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

JUN 17 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of four mutagenicity assays for MCPB Acid received by Data Call-In

TO: Gerry Werdig/F. Rubis
Data Call-In Staff
PM 50
Registration Division (TS-767)

FROM: Margaret L. Jones MJ Jones 17 June 1988
Review Section III
Toxicology Branch
Hazard Evaluation Division (TS-769)

THROUGH: Marcia van Gemert, Ph.D., Head
Review Section III M. van Gemert 6/17/88
Toxicology Branch

and Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division

Chemical: MCPB; 4-(2-Methyl-4-chlorophenoxy)butyric acid
CAS 94-81-5
Tox. Chem: 558
Record No: 220770
Registrant: Rhone Poulenc

Action Requested: Review four mutagenicity assays received as a result of the Data Call-In notice for MCPB.

Conclusions: The four studies were non-acceptable as received, primarily due to the absence of product indentification information. Several of the studies can be easily upgraded with this information.

AMES ASSAY: MCPB technical did not induce reverse mutations when tested at 5.00, 16.7, 50.0, 167, 500, and 1670 ug/plate with and without metabolic activation. The study was done using 3 plates per dose and the result should be fully acceptable once test article information is received, as described below. Cytotoxicity (testing was done without S9) was demonstrated at 500 and 1670 ug/plate as reduced background lawn; complete inhibition of growth was observed at 5000 ug/plate.

Study: Non-acceptable. Test article was not analysed for stability. Purity was reported, however, impurities and/or contaminants were not reported. Rationale for changing solvents from DMSO for the preliminary cytotoxicity assay to EtOH for the full assay was not reported.

FORWARD MUTATION/CHO CELLS: MCPB Acid Technical was tested in Chinese hamster ovary cells (HGPRT locus)[in vitro] at concentrations of 50.0, 100, 250, 500, 1000, 1500 and 2000 ug/ml with and without S9. In an assay to confirm or refute results of the first assay (portion done with S9) , which indicated the test substance is a suspect mutagen, concentrations were tested as follows: 50.0, 100, 250, 500, 600, 700, 800, 900, and 1000 ug/ml with S9. Negative and solvent (EtOH, 10.0 ug/ml) controls and positive controls with S9 (dimethylnitrosamine, 200 ug/ml) and without S9 (ethylmethanesulfonate, 100 ug/ml) were used in initial and confirmatory mutagenicity assays. Results showed MCPB acid technical was not mutagenic in Chinese hamster ovary cells at the HGPRT locus.

Study: Non-acceptable. Test substance analysis with purity, contaminants, and stability information was not found in the test report.

CHROMOSOMAL ABERRATIONS: MCPB Acid was tested in Chinese hamster ovary cells (CHO) at 100, 600, and 1000 ug/ml (-S9) and at 75, 350, and 750 ug/ml (+S9). Statistically significant increases in numbers of aberrations per cell were observed at 750 ug/ml with S9. At this dose, average proliferation time was lengthened by 21% (about 64% of cells replicated between 1-2 cycles in the presence of BrdUrd). MCPB acid produces chromosome anomalies at 750 ug/plate (with S9) when tested as described in the report.

Classification: Non-acceptable. Test substance analysis with stability and contaminant information and cell line maintenance information will be necessary in order to upgrade the study.

DNA REPAIR: Propachlor was tested in rat primary hepatocyte cultures at doses from 0.01 to 3000 ug/ml. Cytotoxicity was observed at 3.0, 10, 30, 100, 300, 1000, and 3000 ug/ml. The high dose selected for the full assay was therefore 1.0 ug/ml. Doses scored were 0.01, 0.03, 0.1, 0.3, and 1.0 ug/ml. Positive controls demonstrated the sensitivity of the test system. None of the dose levels appeared to produce unscheduled DNA synthesis in the cells tested.

Study: Non-acceptable. Test substance analysis was not included in the study report. Information verifying reported purity and on stability and contaminants in the technical chemical will be necessary in order to upgrade the study.

