-Dawley Crl:CD(SD)BR strain rats were used. They weighed from 138 to 150 g.

Dosage form: The test material as described in item 2., above was prepared as a mixture of radiolabelled and unlabelled paclobutrazol (specific activity = 32.16 uCi/mg). The mixture was disolved in polyethylene glycol 600 for administration to test animals.

Analytical methods: Tissue samples, cage debris, and gastro-intestinal tract contents were homogenized in water, and fecal samples were homogenized in methanol. Measured amounts of homogenates were added to ashless floc and combusted. The combustion products were absorbed in Carbosorb™ which was subsequently mixed with liquid scintillant (Permaflour V) for determination of radioactivity.

Cage washings, urine, and methanol extracts of fecal samples were added directly to liquid scintillant (Fisoflour) for counting.

The limit of detection for the combustion analyses was defined as 1.5 times background. The background level of radioactivity was determined by combustion of ashless floc under conditions similar to those used for test samples. Limits for urine and fecal extracts were defined as 1.5 times the background disintegration rate determined by counting the appropriate solvent (water or methanol) in liquid scintillant. These limits were reported as follows:

Sample	Range of ug equivalents/g
Fat homogenate	0.012-0.041
Other tissues or homogenates	0.003-0.005

Limits in g equivalents were not stated for urine samples or samples of fecal extracts.

Experimental procedure: Four male and four female rats were given a single dose of 5 mg test substance per kg body weight by oral intubation. Samples of urine and feces were collected at 24 hour intervals for the 7-day period immediately following dosing. At the end of that time, test animals were sacrificed, and samples of brain, bone, liver, kidney, spleen, muscle,

8. MATERIALS AND METHODS (continued)

heart, and gastrointestinal tract contents were analyzed for radioactivity.

9. REPORTED RESULTS

Reported mean percentages of the administered dose recovered in the urine and feces are summarized as follows:

Time of Sample	Urine		Feces	
	Males	Females	Males	Females
24	43.5	40.6	13.1	8.4
48	8.9	11.7	14.6	7.8
72	2.8	4.7	4.6	7.5
96	1.0	0.9	2.6	2.3
120	0.6	0.7	1.9	1.3
144	0.7	0.8	0.9	0.4
168	0.3	0.4	0.4	0.6
Total	57.8	69.8	38.7	28.3

The group mean ug equivalents/g of tissue found in the gastrointestinal tract contents for males and females was reported as 0.025 and 0.012, respectively. The respective amounts for liver samples was reported to be 0.017 and 0.028 ug equivalents/g liver tissue.

10. DISCUSSION

The authors noted that most of the 5 mg/kg dose was excreted during the 48 hours immediately following dosing.

The organs involved in metabolism (liver) or excretion (gastrointestinal tract contents) were found to contain low levels of radioactivity. Other organs had no detectable radioactivity according to the report.

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Radiolabelled paclobutrazol was used [(2RS, 3RS)1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 (^{14}C)-triazole -1yl)-pentan-3-ol; specific activity = 158.6 uCi/mg). Labelled paclobutrazol was mixed with the unlabelled compound for use in the experiment.

3. STUDY/ACTION TYPE: Metabolism - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Cresswell, D.G., J. Ward, and R. Hopkins. February, 1984. (^{14}C)-Paclobutrazol: Excretion and tissue retention of a single oral dose (250 mg/kg) in the rat. Unpublished report no. 3268-72/268 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 25486%.

5. REVIEWED BY:

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6. APPROVED BY:

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Date: 3/13/85

7. CONCLUSIONS: Most of the 250 mg/kg dose was excreted within 72 hours after treatment, and there was no significant retention in tissues. Fecal excretion rates were slightly slower in male rats than in females.

8. MATERIALS AND METHODS:

Test species: Eight to twelve-week old male and female Sprague-Dawley Crl:CD(SD)BR strain rats were used. The males weighed from 199 to 207 g, and the females weighed from 140 to 155 g on receipt at the laboratory.

Dosage form: The test material as described in item 2., above was prepared as a mixture of radiolabelled and unlabelled paclobutrazol (specific activity = 32.16 uCi/mg). The mixture was disolved in polyethylene glycol 600 for administration to test animals.

Analytical methods: Tissue samples, cage debris, and gastro-intestinal tract contents were homogenized in water, and fecal samples were homogenized in methanol. Measured amounts of homogenates were added to ashless floc and combusted. The combustion products were absorbed in Carbosorb™ which was subsequently mixed with liquid scintillant (Permaflour V) for determination of radioactivity.

