US EPA ARCHIVE DOCUMENT

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

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FEB 10 1989

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: p-Dichlorobenzene (p-DCB) - Review of Toxicology Studies Submitted by the Registrant in Response to the October 21, 1987 Data Call-In Notice

TOX Chem No.: 632
Project No.: 8-0774
Record No.: 221172
MRID Nos.: 405210-01 to 405210-14

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Y.M. Ioannou 2-3-89

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THRU: Marion P. Copley, D.V.M., Section Head
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Marion Copley 2/3/89

and

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Registrant: Chlorobenzene Producers Association

Toxicology Branch I (TB-I) has reviewed a number of toxicity studies submitted by the registrant in response to the October 21, 1987 Data Call-In Notice on p-dichlorobenzene (p-DCB).

1 of 227

007021

The following mutagenicity studies have been evaluated and found to be acceptable or unacceptable as follows (see also attached DERs):

A. Gene Mutations

1. Salmonella/Mammalian Microsome Mutagenicity Assay (MRID No. 405210-04) - This study was found to be Unacceptable (due to the fact that the positive control was cytotoxic but not mutagenic). However, published data indicate that p-DCB is not mutagenic with or without metabolic activation in S. typhimurium strains.

2. Mutagenicity - Salmonella/Mammalian Microsome Assay (MRID No. 405680-02) - p-DCB was not mutagenic in S. typhimurium strains with or without metabolic activation at dose levels ranging from 1 to 100 ug/plate. The study published in "Environmental Mutagenesis Supplement 1, 1983" by S. Haworth et al. and titled: "Salmonella Mutagenicity Test Results for 250 Chemicals" appears to be Adequate and can be used as supportive data in evaluating p-DCB mutagenicity.

3. Mutagenicity - Salmonella/Mammalian Microsome Assay (MRID No. 405680-03) - p-DCB was not mutagenic in S. typhimurium strains in the presence or absence of metabolic activation at dose levels ranging from 51.2 to 13105.2 ug/plate. The study, published is "Mutation Research, 116 (1983)" by M. Shimiza et al., and titled: "Structural Specificity of Aromatic Compounds with Special Reference to Mutagenic Activity in Salmonella typhimurium - a Series of Chloro- or Fluoro-Nitrobenzene Derivatives," appears to be Adequate and can be used as supportive data in evaluating p-DCB mutagenicity.

4. Mutagenicity - Chinese Hamster Ovary (CHO) Cell/HGPRT Forward Mutation (MRID No. 405210-13) - p-DCB was found to be negative for forward gene mutations with or without metabolic activation at dose levels ranging from 50 to 400 ug/mL. The study was found to be Acceptable.

5. Mutagenicity - Forward Gene Mutation In Vitro (CHO/HGPRT) (MRID No. 405210-07) - p-DCB did not induce a significant increase in HGPRT mutants in CHO cells with or without activation at dose levels ranging from 25 to 250 ug/mL. The study was found to be Acceptable.

6. Mutagenicity- Gene Mutations in Insects (Drosophila/SLRL) (MRID No. 405210-07) - P-DCB was negative for the induction of sex-linked recessive lethals in Drosophila melanogaster adult males at concentrations ranging from 6,000 to 13,300 ppm/hour (inhalation route). The study was found to be Acceptable.

B. Structural Chromosome Aberrations

1. Mutagenicity - In Vivo Cytogenetic Study in Rats (MRID No. 405210-10) - p-DCB was not clastogenic in male or female rats at dose levels of 299 to 682 ppm (acute exposure) or 75 and 500 ppm (subacute and subchronic exposures). The study was found to be Unacceptable due mainly to the fact that no animal toxicity or cytotoxicity to the target cells was seen.

2. Mutagenicity - Dominant Lethal Assay in Mice (MRID No. 405210-11) - p-DCB was found to be negative for dominant lethal mutations in mice at exposure levels of 75 to 450 ppm (via the inhalation route). The study was found to be Unacceptable mainly due to the fact that no cytotoxicity was observed.

