(4-10-92) 011061

Guideline Series 84: **MUTAGENICITY**

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DATA EVALUATION REPORT

CHEMICAL: Paclobutrazol Tox. Chem. No.: 628C

STUDY TYPE: In Vivo Unscheduled DNA Synthesis Assay in Rat Hepatocytes

ACCESSION or MRID NUMBER: 407343-04

SPONSOR: Imperial Chemicals Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Paclobutrazol: Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo

AUTHOR(S): R. W. Trueman

STUDY NUMBER(S): Lab Project No. CTL/P/1608

REPORT ISSUED: October 21, 1986

CONCLUSION(S) - Executive Summary: Paclobutrazol was tested for capability to induce unscheduled DNA synthesis in vivo in rat hepatocytes. Rats were orally dosed with 40, 200 or 400 mg/kg bw paclobutrazol and hepatocytes were isolated from the animals at either 4 or 12 hours post dosing. UDS was measured and calculated from the cells and compared to both vehicle and positive controls. Paclobutrazol was not found to induce unscheduled DNA synthesis in rat hepatocytes exposed in vivo.

Classification: This study utilizes a procedure which is not a standard protocol normally seen by the Agency. The study cannot be graded for regulatory purposes at this time until we receive additional explanations on the protocol (see discussion).

TESTING GUIDELINE SATISFIED: None.

A. MATERIALS

1. Test Material: Name: (2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-(1,2,4-triazol-1-yl)pentan-3-ol

Description (e.g. technical, nature, color, stability): Buff colored powder, stable in sealed container at room temperature for a period in excess of 4 years.

Batch #: CTL ref. no. Y00001/001/017 Purity: 92.4%
Contaminants: if reported, list in CBI appendix
Solvent used: corn oil
Other comments: The ^{3}H-thymidine solution (37 MBq/ml; TRK 120) was obtained from Amersham International PLC. 100µl (3.7MBq; 100µCi) was added to each 10 ml WE-incomplete solution.

2. Control Materials:
Negative (if not vehicle)/Route of administration: Corn oil/gavage

Vehicle/Final volume/Route of administration: 10 ml/kg/gavage

Positive/Final dose(s)/Route of administration: 6-dimethyl-aminophenyl-azobenzthiazole/gavage/40 mg/kg bodyweight in corn oil (6BT).

3. Test compound:
Volume of test substance administered: 10 ml/kg
Route of administration: gavage
Dose levels used: 40, 200, and 400 mg/kg

4. Test animals:
a. Species male Alderley Park (SPF) Strain ALpk:AP albino
Age 7-10 weeks
Weight: male 240-343 g female N/A
Source: Animal Breeding Unit, ICI PLC, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire UK

b. No. animals used per dose: 2 (controls), 4 or 5 depending on experiment number.
c. Properly maintained? Yes

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
   a. Test compound
      Dosing: __1__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): __x__ 4 hr __x__ 12 hr
      _____ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
      _____ other (describe):
   b. Negative and/or vehicle control
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): __x__ 4 hr __x__ 12 hr
      _____ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
      _____ other (describe):

   c. Positive control
      Dosing: __x__ once _____ twice (24 hr apart)
      _____ other (describe):

      Sampling (after last dose): __x__ 4 hr __x__ 12 hr
      _____ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
      _____ other (describe):

2. Tissues and Cells Examined:

   _____ bone marrow __x__ other (list): hepatocytes

3. Details of slide preparation: The report stated that "Suspensions of hepatocytes were prepared from animals dosed with paclobutrazol, corn oil or 6BT. The suspension of hepatocytes was diluted in order to give a concentration of 1.5×10^5 viable cells per ml and the cells allowed to attach to a series of plastic coverslips.

   When the cells were attached (1.5h) the supernatant medium was removed and replaced with medium containing ^{3}H-thymidine. The hepatocytes were then incubated in a 5% CO_2/air atmosphere for 4 hours. After this period the supernatant containing radio-label was removed, the cells washed three times and then incubated overnight with medium containing unlabelled thymidine. The cells were then washed, fixed, dried and mounted.