Cage washings, urine, and methanol extracts of fecal samples were added directly to liquid scintillant (Fisoflour) for counting.

The limit of detection for the combustion analyses was defined as 1.5 times background. The background level of radioactivity was determined by combustion of ashless floc under conditions similar to those used for test samples. Limits for urine and fecal extracts were defined as 1.5 times the background disintegration rate determined by counting the appropriate solvent (water or methanol) in liquid scintillant. These limits were reported as follows:

Sample	Range of ug equivalents/g
Carcass homogenate	0.145-0.190
Fat homogenate	0.149-0.252
Other tissues or homogenates	0.087-0.133

Limits in g equivalents were not stated for urine samples or samples of fecal extracts.

Experimental procedure: Four male and four female rats were given a single dose of 250 mg test substance per kg body weight by oral intubation. Samples of urine and feces were

8. MATERIALS AND METHODS (continued)

collected at 24 hour intervals for the 7-day period immediately following dosing. At the end of that time, test animals were sacrificed, and samples of brain, bone, liver, kidney, spleen, muscle, heart, and gastrointestinal tract contents were analyzed for radioactivity.

9. REPORTED RESULTS

Reported mean percentages of the administered dose recovered in the urine and feces are summarized as follows:

Time of Sample	Urine		Feces	
	Males	Females	Males	Females
24	16.5	4.3	4.2	3.6
48	23.6	21.7	12.2	16.2
72	3.3	7.4	13.3	19.9
96	3.1	2.9	9.1	4.1
120	1.3	1.0	2.2	0.5
144	0.6	0.8	1.1	0.3
168	0.7	1.3	0.6	0.2
Total	47.1	56.4	42.7	44.7

The group mean percentage of the administered radioactivity recovered in the cage washings 7 days after treatment was reported to be 1.2 and 1.4 for male and female rats, respectively.

The group mean ug equivalents/g of tissue found in the gastrointestinal tract contentsfor males and females was reported as 1.461 and 0.303, respectively. The respective amounts for liver samples was reported to be 0.848 and 0.444 ug equivalents/g liver tissue.

10. DISCUSSION

The authors noted that most of the 250 mg/kg dose was excreted during the 72 hours immediately following dosing. The only difference between the sexes was reported to be a slower fecal excretion rate in males.

The organs involved in metabolism (liver) or excretion (kidneys and gastrointestinal tract contents) were found to contain low levels of radioactivity. Other organs had no detectable radioactivity according to the report. The investigators concluded that the slightly higher levels of radioactivity in tissues from males as compared with those for females was the result of the slower fecal excretion rate.

004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Radiolabelled paclobutrazol was used [(2RS, 3RS)1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 (^{14}C)-triazole -1yl)-pentan-3-ol; specific activity = 158.6 uCi/mg). Labelled paclobutrazol was mixed with the unlabelled compound for use in the experiment.

3. STUDY/ACTION TYPE: Metabolism - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Greenslade, D., J. Vickers, and R. Hopkins. May, 1984. (^{14}C)-Paclobutrazol: Bioaccumulation of repeated oral doses (5 mg/kg) in the rat. Unpublished report no. 3743-72/269 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254865.

5. REVIEWED BY:

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6. APPROVED BY:

Name: Jane Harris, Ph. D.
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Organization: Review Section 6
Toxicology Branch
Signature: Jane E. Harris
Date: 3/13/85

7. CONCLUSIONS:

Concentrations of radioactivity plateaued after 28 daily doses of 5 mg ^{14}C-paclobutrazol per kg body weight. Blood levels gradually increased throughout the 49 day dosing period, and fat levels remained at or slightly above the limit of detection during the experiment. The rapid decline of tissue levels after dosing was stopped indicated that there was no retention of the test substance.

004352

8. MATERIALS AND METHODS

Test species: Weanling male Sprague-Dawley Crl:CD(SD)BR strain rats were used.

Dosage form: The test material as described in item 2., above was prepared as a mixture of radiolabelled and unlabelled paclobutrazol (specific activity = 32.16 uCi/mg). The mixture was disolved in polyethylene glycol 600 for administration to test animals.