3. Mutagenicity - In Vivo Micronucleus Assay in Mice (MRID No. 405210-12) - p-DCB was not genotoxic in male and female mice at the dose level of 2500 mg/kg. The study was found to be Acceptable.

4. Mutagenicity - In Vitro Cytogenetics (CHO/SCE) (MRID No. 405210-07) - p-DCB was negative for induction of sister chromatid exchanges in cultured CHO cells in the presence or absence of metabolic activation at dose levels ranging from 20 to 120 ug/mL. The study was found to be Acceptable.

5. Mutagenicity - In Vitro Cytogenetics (CHO/CA) - (MRID No. 405210-07) - p-DCB was not clastogenic to CHO cells in vitro with or without metabolic activation at dose levels ranging from 20 to 300 ug/mL. The study was found to be Acceptable.

6. Mutagenicity- In Vivo Cytogenetics (Rat BM/CA) (MRID No. 405210-07) - p-DCB did not increase chromosomal aberrations in rat bone marrow at dose levels ranging from 200 to 800 mg/kg. The study was found to be Acceptable.

007021

C. Other Genotoxic Tests

1. Mutagenicity - In Vivo/In Vitro Unscheduled and Scheduled DNA Synthesis Assay in Mouse Hepatocytes (MRID No. 405210-08) - p-DCB was not genotoxic in male or female mice at dose levels between 300 and 1000 mg/kg. The study was found to be Acceptable.

2. Mutagenicity - In Vivo/In Vitro Unscheduled and Scheduled DNA Synthesis Assay in Rat Kidney Cells (MRID No. 405210-09) - p-DCB was not genotoxic to male or female rat kidney cells at dose levels of 300 to 1000 mg/kg. The study was found to be Acceptable in male but Unacceptable in female rats.

3. Mutagenicity - BALB/3T3 Cell Transformation Assay (MRID No. 405210-14) - p-DCB was negative for morphological transformations at dose levels ranging from 60 to 140 ug/mL. The study was found to be Unacceptable due to the high contamination observed in the solvent control flasks.

Based on the fact that the registrant submitted a battery of mutagenicity studies of acceptable quality for each of the three categories listed above, and the fact that none of the studies classified as unacceptable were positive for mutagenicity, the registrant is not required to submit to the Agency additional data on p-DCB mutagenicity.

The following studies were also reviewed by TB-I. Comments pertaining to each study are given below (see also DERs):

1. Teratogenicity Study in Rats (MRID No. 405210-02) - p-DCB does not appear to be teratogenic in specific pathogen-free rats when administered via the inhalation route on days 6 through 15 of gestation at dose levels of 75, 200 or 500 ppm. However, the study was classified as Core-Supplementary based on the fact that the HDT was not high enough to elicit maternal and/or developmental toxicity. Therefore, the study should be repeated.

2. Teratogenicity Study in Rabbits (MRID No. 405680-01) - p-DCB does not appear to be teratogenic in New Zealand White rabbits when administered via the inhalation route on days 6 through 18 of gestation at dose levels of 100, 300 or 800 ppm. The LEL for maternal toxicity was considered to be the HDT (800 ppm) based on depression of body weight gains. The NOEL was found to be the MDT (300 ppm). The LEL for developmental toxicity (retroesophageal right subclavian artery) was 800 ppm and the NOEL 300 ppm. The study was classified as Core-Minimum.

3. Teratogenic Evaluation in CD Rats (MRID No. 405210-03) - p-DCB was not teratogenic in CD rats when administered by oral gavage on days 6 through 15 of gestation at dose levels of 250, 500, 750 or 1000 mg/kg/day. Maternal toxicity (depression of body weight gains) was seen only during the exposure period (days 6 to 15 of gestation) at all p-DCB dose levels tested, reaching statistical significance at the dose of 500 mg/kg/day or higher. This lower body weight gain was associated with a decrease in food consumption during the same exposure period in all p-DCB treated groups. Developmental toxicity (depression of body weight) was observed only in the HDT. This study, published in "Bulletin of Environmental Contamination and Toxicology, 37, 1986" by E. Giavini, et al., does not appear to satisfy Guideline requirements due to the limited data provided by the authors. The study was classified as Core-Supplementary and therefore needs to be repeated.