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Guideline Series 84: MUTAGENCITY

Reviewed by: Margaret L. Jones M. L. Jones 17 June 1988
Section III, Tox Branch (TS-769)
Secondary reviewer: Irving Mauer, Ph.D. Irving Mauer, 06/17/88
Review Section VI, Tox Branch (TS-769)
Date:

DATA EVALUATION REPORT

CHEMICAL: 2-Methyl-4-chlorophenoxybutyric acid Tox. Chem. No.: 558

EPA File Symbol:

STUDY TYPE: Mammalian cells in culture cytogenetics assay in Chinese hamster ovary cells

ACCESSION NUMBER: 405643-01

SYNONYMS/CAS No.: MCPB Acid; 94-81-5

SPONSOR: Rhone-Poulenc

TESTING FACILITY: Pharmakon Research International, Inc.
P.O. Box 313
Waverly, Pa. 18471

TITLE OF REPORT: 2-Methyl-4-chlorophenoxybutyric acid (MCPB acid) In Vitro Chromosome Aberration Analysis in Chinese Hamster Ovary (CHO) Cells

AUTHOR(S): SanSebastian, J.R.

STUDY NUMBER(S): PH 320-RP-001-87

REPORT ISSUED: February 22, 1988; study completed on October 30, 1987

CONCLUSIONS(S) - Executive Summary: MCPB Acid was tested in Chinese hamster ovary cells (CHO) at 100, 600, and 1000 ug/ml (- S9) and at 75, 350, and 750 ug/ml (+S9). Statistically significant increases in numbers of aberrations per cell were observed at 750 ug/ml with S9. At this dose, average proliferation time was lengthened by 21% (about 64% of cells replicated between 1-2 cycles in the presence of BrdUrd). MCPB acid produces chromosome anomalies at 750 ug/plate (with S9) when tested as described in the report.

Classification: Non-acceptable. Test substance analysis with stability and contaminant information and cell line maintenance information will be necessary in order to upgrade the study.

A. MATERIALS

1. Test Material:
Name: 2-Methyl-4-chlorophenoxybutyric acid (MCPB acid);
Description: technical, white to off-white powder)
Lot # NPD-X094R
Stability: unreported
Purity: 97.6% (not accompanied by analysis sheet)
Contaminants: unreported
Solvent: dimethylsulfoxide (DMSO)

2. Control Materials:
Negative:
Solvent/final concentration: DMSO 100 ul at final concentration of 1%

Positive: Non-activation: N-Methyl-N-nitro-N-nitrosoguanidine (MNNG); Aldrich Chem. Lot# 030277; dissolved in ethanol and added in a 20 ul volume for final concentration of 2.0 ug/ (1.7×10^{-5}M)

Activation: N-Nitrosodimethylamine (DMN); Aldrich chem. Lot# 80383KM; dissolved in distilled water and added in a 100 ml volume for final concentration of 1000 ug/ml (1.35×10^{-2}M)

3. Activation: S9 derived from Aroclor 1254 induced rat liver

S9 mix composition: 10 mM $MgCL_2$, 10 mM $CaCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP (disodium salt), 50 mM sodium phosphate buffer (pH 7.4) and 0.1 ml of microsomal preparation (from Aroclor 1254 induced rat liver) with 44.2 mg protein/ml.

4. Test compound concentrations used:
Non-activated conditions: 100, 600, 1000 ug/ml
Activated conditions: 75, 350, and 750 ug/ml

5. Test cells: mammalian cells in culture

CHO-K1-BH4 cells, Lot # A-12, a continuous cell line with the modal number of 20 chromosomes and a population doubling time of 12-14 hours. Cell line was obtained from Dr. Abraham W. Hsie, Biology Division, Oak Ridge National Laboratories, Oak Ridge, Tenn.

Properly maintained? not reported

Cell line or strain periodically checked for Mycoplasma contamination? not reported

Cell line or strain periodically checked or karyotype stability? not reported

IN VITRO MAMMALIAN CYTOGENETICS

B. TEST PERFORMANCE

1. Cell treatment:
   a. Cells exposed to test compound for:
      5 hours (non-activated) 5 hours (activated)

   b. Cells exposed to positive controls for:
      5 hours (non-activated) 5 hours (activated)

   c. Cells exposed to solvent controls for:
      5 hours (non-activated) 5 hours (activated)
      (negative control without solvent was not in assay)