Analytical methods: Tissue samples, cage debris, and gastro-intestinal tract contents were homogenized in water, and fecal samples were homogenized in methanol. Measured amounts of homogenates were added to ashless floc and combusted. The combustion products were absorbed in Carbosorb™ which was subsequently mixed with liquid scintillant (Permaflour V) for determination of radioactivity.

Cage washings, urine, and methanol extracts of fecal samples were added directly to liquid scintillant (Fisoflour) for counting.

The limit of detection for the combustion analyses was defined as 1.5 times background. The background level of radioactivity was determined by combustion of ashless floc under conditions similar to those used for test samples. Limits for urine and fecal extracts were defined as 1.5 times the background disintegration rate determined by counting the appropriate solvent (water or methanol) in liquid scintillant. These limits were reported as follows:

Sample	Range of ug equivalents/g
Fat	0.040-0.129
Liver	0.013-0.126
Kidney	0.019-0.122
Blood	0.025-0.031

The variability was attributed to the weight of the sample used in the analysis.

Limits in g equivalents were not stated for urine samples or samples of fecal extracts.

8. MATERIALS AND METHODS (continued)

Experimental procedure: Rats were given single daily doses of 5 mg radiolabelled paclobutrazol per kg body weight according to the following schedule:

Animal numbers	Number of doses	Number of vehicle doses	Sacrifice (days after last dose
1-3	3		1
5-7	7		1
9-11	14		1
4, 8, 12		3, 7, 14	1
13-15	21		1
17-19	28		1
21-23	35		1
16, 20, 24		21, 28, 35	1
25-27	42		1
29-31	49		1
33-35	49		3
28, 32, 36		42, 49, 49	1, 1, 3
37-39	49		7
41-43	49		14
45-47	49		21
40, 44, 48		49	7, 14, 21
49-51	49		28
53-55	49		35
52, 56		49	28, 35

Animals numbered 29 through 31 were maintained in metabolism cages for 24 hours following the first and 49th doses so that urine and fecal samples could be collected for analysis.

After sacrifice, samples of liver, kidney, perirenal fat, and blood were taken for determination of concentrations of radioactivity.

9. REPORTED RESULTS

The investigators noted no treatment-related signs of toxicity in test animals.

In the excretion phase of the study the recovery of radioactivity in the urine and feces averaged 41.43 ~~and~~ and 28.76% of the dose

9. REPORTED RESULTS (continued)

the first day, respectively. The respective recoveries in urine and feces after the 49th dose were 43.82 and 14.14%.

In the bioaccumulation phase of the study, the authors noted that higher concentrations of radiolabel were found in the liver and kidney than in the blood or fat, and maximum levels were found in tissues after the 49th dose. Mean concentrations were reported as follows:

Number of doses	Concentration (ug equiv./g) in Liver	Kidney	Blood	Fat
3	0.952	0.534	0.059	0.148
7	1.30	0.539	0.087	<0.081
14	1.30	0.580	0.057	<0.079
21	2.07	0.746	0.092	<0.086
28	2.22	1.05	0.106	0.116
35	1.62	0.541	0.077	0.046
42	2.55	0.923	0.158	0.061
49	4.76	2.73	0.235	0.138

After dosing was stopped, tissue levels were reported to decline below detectable levels after 28 days. Mean concentrations for that phase of the study are summarized as follows:

Days after dosing	Concentration (ug equiv./g) in Liver	Kidney	Blood	Fat
1	4.76	2.73	0.235	0.138
3	0.720	0.207	0.039	<0.044
7	0.115	0.057	0.035	0.088
14	0.050	0.053	0.056	0.090
21	0.027	0.020	<0.027	<0.043
28	<0.015	<0.021	<0.028	<0.043
35	<0.016	<0.022	<0.028	0.058

The investigators described the tissue concentrations observed after the 49th dose as artificially high, and they speculated that the stress of housing the three rats in metabolism cages caused the observed increases. The three rats were also reported to have decreased body weights resulting from their change in housing. On that basis, the results observed after the 42nd dose were used in the calculation of half lives for the tissues.

When concentrations of radioactivity were presented graphically (see Appendix), the authors concluded that elimination from the

9. REPORTED RESULTS (continued)

liver and kidney followed a biphasic exponential curve. They used least spuares linear regression analysis to determine the half times for the two phases, and the results are summarized as follows:

	Half life in days	
Tissue	1st phase	2nd phase
Liver	1.36	6.69
Kidney	1.56	9.26

The half life for radioactivity in the blood was 3.16 days, and because of the nearly undetectable levels in fat a half life was not established for that tissue.