4. Subchronic Toxicity Study - Para-Dichlorobenzene (C54955), Fischer 344 Rats (MRID No. 405680-07) - p-DCB was administered to Fischer 344 rats by gavage at dose levels of 300, 600, 900, 1200, and 1500 mg/kg/day for 90 days. The dose levels of 1200 and 1500 mg/kg/day were very toxic to male rats (high mortality) and the dose level of 1500 mg/kg/day was very toxic to female rats (high mortality). The LEL for systemic toxicity (body weight gain depression) was 600 mg/kg/day for male rats and 900 mg/kg/day for female rats. The NOEL for male rats was 300 mg/kg/day and for females was found to be 600 mg/kg/day. However, this study was classified as Core-Supplementary due to the following deficiencies:

   a. No statistical analysis was performed on any data reported in this study.

   b. No summary tables on the clinical signs of toxicity were reported.

   c. No summary tables of urinalysis measurements were reported.

   d. No individual animal body weights were reported.

   e. No individual animal organ weights were reported.

007021

f. Organ to body weight ratios (relative organ weights) and organ to brain weight ratios were not reported.

g. No individual animal data or summary tables for gross pathology were reported.

h. The individual animal histopathology data were presented in a handwritten (mostly nonlegible) form; no summary tables for the histopathological lesions were reported.

5. Subchronic Toxicity Study: Paradichlorobenzene (C54955) B6C3F1 Mice (Rerun) (MRID No. 405680-06) - p-DCB was administered (by gavage) to male and female B6C3F1 mice at dose levels of 0, 84.4, 168.8, 337.5, 675, and 900 mg/kg/day for 90 days. The available data were insufficient to allow full evaluation of this study. The study was classified as Core-Supplementary. Additional data are required as follows:

   a. Provide the Agency with hematology, clinical chemistry, and urinalysis data in summary tables as well as individual animal data.

   b. Provide the Agency with mean organ weights and individual animal organ weights for those organs required by EPA Guidelines. Calculate organ-to-body weight ratios.

   c. Provide individual animal histopathology data as well as summary tables for all tissues (for all treatment groups) in male and female mice.

   d. Carry out statistical analyses on all data.

6. Paradichlorobenzene: Long-Term Inhalation Study in the Rat (MRID No. 405680-08) - This study has investigated the chronic toxicity and oncogenicity of p-DCB to male and female Wistar rats via the inhalation route at dose levels of 0, 75, and 500 ppm. Data presented by the authors were insufficient for a complete evaluation of the study. The study was classified as Core-Supplementary. Additional data are required as follows:

   a. Report the range of temperature and relative humidity in the exposure chamber.

b. Report all clinical symptoms of toxicity for all animals throughout the study.

c. Report all gross pathology findings in summary tables and as individual animal data.

d. Report individual animal data concerning:

1) Body weight;

2) Food consumption;

3) Clinical chemistry values;

4) Hematology values;

5) Urinalysis values;

6) Organ weights (absolute and relative); and

7) Histopathology.

e. For histopathology summary tables, specify the total number of tissues examined for each observation.

f. Evaluate additional clinical chemistry, hematology, and urinalysis parameters as specified in the EPA Guidelines.

g. Supply Table 22 (mean blood glucose values in females).

h. Clarify Tables 33 and 34 (no values are given).

i. Perform statistical analyses for all urinalysis data.

j. Report the standard deviation (± SD) for all mean values.