2. Protocol:

See appended pages 1-2 (pages 8-9 from test report PH 320-RP-001-87)
Number of cell cultures: two for each dose group and controls
Medium: F12 (ham's, K.C. Biological Co.)
Incubation times: 5 hours after treatment; 18 hours after washing and adding more medium; 2-3 hours after adding colcemid
Cell density during treatment: not reported, however density when growth phase began was 8 x 10^5 cells/80 cm^2; cells allowed to grow for 16-24 hours
Harvest times: 5 hours, 20-21 hours
Spindle inhibitor: colcemid 2-3 hours at end of incubation
Chromosome preparation and analysis: see appended page 2
Number of cells/culture analyzed: 100 "well spread" metaphases were scored for each data point (50 per culture)
Statistics: Chi-square to analyse cells with $\geq$ 1 aberration; *t* test to analyse aberration numbers in treated compared to controls;

3. Preliminary cytotoxicity assay

Doses of 5, 25, 50, 100, 250, 500, 750, 1000, 2500, and 5000 ug/ml were evaluated with and without S9. 1500 ug/ml without S9 was selected after observing that 2500 ug/ml without S9 had too few metaphases to score, 5000 ug/ml without S9 had no cell survival, and 2000 ug/ml had only a 20.73% increase in average proliferation time. In S9-treated cells, there was no cell survival at 2500 or 5000 ug/ml. At 1000 ug/ml, 80.04% increase in average proliferation time was noted and at 750 only a 37.34% increase in this parameter was noted, therefore 850 was selected as the high dose for the assay with S9. Rationale for determining harvest times was not reported.

4. Cytogenetics assay with study author's conclusions

Results appear on appended pages 3-5 (pages 13-15 from test report PH 320-RP-001-87). These pages include a legend which defines chromosome aberrations. Gaps were not included in the scoring of chromosomal aberrations but were reported separately.

In a screen for cytotoxicity in the full assay, it was discovered the 850 ug/ml (+S9) and 1500 ug/ml (-S9) doses had low numbers of metaphases and abnormal interface nuclei, therefore these dose levels were not included in analysis.

The study author concluded the test substance did not induce a statistically significant increase in aberrations/cell or in the proportion of aberrant metaphases at any dose evaluated without S9 mix. At 750 ug/ml with S9 mix there were statistically significant increases in aberrations/cell and in proportion of aberrant metaphases. Dose related trends were noted at the low and mid doses (75 and 350 ug/ml).

Positive controls demonstrated the sensitivicy of the test system with and without metabolic activation.

5. Reviewer's discussion/conclusions

Toxicology Branch agrees with the study author's conclusion. The large proportion of aberrations were apparently due to chromatid deletions and to interchanges in chromatids from different chromosomes.

Certain information was not found in the study report, which otherwise is generally acceptable. Product analysis should be provided, with stability information and contaminants or impurities in the technical formula. Information about treatment of cell line should also be provided, including whether the strain was checked for Mycoplasma, and karyotype stability.

MCPB toxicology review

Page _____ is not included in this copy.

Pages 7 through 11 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

___ Information about a pending registration action

X FIFRA registration data

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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.

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Guideline Series 84: MUTAGENICITY

iewed by: Margaret L. Jones M. L. Jones 17 June 1988
tion III, Tox. Branch (TS-769)
ondary reviewer: Irving Mauer, Ph.D.
iew Section VI, Toxicology Branch (TS-769)

DATA EVALUATION REPORT

MICAL: 2-Methyl-4-chlorophenoxybutyric acid
Tox. Chem. No.: 558
EPA file symbol:

DY TYPE: Mammalian cells in culture/ DNA Repair test

ESSION NUMBER: 405643-04

ONYMS/CAS No.: MCPB Acid; 94-81-5

NSOR: Rhone-Poulenc

TING FACILITY: Pharmakon Research International, Inc.
Waverly, Pa. 18471

LE OF REPORT: 2-Methyl-4-chlorophenoxybutyric acid (MCPB Acid)
Rat Hepatocyte Primary Culture/ DNA Repair Test

HOR: Barfknecht, T.R., Ph.D.