10. DISCUSSION

There were adequate data presented to support the authors' conclusions that concentrations of radioactivity plateaued after 28 daily doses of 5 mg ^{14}C-paclobutrazol per kg body weight. Blood levels gradually increased throughout the 49 day dosing period, and fat levels remained at or slightly above the limit of detection during the experiment. The rapid decline of tissue levels after dosing was stopped indicated that there was no bioretention according to the authors.

With respect to the relatively high tissue levels observed in the rats sacrificed after the 49th dose was given, the investigators noted that recovery of radioactivity in the urine and feces was less than that reported in a single dose experiment (reviewed elsewhere). The increased levels in liver and kidney were also inconsistent with the trends suggested by results from previously sacrificed animals which further suggests that circumstances of the experiment other than a treatment related effect are responsible for the observation after the 49th dose was given.

APPENDIX

Elimination curves for the tissues
sampled from rats given repeated oral doses
of 5 mg ^{14}C labelled paclobutrazol per kg body weight

Paclobutrazol scientific review

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___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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___ The document is not responsive to the request

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004352

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (±)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Paclobutrazol (99%) was used (see Item 1.) The radiolabel (^{14}C) was in the triazole ring, and the stated specific activity was 1.68GBq/mM.

3. STUDY/ACTION TYPE: Metabolism study - rats; (EUP for new chemical)

4. STUDY IDENTIFICATION: Jones, B. K., R. M. Ladd,and J. Galvin. May 31, 1984. Paclobutrazol: Biotransformation in the rat. Unpublished report no. CTL/P/1036 prepared by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Submitted by ICI Americas Inc. EPA Acc. No. 251747.

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7. CONCLUSION: A single oral dose (5 or 250 mg/kg) of paclobutrazol was absorbed almost completely by male and female rats. There was no metabolism of the triazole or halogenated phenyl moieties of the molecule (see Appendix 3 below). Paclobutrazol is oxidized in rats to the diol or carboxylic acid and excreted in conjugated or unconjugated forms. Its metabolism is sex and dose-dependent with males excreting more of the carboxylic acid metabolites than females. However, female rats excrete more of the acid when they receive a low dose (5 mg/kg).

8. MATERIALS AND METHODS

Test species: Seven- to eleven-week old male and female Alpk/AP strain rats were used.

Dosing solutions: Two polyethylene glycol 600 solutions were prepared so that the specific activities for each experiment (see below) were 1.68 GBq/mM and 6.107 KBq/mM.

Experimental procedure: In the first experiment 10 female rats were given a single dose of 250 mg/kg by gavage. The animals were then placed in metabolism cages, and urine and feces were collected at 24 hour intervals for three days following treatment.

In the second experiment the bile ducts of two male and two female rats were canulated. Following recovery each of these rats was given a 250 mg/kg dose by gavage. The animals were subsequently placed in "restraining" cages, and samples of bile, urine, and feces were collected at 24-hour intervals for 4 days following dosing.

At the end of the post-dosing observation periods the animals were weighed and sacrificed. The carcasses were stored at -20° C. Cages for four of the rats from the first experiment were washed with a known volume of methanol:water (1:1), and the washings were saved for radioassay.

Analytical procedures: Fecal samples were homogenized in methanol. Measured amounts of homogenates were combusted, and combustion products were absorbed in Carbosorb™ which was subsequently mixed with liquid scintillant for determination of radioactivity.

Cage washings, urine, and methanol extracts of fecal samples were added directly to liquid scintillant (Fisoflour) for counting.

For purposes of metabolite identification, samples of bile, fecal extracts, and urine were concentrated by lyophilization or rotary film evaporation. The residues from lyophilized urine and bile samples were extracted with methanol, and the extracts were concentrated by rotary film evaporation.

Urine samples and acid hydrolyzed urine and bile samples were adjusted to pH 1.0 with hydrochloric acid and extracted with diethyl ether.

Bile and urine samples as well as concentrated ether extracts

8. MATERIALS AND METHODS (continued)

of urine were incubated in phosphate buffer (pH 7.4) with beta-glucuronidase or sulphatase for 72 hours at 37° C before analysis. These three types of samples were also subjected to acid hydrolysis (5 M HCl refluxed for 5 hours at 100° C).