7. Toxicology and Carcinogenesis Studies of p-DCB in F344/N Rats (MRID No. 405210-05) - Study conducted by the National Toxicology Program (NTP) - p-DCB, dissolved in corn oil, was administered by gavage to male F344/N rats at dose levels of 0, 150 or 300 mg/kg/day and to female F344/N rats at dose levels of 0, 300 or 600 mg/kg/day (5 days/week) for 2 years (50 animals/group). Male rats dosed with 300 mg/kg of p-DCB had a significantly lower survival rate than controls (after week 97) and body weights were 5 to 8 percent lower than controls (after week 38). Body weights in female rats dosed with 600

mg/kg were 5 to 7 percent lower than controls (after week 55). Administration of p-DCB to male rats increased the average severity of nephropathy and caused epithelial hyperplasia of the renal pelvis (1/50; 30/50; 31/50), mineralization of the collecting tubules in the renal medulla (4/50; 46/50; 47/50), and focal hyperplasia of renal tubular epithelium (0/50; 1/50; 9/50). There were increased incidences of nephropathy in both low and high dose female rats compared with vehicle controls (21/49; 32/50; 41/49). p-DCB produced a dose-related increase in the incidence of tubular cell adenocarcinomas of the kidney in male rats (1/50; 3/50; 7/50); one tubular cell adenoma was observed in a high dose male rat. These malignant tumors are uncommon in male F344/N rats. They have been diagnosed in only 4/1098 (0.4%) corn oil gavage controls in previous NTP studies. There were no tubular cell tumors in dosed or vehicle control female rats. There was a marginal increase in the incidence of mononuclear cell leukemia in dosed male rats compared with that in vehicle controls (5/50; 7/50; 11/50). The LEL for systemic toxicity (depression of body weight) was 300 mg/kg/day in male rats and 600 mg/kg/day in female rats. The NOEL was 150 mg/kg/day in male rats and 300 mg/kg/day in female rats. p-DCB was found to be oncogenic in male rats inducing tubular cell adenocarcinomas of the kidney. The study was given a Core-Reserved classification pending full evaluation prior to peer review.

8. Toxicology and Carcinogenesis Studies of p-DCB in B6C3F1 Mice (MRID No. 405210-05) - This study was conducted by the NTP - p-DCB dissolved in corn oil was administered by gavage to male and female B6C3F1 mice at dose levels of 300 and 600 mg/kg/day (5 days a week) for 2 years. Survival of male and female mice and mean body weights were comparable between the p-DCB treated animals and the controls throughout the study. p-DCB increased the incidences of nonneoplastic liver lesions in male and female mice, including alteration in cell size (cytomegaly and karyomegaly), hepatocellular degeneration, and individual cell necrosis. p-DCB also increased the incidences of nephropathy in male mice and renal tubular regeneration in female mice. p-DCB increased the incidences of hepatocellular carcinomas in high dose male (14/50; 11/49; 32/50) and female (5/50; 5/48; 19/50) mice and hepatocellular adenomas in dosed male (5/50; 13/49; 16/50) and high dose female (10/50; 6/48; 21/50) mice. Hepatoblastomas were observed in four high dose male mice but not in vehicle controls. This rare tumor has not occurred in 1091 male vehicle control mice in NTP studies. An increase in thyroid gland follicular cell hyperplasia was observed in dosed male mice (1/47; 4/48; 10/47), and there was a

marginal positive trend in the incidence of follicular cell adenomas of the thyroid gland in female mice (0/48; 0/45; 3/46). Pheochromocytomas (benign or malignant, combined) of the adrenal gland occurred with a positive trend in dosed male mice, and the incidence in the high dose group was significantly greater than in the vehicle controls (0/47; 2/48; 4/49). The incidence of adrenal gland medullary hyperplasia in male mice was 2/47, 4/48, and 4/49. Focal hyperplasia of the adrenal gland capsule was also observed in dosed male mice (11/47; 21/48; 28/49). The results indicate that there was clear evidence of carcinogenicity of p-DCB in male and female B6C3F1 mice mainly by inducing the formation of hepatocellular carcinomas and adenomas. The LEL for systemic toxicity in male and female mice was not established in this study. Systemic toxicity was observed in both sexes receiving either the low or the high dose and consisted of nephropathy, hepatocellular degeneration with cell necrosis and liver cell size alteration (cytomegaly and karyomegaly). The selection of the dose levels tested in this study was based on a 90-day study which established that dose levels of 675 mg/kg/day or higher resulted in systemic toxicity while the low dose of 375 mg/kg/day was a NOEL. The study was given a Core-Reserved classification pending full evaluation prior to peer review.