DY NUMBER: PH 31-RP-001-87

ORT ISSUED: February 22, 1988; Study completed: November 3, 1987

CLUSIONS: Executive Summa : Propachlor was tested in rat mary hepatocyte cultures at doses from 0.01 to 3000 ug/ml. otoxicity was observed at 3.0, 10, 30, 100, 300, 1000, and 0 ug/ml. The high dose selected for the full assay was refore 1.0 ug/ml. Doses scored were 0.01, 0.03, 0.1, 0.3, 1.0 ug/ml. Positive controls demonstrated the sensitivity the test system. None of the dose levels appeared to duce unscheduled DNA synthesis in the cells tested.

dy: Non-acceptable. Test substance analysis was not luded in the study report. Information verifying reported ity and on stability and contaminants in the technical nical will be necessary in order to upgrade the study.

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PRIMARY CULTURE: DNA Repair

A. MATERIALS

1. Test Material:
Name: 2- Methyl-4-chlorophenoxybutyric acid;
Description: technical; white to off-white powder; Purity: 97.6%.
Lot #: NPD-X094R, received 9/1/87
Contaminants: not reported
Solvent used: ethanol, Lot #: 87H12A-71
Other comments: Test substance analysis was not included in the study report. Information about purity, stability, and homogeneity of the test substance will be necessary in order to uprade the study.

2. Control Materials:
Negative: a negative control was not used
Solvent/final concentration: 1% ethanol
Positive: 2-Acetamidofluorene (2AAF) at $1X10^{-7}$M final concentration

3. Test cells:
Rat (Fischer 344) primary hepatocytes; Method of collection described in protocol

4. Test compound concentrations used:
0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 30, 100, 300, 1000, and 3000 ug/ml in ethanol were tested to ensure sufficient dose levels for scoring

B. TEST PERFORMANCE

1. Cell treatment:
Triplicate cultures were seeded with $1X10^5$ viable cells and were treated with 20 ul of MCPB Acid at doses given above

2. Protocol:
A modification of the method of Williams as described in appended pages 1-3 (pages 7-9 of test report PH-RP-001-87)

3. Preliminary cytotoxicity assay:
A preliminary cytotoxicity assay using acetone as solvent and doses ranging from 3.0 to 3000 ug/ml produced a "high degree of cytotoxicity", therefore a retest was planned using ethanol as solvent. In the repeat assay, doses from 0.0* to 3000 were used, however, cytotoxicity was again observed at the high doses and doses selected for scoring were 0.01, 0.03, 0.1, 0.3, and 1.0.

4. Mutagenicity assay:
Cells were prepared as described. Viable hepatocytes ($1X10^5$) were inoculated into 12 well cluster dishes containing 15 mm diameter Thermanox plastic coverslips. Cells were allowed to attach (in medium- WMES) for 2 hours in a 37°incubator. After two hours growth, cultures were washed and new serum-free medium with test compound with 10 uC/ml of ^{3}H-Thymidine were added to each culture and allowed 18 to 20 hours exposure. Cells were then fixed and stained for examination.

Cells counted:
150 cells per dose were counted for determination of unscheduled DNA synthesis (UDS).

Correction coefficient:
Calculated by obtaining an area/grain-ratio by visually scoring an area of each slide with 3-5 nuclear grains and then using the Artek Model 880 Colony Counter to obtain an object area count. Five areas were scored and the mean value used as correction coefficient.

Cytoplasmic grain count, nuclear grain count and net grain count:
Cytoplasmic count obtained by randomly selecting three nuclear sized areas adjacent to the nucleus. Net nuclear grain count was obtained by subtracting the largest of three cytoplasmic counts from the uncorrected nuclear count.

Criteria for positive result: Minimum net grain count of 3 per nucleus is consistently observed in three wells and a dose response is observed.

Results:
Positive controls demonstrated the sensitivity of the test system. Solvent control was negative. Cytotoxicity was observed at doses of 3.0 to 3000 ug/ml and doses scored were 0.03, 0.1, 0.3, 1.0 ug/ml. None of the treated cultures produced mean net nuclear grain counts greater than solvent control.

5. Reviewer's discussion and conclusions:
The study appears to have been properly conducted. The criteria for positive result in this assay are more conservative than other available protocols (the criteria for positive result here was 3 net grains per nucleus and in other protocols values of 5 and 6 were found). The test substance does not appear to produce DNA repair in rat primary hepatocyte cultures. When test substance analysis is received the study can be upgraded to acceptable.