The urine, bile, and fecal samples as well as their hydrolysates and extracts were subjected to thin layer chromatography (TLC) on silica gel in the following solvent systems:

(i) chloroform:methanol:acetic acid (18:1:1 v/v/v)
(ii) butanol:acetic acid:water (60:15:25 v/v/v)
(iii) butanol:acetic acid:water (85:2:13 v/v/v)

Developed TLC plates were autoradiographed, and radioactivity was quantified by scanner or removal of radioactive bands followed by counting.

Ether extracts of hydrolyzed and unhydrolyzed urine were dried and the residues redissolved in chloroform:methanol: acetic acid (18:1:1 v/v/v) for low pressure liquid chromatography. Fractions eluted in this step were assayed for radioactivity, and radioactive fractions were further concentrated and subjected to reverse phase chromatography.

Derivatization, gas-liquid chromatography, and spectroscopic procedures for identification of metabolites are described in the Appendix below.

9. REPORTED RESULTS

Reported mean percentages of the administered dose recovered in the urine and feces of female rats are summarized as follows:

Time of Sample	Female nos.1-4		Female nos.7-12	
	Urine	Feces	Urine	Feces
24	14.56	1.03	16.12	2.85
48	40.28	7.54	25.63	18.59
72	11.53	12.37	6.18	15.61

The mean percentage of the administered radioactivity recovered in the bile, urine, and feces of cannulated rats is summarized as follows:

9. REPORTED RESULTS (continued)

Time of Sample	Bile	urine	Feces
	Males		
24	28.08	7.16	0.45
48	46.32	11.92	1.21
72	0.27	0.82	0.87
96	0.01	0.16	0.21
	Females		
24	9.63	3.89	0.12
48	24.16	11.96	0.34
72	21.10	15.80	3.99
96	0.14	0.93	2.05

TLC results from bile, urine, and fecal samples indicated that there were two groups of metabolites. Two metabolites were extracted from urine with ether and had Rf values of 0.4 to 0.5. One of those metabolites was identified by derivatization and GCMS analysis as of 1-(4-chlorophenyl)-4, 4-dimethyl-3-hydroxy-3-(1H,1,2,4-triazol-1-yl) pentanoic acid (paclobutrazol acid; see Appendix 2 below). The investigators stated that the underivatized metabolite was also identified by NMR spectroscopy. A similar procedure was used to identify the second metabolite as 1-(4-chlorophenyl)-4,4-dimethyl-3-hydroxy-3-(1H,1,2,4-triazol-1-yl) pentan-5-ol (paclobutrazol diol; see Appendix 2 below).

The investigators noted that the polar metabolites were predominant in the bile. Acid hydrolysis was said to result in one major product which co-chromatographed with paclobutrazol diol. The authors stated:

> Incubation of the urinary and biliary polar metabolites with beta-glucuronidase effected some hydrolysis to yield the diol, but left the remaining polar conjugates unhydrolysed. These conjugates were not hydrolysed with sulphatase and further attempts to identify the intact conjugates were unsuccessful. Hence, on the basis of acid hydrolysis data, these metabolites were designated as unidentified conjugates of paclobutrazol diol and paclobutrazol acid.

The authors noted that quantification of metabolites was attempted with pooled samples from rats of two other studies

9. REPORTED RESULTS (continued)

which have been reviewed elsewhere.

The proportions of each metabolite identified in the urine of male rats given a 5 or 250 mg/kg dose was reported as follows:

Metabolite	% activity in sample	% of dose
Paclobutrazol diol	4*	2*
Paclobutrazol acid	75-78	39-44
Diol/glucuronide conjugate	3-5	1-3
Diol conjugate (unknown acid labile)	7-8	3-5

*Found only in males given the 5 mg/kg dose.

The urine from bile cannulated male rats contained primarily free paclobutrazol acid (98% of urinary radioactivity and 20% of the administered dose).

The levels of urinary metabolites in samples from female rats in the two studies reviewed elsewhare appeared to be dose related. Those results are summarized as follows:

	5 mg/kg dose*		250 mg/kg dose*	
Metabolite	% activity in sample	% of dose	% activity in sample	% of dose
Paclobutrazol diol	8	5	-	-
Paclobutrazol acid	52	32	28	14
Diol/glucuronide conjugate	6-14**	3-7**	-**	-**
Diol conjugate (unknown acid labile	27	17	51	25

**The urine was pooled from animals in other studies which are reviewed elsewhere.
*Not separated according to dose in the report.