The following acute toxicity studies were also evaluated.

1. Acute Oral LD_{50} in Rats (MRID No. 405210-01) - The $AOLD_{50}$ of p-DCB in Sherman rats was found to be 3863 mg/kg (with a 95% CL of 3561 to 4153 mg/kg) for males and 3790 mg/kg (with a 95% CL of 3425 to 4277 mg/kg) for females. Toxicity Category III. The study is considered of Core-Minimum classification.

2. Acute Oral LD_{50} in Rats (MRID No. 405680-04) - The $AOLD_{50}$ of p-DCB is male and female rats (Sprague-Dawley strain) was reported to be 1860 mg/kg (95% CL = 1690 to 2050 mg/kg). The study is considered of Core-Supplementary classification (only 2 to 3 rats were used/dose/sex; the purity of p-DCB used was not specified). Toxicity Category III.

3. Acute Dermal LD_{50} in Rats (MRID No. 405210-01) - The $ADLD_{50}$ of p-DCB in Sherman rats was found to be greater than 6000 mg/kg in both sexes. Toxicity Category III. The study is considered to be of Core-Minimum classification.

4. Acute Dermal LD_{50} in Rabbits (MRID No. 405680-04) - The $ADLD_{50}$ of p-DCB in male and female New Zealand albino rabbits was found to be greater than 5010 mg/kg. The study was classified as Core-Supplementary

because only one rabbit was used/dose/sex. The purity of p-DCB used was not specified. Toxicity Category III.

5. Acute Eye Irritation in New Zealand Albino Rabbits (MRID No. 405680-04) - The primary irritation score (24 to 72 hours) was 13.7. Light irritation on day 3 (72 hours); all irritation disappeared by day 7. Toxicity Category III. The study is classified as Core-Supplementary due to the fact that: the p-DCB purity was not specified; the animal's sex was not specified and the study was presented in summary form.

6. Acute Skin Irritation in New Zealand Albino Rabbits (MRID No. 405680-04) - The primary irritation score was 1.0. No irritation was reported after 24 hours. Toxicity Category IV. The study is classified as Core-Supplementary due to the fact that the p-DCB purity was not specified; the animal's sex was not specified; and only summary of results was presented.

Attachments

53041:I:Ioannou:C.Disk:KENCO:12/28/88:DD:VO:CT
R:53042:Ioannou:C.Disk:KENCO:1/9/89:rw:VO:AS

007021

TRADE SECRET OR CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12356)

EPA: 68D80056
DYNAMAC No. 121-A
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--Salmonella typhimurium Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: 12-21-88

007021

EPA: 68D80056
DYNAMAC No. 121-A
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--Salmonella typhimurium Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

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D.A.B.T.
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Date: 1/30/89

13

007021

DATA EVALUATION RECORD

CHEMICAL: PDCB (para-Dichlorobenzene).

STUDY TYPE: Salmonella/mammalian microsome mutagenicity assay.

MRID: 405210-04.

SYNONYMS/CAS no.: Santachlor; benzene, p-dichloro.

SPONSOR: Monsanto Company, St. Louis, MO.

TESTING FACILITY: Monsanto Company Environmental Health Laboratory, St. Louis, MO.

TITLE OF REPORT: para-Dichlorobenzene--Final Report on Salmonella Mutagenicity Assay of dichlorobenzene (Technical).

AUTHOR(S): Flowers, L. J., and Kier, L. D.

STUDY NO.: LF-78-145.

REPORT ISSUED: July 7, 1978.

007021

CONCLUSIONS - Executive Summary: para-Dichlorobenzene, over a concentration range of 0.6 to 600 μg/plate either with or without S9 activation, was not mutagenic in S. typhimurium TA98, TA100, or TA1537. The highest assayed dose was cytotoxic in all strains except for TA1537. Due to extremely low solvent-control mutant frequencies for strain TA1535, ≥ 2-fold increases in revertant colonies were seen at all noncytotoxic doses of the test material. These increases, however, did not exceed the generally accepted spontaneous revertant counts for the strain.[1] This portion of the assay should have been repeated to resolve the questionable increases in TA1535 and also because the S9-activated positive control for TA1535, tris-(2,3-dibromopropyl) phosphate [Tris-(DBP)], was cytotoxic but not mutagenic. However, the results of Haworth et al.[2] from well-controlled S. typhimurium mammalian preincubation assays indicated that five concentrations of para-dichlorobenzene (1.0 to 100 μg/plate) were not mutagenic; the high dose, both with and without S9 activation, was cytotoxic in S. typhimurium TA100.