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

7. CBI appendix attached? YES

MCPB toxicology review

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Pages 16 through 18 are not included in this copy.

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___ 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

___ Information about a pending registration action

X FIFRA registration data

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Guideline Series 84: Mutagenicity

006896

Reviewed by: Margaret L. Jones M-L. Jones 17 June 1988
Section III, Toxicology Branch (TS-769)
Secondary reviewer: Irving Mauer, Ph.D.
Review Section VI Toxicology Branch (TS-769)
Date:

DATA EVALUATION REPORT

CHEMICAL: 2-methyl-4-chlorophenoxybutyric acid; MCPB Acid

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER: 405643-02

SYNONYMS/CAS NO.:

SPONSOR: Rhone-Poulenc AG Company

TESTING FACILITY: Pharmakon Research International, Inc.

TITLE OF REPORT: 2-Methyl-4-clorophenoxybutyric acid (MCPB Acid) Ames/*Salmonella* Plate Incorporation Assay

AUTHOR: Leon F. Stankowski, Jr., Ph.D.

STUDY NUMBER: PH 301-RP-001-87

REPORT ISSUED: February 22, 1988; study completed October 18, 1987

CONCLUSIONS: Executive Summary: MCPB technical did not induce reverse mutations when tested at 5.00, 16.7, 50.0, 167, 500, and 1670 ug/plate with and without metabolic activation. The study was done using 3 plates per dose and the result should be fully acceptable once test article information is received, as described below. Cytotoxicity (testing was done without S9) was demonstrated at 500 and 1670 ug/plate as reduced background lawn; complete inhibition of growth was observed at 5000 ug/plate.

Study: Non-acceptable. Test article was not analysed for stability. Purity was reported, however, impurities and/or contaminants were not reported. Rationale for changing solvents from DMSO for the preliminary cytotoxicity assay to EtOH for the full assay was not reported.

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A. MATERIALS

1. Test Material: Name: 2-methyl-4-chlorophenoxybutyric acid (MCPB Acid); Description: technical grade MCPB; Lot No.:NPD-X094R; white/off white powder; Purity: 97.6%; Analysis of test substance for impurities and stability was not reported; Contaminants, if any, are unknown; Solvents used: dimethyl sulfoxide (DMSO), Lot # 851283 or ethanol (EtOH), Lot # 87A14A-18. EtOH was used in the full assay. DMSO was apparently used in the preliminary cytotoxicity assay.

2. Control Materials:
Negative Control: solvent with and without S-9 activation in triplicate plates for each strain, DMSO (1.7 mL) was used in the preliminary cytotoxicity assay, and EtOH (10.87 mL) was used in the mutagenicity assay.
Positive Control: known positive controls were used to test each strain with and without S-9 activation, as follows:
TA1535 and TA100, sodium azide, 10.0 ug/plate, without S-9
TA1537, 9-aminoacridine, 150 ug/plate, without S-9
TA1538 and TA98, 2-nitrofluorene, 5.00 ug/plate, without S-9
All tester strains, 2-anthramine, 2.5 ug/plate, with S-9

3. Activation: S9 derived from Aroclor 1254 induced male Sprague-Dawley rat liver homogenate. S9 mix contained 8mM $MgCl_2$, 33mM KCl, 4mM NADP, 5mM glucose-6-phosphate, 100mM Na_2HPO_4 (pH 7.4) and 6% (v/v) Aroclor 1254-liver homogenate, as described above.

4. Test organisms:
TA98, TA100, TA1535, TA1537, TA1538 strains of S. typhimurium from Dr. Bruce N. Ames of U. CAL, Berkeley, CA; Tester strains were checked monthly for the presence of appropriate genetic markers (uvrB deletion mutation, rfa mutation, hisG locus, hisC locus, hisD locus, depending on the particular strain) [method of checking not described]; Cultures appeared to be properly maintained.

5. Test concentrations used:
Non-activated conditions: 5.00, 16.7, 50.0, 167, 500, 1670 ug/plate
Activated conditions: same concentrations

B. TEST PERFORMANCE

1. Type of Salmonella assay: standard plate assay

a. Protocol: See appended pages 1-2 (pages 8 and 9 of report PH 301-RP-001-87

b. Evaluation criteria: See appended page 2.