The report stated that results from bile duct cannulated female rats showed similar results to those shown above, but the proportion of the administered radioactivity was smaller because of the reduced urinary excretion.

9. REPORTED RESULTS (continued)

The results obtained from urine samples collected from female rats given the 250 mg/kg dose in the first experiments described on page 2 above showed the following:

Metabolite	% activity in sample	% of dose
Paclobutrazol diol	11	7
Paclobutrazol acid	54	37
Diol/glucuronide conjugate	7	5
Diol conjugate (unknown acid labile)	23	16

The authors noted that most of the methanol extractable radioactivity in bile samples was associated with conjugated metabolites. They further stated:

> ...for both sexes, a small amount of paclobutrazol acid was present (males - 8% of biliary radioactivity, 6% of the dose; females - 5% of the biliary label, 3$ of the dose).
>
> Following acid hydrolysis of male bile, 21% of total biliary radioactivity (16% of dose) was shown to correspond to paclobutrazol acid and 67% (50% of the dose) to the diol. Therefore, 13% of the biliary ^{14}C-label (21 - 8%) corresponded to a conjugate of paclobutrazol acid and 67% to the glucuronide and unidentified conjugates of the diol. During enzyme hydrolyses of male biliary conjugates, the control samples were found to be labile under the incubation conditions used, hence, it was not possible to characterise the conjugates further by this procedure. However, chromatographic characteristics of the intact conjugates indicated that the major component was the glucuronide conjugate of the diol.

The authors noted that 95% of the radioactivity in bile from female rats was associated with acid hydrolyzable conjugates which formed the diol aglycone. Seventy-one per cent of the biliary radioactivity (39% of the dose) was attributed to the glucuronide conjugate and 24% (13% of the dose) to the unknown conjugate of the diol.

The authors indicated that approximately 50 to 90% of the radioactivity in fecal samples was extracted in methanol, and the metabolic profile was limited to analysis of that fraction

9. REPORTED RESULTS (continued)

rather than the unextractable residues. The relative proportions of metabolites (% of the total radioactivity in the sample) were reported as follows:

Metabolite	% activity in sample
Paclobutrazol	6-8*
Paclobutrazol diol	6-15
Paclobutrazol acid	12-31
Diol/glucuronide or unknown (acid labile) conjugates combined	35-65

*Approximately 5% of the dose.

There was no appreciable difference with respect to sex of the test animals according to the report.

Fecal samples from one rat of each sex with bile duct cannulation had 67 to 88% of the radioactivity extracted. All of that activity was attributed to unchanged paclobutrazol.

10. DISCUSSION

Adequate data were presented to support the conclusions of the investigators that single oral doses (5 or 250 mg/kg) of paclobutrazol are absorbed almost completely by male and female rats. There was no metabolism involving the triazole or halogenated phenyl moieties of the molecule (see Appendix 3 below). Paclobutrazol is oxidized in rats to the diol or carboxylic acid and excreted in conjugated or unconjugated forms, and its metabolism is sex and dose-dependent.

After the chemical is metabolized in the liver (first pass metabolism), the authors concluded that male rats eliminate approximately 20% of the absorbed dose (250 mg/kg) in urine as paclobutrazol acid. Female rats excreted 11% of the dose as the acid and 19% as free and conjugated paclobutrazol diol. Female rats also metabolized more of a low dose to the acid.

APPENDIX 1

Derivatization Procedures and Spectroscopy

Paclobutrazol scientific review

Page _____ is not included in this copy.

Pages 182 through 184 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 product 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