Classification:

The study is technically unacceptable; however, published results from well-controlled studies[3] indicate that the test material is not mutagenic in S. typhimurium. We, therefore, do not recommend that the assay be repeated.

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[1]de Serres, F. J. and Shelby, M. D. Recommendations on data production and analysis using the Salmonella microsomal mutagenicity assay. Mutat. Res. (1979)64: 159-165.

[2]Haworth, S., Lawlar, T., Mortelmans, K., Speck, W., and Zeiger, E. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagenesis (1983) 5, (Suppl. 1):3-142.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

00702

A. MATERIALS:

1. Test Material: Name: para-Dichlorobenzene
   Description: White crystalline solid
   Lot No.: KK04-7059; NB1066146
   Purity: >99.5%
   Contaminants: [redacted]
   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: The test material at 100 mg/mL formed an orange solution in DMSO.

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: 100 µL/plate
   Positive:

   Nonactivation:

Sodium nitrate	100	µg/plate, TA1535
4-Nitroquinoline-N-oxide (4-NQO)	0.1	µg/plate, TA98
	0.5	µg/plate, TA100
9-Aminoacridine (9-AA)	30	µg/plate, TA1537

   Activation:

Tris-(DBP)	30	µg/plate, TA1535
2-Acetamidofluorene(2-AAF)	30	µg/plate, TA98
Benzo(a)pyrene (BaP)	2	µg/plate, TA100
2-Aminoanthracene (2-AA)	30	µg/plate, TA1537

3. Activation: S9 derived from:

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

   If other, describe below. Describe S9 composition (if purchased, give details): The S9 from lot No. IRL-48 was purchased from Litton Bionetics, Inc., Kensington, MD.

4. Test Organism Used: S. typhimurium strains

   ___ TA97 __x__ TA98 __x__ TA100 ____ TA102 ____ TA104
   __x__ TA1535 __x__ TA1537 ___ TA1538; list any others: None.
   Test organisms were properly maintained: Yes.
   Check for appropriate genetic markers (rfa mutation, R factor): Yes.

5. Test Compound Concentrations Used:

   a. Cytotoxicity assay: 0.01, 0.04, 0.2, 1, 3, and 10 mg/plate +/- rat S9.
   b. Spot test: 10 mg/plate +/-S9 rat and mouse S9 (mouse S9 was not described).
   c. Plate incorporation assay: 0.6, 2.4, 12, 60, 180, and 600 µg/plate +/- rat S9.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
   - x Standard plate test
   - ____ Pre-incubation (_) minutes
   - ____ "Prival" modification
   - x Spot test
   - ____ Other (describe)

2. Preliminary Cytotoxicity Assay: (include the concentration range, strain(s) used, reported results): The report stated that six nonactivated and six S9-activated doses (0.1 to 10 mg/plate) were assayed using S. typhimurium TA100; results were only presented for 0.4, 0.2, 1, and 3 mg/plate. Based on the absence of or a marked reduction in the background lawn of growth, 0.2 mg/plate/-S9 was marginally cytotoxic; above this concentration, marked cytotoxicity was induced. In the presence of S9 activation, doses $\geq$ 1 mg/plate were cytotoxic; no cytotoxicity occurred at 0.2 mg/plate.

3. Spot Test: Ten milligrams/plate of the test material either with or without S9 microsomes derived from rat and mouse livers were neither cytotoxic nor mutagenic in the four tester strains.