2. Preliminary cytotoxicity assay Test article was evaluated at concentrations of 50.0, 167, 500, 1670, and 5000 ug/plate in the absence of S9 to determine growth of background lawn and/or frequency of spontaneous revertants. Each dose was also evaluated in duplicate cultures of strains TA1538 and TA100 and solvent control (DMSO) was also evaluated. Apparently, preliminary testing with S9 activation was not performed.

Results: MCPB was not toxic to TA1538 or TA100 at doses of 50.0 and 167 ug/plate without S9 activation. Inhibition of growth (reduced background lawn and/or presence of pindot colonies) was observed at 500 and 1670 ug/plate in both strains and at 5000 ug/plate in TA100. Complete toxicity (no growth) was found in TA1538 at 5000 ug/plate.

3. Mutagenicity assay - Appended page 3 (page 11 of test report PH 301-RP-001-87) shows the summary of results obtained from three plates per dose with and without metabolic activation.

Results: Revertant frequencies in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100 treated with 5.00, 16.7, 50.0, 167, 500, and 1670 ug/plate with and without metabolic activation were in the range of concurrent and historical controls (appended page 2) in the assay. Positive controls demonstrated the sensitivity of the tester strains and negative (solvent-EtOH) controls showed results similar to concurrent and historical controls.

4. Toxicology Branch discussion/conclusions:

According to the reported results, MCPB technical was not mutagenic in Salmonella tester strains TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation. The assay was performed using three plates per dose and a replicate assay will not be required. However, the rationale for changing solvents from DMSO in the preliminary cytotoxicity assay to EtOH in the mutagenicity assay was not explained. Test substance analysis should be reported including purity, contaminants, and stability of test substance. The assay should be fully acceptable when the missing information is provided.

5. The assay was performed under GLP Regulations and quality assurance and GLP compliance statements were included in the report.

6. CBI appendix not attached.

MCPB toxicology review

Page _____ is not included in this copy.

Pages 22 through 23 are not included in this copy.

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___ Identity of product inert ingredients

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Guideline Series 84: MUTAGENICITY

Reviewed by: Margaret L. Jones M. L. Jones 17 June 1988
Section III, Toxicology Branch (TS-769C)
Secondary reviewer: Irving Mauer, Ph.D.
Review Section VI, Toxicology Branch (TS-769C)
Date:

006896

DATA EVALUATION REPORT

CHEMICAL: 2-methyl-4-chlorophenoxybutyric acid; MCPB Acid Technical

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster ovary cells (HGPRT locus)

ACCESSION NO.: 405643-03

SYNONYMS/CAS NO.:

SPONSOR: Rhone-Poulenc AG Company

TESTING FACILITY: Pharmakon Research International, Inc.
Waverly, Pa. 18471

TITLE OF REPORT: 2-methyl-4-chlorophenoxybutyric acid (MCBP Acid) CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay

AUTHORS: Stankowski, L., Tuman, W., Bieszczad, M.

STUDY NUMBERS: PH 314-RP-001-87

REPORT ISSUED: February 22, 1988, Study completed: January 8, 1988

CONCLUSIONS: Executive Summary: MCPB Acid Technical was tested in Chinese hamster ovary cells (HGPRT locus)[in vitro] at concentrations of 50.0, 100, 250, 500, 1000, 1500 and 2000 ug/ml with and without S9. In an assay to confirm or refute results of the first assay (portion done with S9) , which indicated the test substance is a suspect mutagen, concentrations were tested as follows: 50.0, 100, 250, 500, 600, 700, 800, 900, and 1000 ug/ml with S9. Negative and solvent (EtOH, 10.0 ug/ml) controls and positive controls with S9 (dimethylnitrosamine, 200 ug/ml) and without S9 (ethylmethanesulfonate, 100 ug/ml) were used in initial and confirmatory mutagenicity assays. Results showed MCPB acid technical was not mutagenic in Chinese hamster ovary cells at the HGPRT locus.