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X FIFRA registration data

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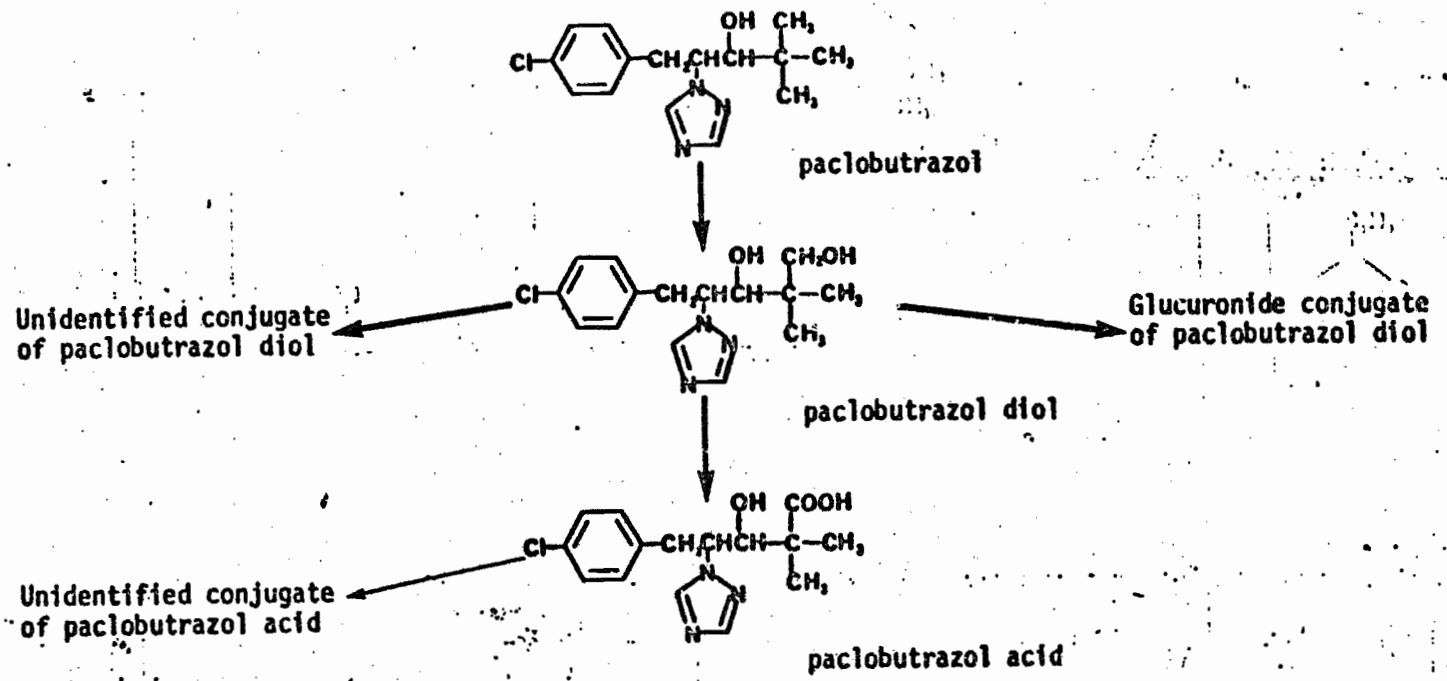
APPENDIX 3

Chemical Structures and Metabolic Pathway

PACLOBUTRAZOL: BIOTRANSFORMATION IN THE RAT

FIGURE 5S

PROPOSED ROUTE OF BIOTRANSFORMATION



DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol (+)-(R*,R*)-beta-[(4-chlorophenyl)methyl]-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol or (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazole-1-yl)-pentan-3-ol

2. TEST MATERIAL: Radiolabelled paclobutrazol was used [(2RS, 3RS)1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 (^{14}C)-triazole -1yl)-pentan-3-ol; specific activity = 158.6 uCi/mg). Labelled paclobutrazol was mixed with the unlabelled compound for use in the experiment.

3. STUDY/ACTION TYPE: Metabolism - dogs; (EUP for new chemical)

4. STUDY IDENTIFICATION: Cresswell, D.G., J. Ward, and R. Hopkins. February, 1984. (^{14}C)-Paclobutrazol: Absorption, excretion, and tissue retention of a single oral dose (5 mg/kg) in the dog. Unpublished report no. 3494-72/270 prepared by Hazleton Laboratories Europe Ltd., Otley Road, Harrogate, North Yorkshire, UK. Submitted by ICI Americas. EPA Acc. No. 254865.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 3/13/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 3/13/85

7. CONCLUSIONS: Paclobutrazol is rapidly absorbed in the dog after a single oral dose of 5 mg/kg is administered. Peak plasma and blood levels were observed 1.5 hours after dosing, and during the 24 hours following treatment approximately 80-85% of the administered radioactivity was recovered in the urine (approximately 50% of the dose) and feces (approximately 40% of the dose). The decline in blood and plasma levels was consistent with

8. CONCLUSION (continued)

the rapid excretion, and along with the absence of detectable radioactivity in the tissues, these results indicated that no significant bioretention occurred. There was no sex difference observed in the test animals.

8. MATERIALS AND METHODS:

Test species: Six-month old male and female beagle dogs were used. The males weighed from 10.2 to 12.9 kg, and the females weighed from 8.5 to 8.82 kg on receipt at the laboratory.