4. Plate Incorporation Assay: Based on the findings from the preliminary cytotoxicity assay, 0.6, 2.4, 12, 60, 180, and 600 µg/plate were selected for the non-activated and S9-activated mutation assays. As shown in Table 1, the highest dose with and without S9 activation was cytotoxic to strains TA98 and TA100. At the remaining noncytotoxic levels, no increases in reversion to histidine prototrophy of these two strains were seen.

   It was noted that the nonactivated solvent-control mutant colony counts for strain TA98 were low; however, the strain responded in an acceptable manner to the nonactivated positive control, 0.1 µg/plate of 4-NQO. Nonactivated assay data presented for strains TA1535 and TA1537 were difficult to follow. It appeared that mutant colony counts in the raw data were misaligned with the substance column (see CBI p. 12). Based on the findings from the preliminary test and the mutation assay results for strains TA98 and TA100, we can only assume that the term "toxic," which corresponds to the solvent control results in the report, refers to the 600-µg/plate dose level of the test material. This, however, does not explain why there are 10 sets of raw data values and nine categories under the substance column. Nevertheless, the results for strains TA1535

TABLE 1. Representative Results of the Salmonella typhimurium Mutagenicity Assay with Para-dichlorobenzene

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl-sulfoxide	-	--	3.7	4.0	13.7	122.0
	+	--	6.7	16.0	47.7	122.3
Water	-	--	--	--	16.3	135.0
	+	--	--	--	50.7	139.7
Positive Controls						
Sodium nitrate	-	100.0	>2000	--	--	--
4-Nitroquinoline-N-oxide	-	0.1	--	--	221	--
	-	0.5	--	--	--	1030
9-Aminoacridine	-	30.0	--	41	--	--
Tris-(2,3-dibromo-propyl) phosphate	+	30.0	T[c]	--	--	--
2-Acetamidofluorene	+	30.0	--	--	982	
Benzo(a)pyrene	+	2.0	--	--	--	573
2-Aminoanthracene	+	30.0	--	311	--	--
Test Material						
Para-dichlorobenzene	-	180[b]	3.7	3.7	13.3	93.7
	-	600	T	4.3	T	T
	+	180[b]	17.3	9.3	52.7	102.7
	+	600	T	11.0	T	T

[a]Average of three plates; calculated by our reviewers.

[b]Results for lower doses (60, 12, 2.4, and 0.6 µg/plate) with strains TA98, TA100, and TA1537 either with or without S9 activation and TA1535 without S9 activation were comparable to the corresponding solvent control values.

[c]T = Toxic as reported by the study authors.

00721

and TA1537 with the nonactivated test material and the findings for TA1537 with the S9-activated test material did not indicate a mutagenic response.

It was further noted that background mutant counts (with or without S9 activation) for TA1535 were low. The lower than expected revertant frequency did not affect the outcome in the nonactivated assay. By contrast, the S9-activated counts for all "non-cytotoxic" dose levels of the test material showed ≥ 2-fold increases in mutant colonies of TA1535 compared to the solvent control (Table 2). Although none of the average counts for test material doses exceeded the acceptable spontaneous revertant range for TA1535 (5 to 50, according to deSerres and Shelby[4]; 20, according to Ames et al.[5]), this portion of the assay should have been repeated to resolve the questionable increases and also because the S9-activated positive control for TA1535, tris-(DBP) was cytotoxic but not mutagenic. Hence, the ability of this organism to detect a positive mutagenic response in this assay is in doubt.

The study authors concluded that para-dichlorobenzene (technical) did not induce a significant mutagenic effect in the Salmonella plate incorporation assay.

4. Reviewers' Discussion/Conclusions: We assess that the assay with para-dichlorobenzene is technically unacceptable for the reasons discussed under the reported results. However, there are published results[6] from well-controlled studies that indicate that para-dichlorobenzene over a comparable range of nonactivated and S9-activated doses is not mutagenic in S. typhimurium TA1535, TA1537, TA98, or TA100. Therefore, we assess that the merits of repeating this study are questionable.

5. There was no indication that the test was performed under good laboratory practices; a quality assurance statement was not present.

6. CBI Appendix: Appendix A, Materials and Methods (Protocol), CBI pp. 14-18.

[4]deSerres and Shelby, pp. 159-165.