Study: Non-acceptable. Test substance analysis with purity, contaminants, and stability information was not found in the test report.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

1. Test Material: Name: 2-methyl-4-chlorophenoxybutyric acid (MCPB Acid, technical grade); white powder, stability unreported, purity 97.6% (not accompanied by analysis sheet), Lot # NPD-X094R
Contaminants: not reported
Solvent: ethanol (EtOH) Lot # 87A14A-18, Pharmco Products, dilutions were prepared and used within one hour

2. Control Materials:
Negative: untreated cultures with and without metabolic activation (S9)
Solvent: Ethanol (EtOH), with and without metabolic activation (S9)
Positive: non-activation: Ethylmethanesulfonate (EMS), 200 ug/ml
Positive: activation: Dimethylnitrosamine (DMN), 100 ug/ml

3. Activation: S9 derived from: Aroclor 1254 induced rat liver homogenate, S9 mix was 2% rat liver homogenate with buffer and cofactors

4. Test cells: mammalian cells in culture: Chinese hamster ovary cells [CHO-K1-BH4 (clone K1, subclone BH4)] from Dr. Abraham Hsie, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37831
Cells were properly maintained, periodically checked for Mycoplasma contamination, karyotype stability and "cleansed" against high spontaneous background.

5. Locus examined: hypoxanthine-guanine-phosphoribosyl tranferase (HGPRT) with selection agent: 10uM 6-thioguanine

6. Test compound concentrations used:

Preliminary cytotoxicity assay:
With and without S9: 0.167, 0.500, 1.67, 5.00, 16.7, 50.0, 167, 500, 1670, 5000 ug/ml

Mutagenicity assay:
With and without S9: 50.0, 100, 250, 500, 1000, 1500 and 2000 ug/ml; N.B. two extra concentrations were used with and without S9 in the event of unacceptably high cytotoxicity which was expected, based on the results of cytotoxicity prescreen; The investigators reported significant dose-related increases in average mutant frequencies in the portion with metabolic activation therefore the activation portion of the assay was repeated using the following doses: 50.0, 100, 250, 500, 600, 700, 800, 900, and 1000 ug/ml with S9

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MAMMALIAN CELLS IN CULTURE GENE MUTATION

B. TEST PERFORMANCE

1. Cell treatment:

   a. Cells exposed to test compound for 5 hours (non-activated and activated)
   b. Cells exposed to positive controls for 5 hours (non-activated - DMN, 100 ug/ml, and activated - EMS 200 ug/ml)
   c. Cells exposed to negative and solvent controls (EtOH, 10 ul/ml) for 5 hours (non-activated and activated)
   d. After washing 3 times with 5 ml saline G cells were incubated 7-8 days (expression period) before cell selection
   e. After expression, cells were cultured for 10 days in selection medium to determine numbers of mutants and for 10 days without selection medium to determine cloning efficiency.

2. Protocol: see Appended pages 1-3 (pages 8-10 from report No. PH 314-RP-001-87).

3. Preliminary cytotoxicity assay: Test substance was found to exceed solubility limits in a stock solution of 500 mg/ml in EtOH and was adminstered as a uniform fine suspension. Substance precipitated when added to culture medium at concentrations $\geq$ 500 ug/ml. Results of preliminary cytotoxicity assay appear on Appended page 4 (p 22 from report No. PH 314-RP-001-87).

4. Mutagenicity assay: Results appear in Appended pages 4-8 (pp 13-17 from report No.: PH 314-RP-001-87). The mutagenicity assay was repeated (see Sec. A.6.) due to increased mutation frequencies noted with metabolic activation. Positive controls confirmed the sensitivity of the target cells. The repeat assay with S9 showed no significant mutation frequency.

5. Reviewer's discussion/ conclusions: As the author's states in "Interpretation of Results", (See Appended page 3) there was a dose-dependent increase in average mutation frequency for the mutation assay with S9, making the test substance a suspect mutagen. In the repeat assay, confirmatory evidence was not found. MCPB Acid was therefore not found to be mutagenic in the test system used. Toxicology Branch agrees with the authors' findings.

6. This assay was performed under GLPs with quality assurance statements.

7. CBI appendix not attached.

MCPB toxicology review

Page _____ is not included in this copy.

Pages 27 through 35 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

___ 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.