The prepared slides were coated with photographic emulsion and stored in total darkness at 4°C for 14 days. After the exposure period the slides were developed and the cells stained."

The net grain count was measured and calculated using an automated image analyzer for the actual counting and a computer program for the calculations. If an individual dose level has a mean net grain count of 3 or greater, then that is regarded as a positive response. The effect had to be reproducible.

4. UDS Assay (reported results, appropriateness of negative, solvent and positive control UDS frequencies; appropriateness of dose levels and route; statistical evaluation; include representative table, if appropriate): The complete protocol is provided as an appendix to this report. Hepatocytes were prepared from rats 4 and 12 hours following administration of 40, 200 or 400 mg/kg bodyweight of the test chemical and examined for evidence of unscheduled DNA synthesis. Duplicate tests were conducted. The report stated that "there were no significant increases over controls in either the mean net grain count or the percentage of cells in repair in hepatocytes from animals administered paclobutrazol. The negative and positive control hepatocytes showed the expected responses." At 400 mg/kg bodyweight, there was clear evidence of cytotoxicity in the hepatocytes in that there were pyknotic and deeply staining nuclei. The data from this dose level were included because there were sufficient cells of normal morphology to be examined. At 200 mg/kg, there was no evidence of cytotoxicity, however, at the 12 hour time point, an increase in the number of cells undergoing 'S'-phase (i.e. replicative or scheduled DNA synthesis) was observed. Since these cells were easily recognized, their presence did not interfere with the evaluation of the remainder of the hepatocytes for evidence of UDS. The following table summarizes the results.

PACLOBUTRAZOL: ASSESSMENT FOR THE INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES IN VIVO

Dose (mg/kg)	No. of Animals	N ± SD	C ± SD	Mean (N-C) ± SD	Mean % in Repair (1) ± SD
12 Hours					
Paclobutrazol 40	5	4.54 ± 1.01	7.59 ± 2.97	-3.05 ± 1.98	0.40 ± 0.89
" 200	5	6.91 ± 1.84	11.21 ± 1.93	-4.31 ± 0.87	1.00 ± 1.73
" 400	5	5.58 ± 2.68	9.50 ± 5.18	-3.92 ± 2.62	0.60 ± 1.34
6BT 40	2	27.80 ± 1.61	9.60 ± 5.11	18.20 ± 6.71	87.00 ± 11.31
Corn oil 10ml/kg	2	4.64 ± 0.26	6.64 ± 0.30	-2.00 ± 0.57	0
4 Hours					
Paclobutrazol 40	5	11.90 ± 2.77	19.34 ± 3.78	-7.43 ± 1.13	2.00 ± 1.41
" 200	5	10.72 ± 2.11	20.51 ± 3.25	-9.79 ± 1.55	0.20 ± 0.45
" 400	4	11.86 ± 1.99	22.75 ± 2.88	-10.89 ± 0.94	0.75 ± 0.50
6BT 40	2	36.14 ± 6.17	20.55 ± 0.01	15.59 ± 6.15	85.00 ± 7.07
Corn oil 10ml/kg	2	10.39 ± 4.24	16.68 ± 5.09	-6.30 ± 0.86	1.00 ± 1.41

Legend

N ± SD = mean nuclear grain count ± standard deviation

C ± SD = mean cytoplasmic grain count ± standard deviation

(1) A cell in repair = net grain count $\geq$ 5.

5. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions): There do not appear to be any major discrepancies in these results. The positive and negative controls gave appropriate responses. This protocol is not one of the standard UDS protocols submitted to the Agency. It is a combination in vivo/in vitro assay. Since this is the case, the Toxicology Branch is requesting a justification for the short incubation time in tritiated thymidine (4 hours versus 18-20 hours normally used in the in vitro UDS assay). This study cannot be graded for regulatory purposes until the additional information is provided.

6. Was test performed under GLPs (is a quality assurance statement present)? Yes

7. CBI appendix attached No

Page_____ is not included in this copy.

Pages 7 through 19 are not included.

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