Dosage form: The test material as described in item 2., above was prepared as a mixture of radiolabelled and unlabelled paclobutrazol (specific activity = 32.16 uCi/mg). The mixture was disolved in polyethylene glycol 600 for administration to test animals.

Analytical methods: Tissue samples, cage debris, and gastrointestinal tract contents were homogenized in water, and fecal samples were homogenized in methanol. Measured amounts of homogenates were added to ashless floc and combusted. The combustion products were absorbed in Carbosorb™ which was subsequently mixed with liquid scintillant (Permaflour V) for determination of radioactivity.

Cage washings, urine, and methanol extracts of fecal samples were added directly to liquid scintillant (Fisoflour) for counting.

The limit of detection for the combustion analyses was defined as 1.5 times background. The background level of radioactivity was determined by combustion of ashless floc under conditions similar to those used for test samples. Limits for urine and fecal extracts were defined as 1.5 times the background disintegration rate determined by counting the appropriate solvent (water or methanol) in liquid scintillant.

Experimental procedure: Three male and three female dogs were given a single oral dose of 5 mg test substance per kg body weight. Blood samples were drawn from the jugular vein of each dog just prior to dosing, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours after treatment. Urine and feces were also collected for analysis at 24 hour intervals for 7 days following dosing.

The animals were sacrificed 7 days after treatment and samples of blood, plasma, liver, kidney, heart, lungs, fat, bone,

8. MATERIALS AND METHODS (continued)

brain, gonads, and gastrointestinal tract contents were taken for analysis. The authors noted that only the liver, kidney, blood, plasma, and gastrointestinal contents were analyzed, and the remaining samples were stored (at -20°C) for future reference.

9. REPORTED RESULTS

Reported mean ug equivalents/ml reported in the plasma and blood are summarized as follows:

Time of Sample	Plasma		Blood	
	Males	Females	Males	Females
0.0	<0.016*	<0.013*	<0.024*	<0.018*
0.5	3.949	3.436	2.500	2.153
1.0	4.453	4.106	2.821	2.650
1.5	2.962	3.336	1.852	2.084
2.0	1.878	2.440	1.171	1.522
3.0	0.880	1.181	0.541	0.754
4.0	0.653	0.958	0.402	0.611
6.0	0.570	0.557	0.337	0.344
8.0	0.551	0.409	0.331	0.244
12	0.287	0.291	0.186	0.192
24	0.059	0.043	0.041	0.036
48	<0.016 *	<0.013*	<0.024 *	<0.018*
72	<0.016 *	<0.013*	<0.024 *	<0.018*
168	<0.016 *	<0.013*	<0.024 *	<0.018*

*Limit of detection (see section 8., above)

The reported mean percentage recovery of administered radioactivity in the urine and feces is summarized as follows:

Time of Sample	Urine		Feces	
	Males	Females	Males	Females
24	52.9	48.8	25.2	37.1
48	2.9	1.8	6.1	4.0
72	0.5	0.3	0.9	0.5
96	9.2	0.1	0.3	0.2
120	0.1	0.1	0.2	0.1
144	0.1	0.1	0.2	0.2
168	0.1	<0.1	0.1	0.2
Total	56.6	51.2	33.1	42.3

The group mean percentage of the administered radioactivity recovered in the cage washings 7 days after treatment was

9. REPORTED RESULTS (continued)

reported to be 3.3 and 3.6 for male and female dogs, respectively.

The group mean ug equivalents/g of sample found in the gastrointestinal tract contents and tissue samples from males and females were reported to be less than the limits of detection with the exception of one male whose liver contained 0.057 ug equivalents per g of tissue.

10. DISCUSSION

There were adequate data presented to support the authors' conclusions that paclobutrazol is rapidly absorbed in the dog after a single oral dose of 5 mg/kg is administered. Peak plasma levels were observed 1.5 hours after dosing, and during the 24 hours following treatment approximately 80-85% of the administered radioactivity was recovered in the urine (approximately 50% of the dose) and feces (approximately 40% of the dose). The decline in blood and plasma levels was consistent with the rapid excretion, and along with the absence of detectable radioactivity in the tissues, these results indicated that no significant bioretention occurred. There was no sex difference observed in the test animals.

APPENDIX III

Data Evaluation Record for
the first rat teratogenicity study

004352

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.