[5]Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. (1975) 31:347-364.

[6]Haworth et al., pp. 3-142.

TABLE 2. Fold Increase in Revertant Colony Counts of S. typhimurium TA1535 Following Exposure to S9-Activated para-Dichlorobenzene

Substance	Dose (μg/Plate)	Average Revertants[a]	Fold Increase[b]
Solvent Control			
Dimethyl-sulfoxide	--	6.7	--
Positive Control			
Tris-(2,3-dibromo-propyl) phosphate	30.0	Toxic	--
Test Material			
para-Dichloro-benzene	0.6	14.0	2.1
	2.4	14.3	2.1
	12.0	14.0	2.1
	60.0	16.7	2.5
	180.0	17.3	2.6
	600.0	Toxic	--

[a]Average of three plates; calculated by our reviewers.

[b]Fold Increase = $\frac{\text{Average Count for Treatment Group}}{\text{Average Count for Solvent Control}}$; calculated by our reviewers.

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APPENDIX A

MATERIALS AND METHODS (Protocol)

Paradichlorobenzene toxicology review

Page _____ is not included in this copy.

Pages 22 through 26 are not included in this copy.

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___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

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___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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EPA: 68D80056
DYNAMAC No. 121-B
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--in vivo/in vitro
Unscheduled and Scheduled DNA Synthesis Assay in
Mouse Hepatocytes

STUDY IDENTIFICATION: Steinmetz, K. L., and Spanggord, R. J. para-Dichlorobenzene-Examination of the potential of p-dichlorobenzene to induce unscheduled DNA synthesis or DNA replication in the in vivo - in vitro mouse hepatocyte DNA repair assay. (Unpublished study No. LSC-2603-1 prepared by SRI International, Menlo Park, CA, for Chemical Manufacturers Association, Washington, DC; dated April 1987.) MRID No. 405210-08.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 12-21-88

1. CHEMICAL: PDCB; para-dichlorobenzene.

2. TEST MATERIAL: para-Dichlorobenzene was described as a white crystalline material with a purity of 99.5%.

3. STUDY/ACTION TYPE: Mutagenicity--in vivo/in vitro Unscheduled and scheduled DNA synthesis assay in mouse hepatocytes.

4. STUDY IDENTIFICATION: Steinmetz, K. L., and Spanggord, R. J. para-Dichlorobenzene-Examination of the potential of p-dichlorobenzene to induce unscheduled DNA synthesis or DNA replication in the in vivo - in vitro mouse hepatocyte DNA repair assay. (Unpublished study No. LSC-2603-1 prepared by SRI International, Menlo Park, CA, for Chemical Manufacturers Association, Washington, DC; dated April 1987.) MRID No. 405210-08.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-21-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
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Marion Copley, D.V.M., D.A.B.T.
EPA Acting Section Head,
Section II,
Toxicology Branch I (TS-769C)

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Date: 1/30/89

7. CONCLUSIONS:

A. Three doses (300, 600, and 1000 mg/kg) of para-dichlorobenzene were administered once by gavage to male and female mice. Hepatocytes harvested 16 hours posttreatment were analyzed for unscheduled DNA synthesis (UDS); hepatocytes recovered from animals sacrificed 48 hours postexposure were evaluated for DNA replication, scheduled DNA synthesis, (SDS). Results indicate that 1000 mg/kg paradichlorobenzene induced marked increases in SDS in both sexes, suggesting that the test material is hepatotoxic. However, no increase in UDS was observed in males and females of the three dosed groups.

B. The study is acceptable; para-dichlorobenzene is hepatotoxic but not genotoxic in this test system.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: para-Dichlorobenzene was described as a white crystalline material with a reported purity of 99.5%. Analytical data furnished by the sponsor concluded that the purity of the test material exceeded 97%. The test material was stored at room temperature and was suspended in corn oil. Samples of the dosing solutions were assayed by analytical procedures for test material concentrations.

2. Test Animals: Forty-five male and 46 female B6C3F1 mice weighing between 18 and 20 g were obtained from Simonsen Laboratories Inc., Gilroy, CA.

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[1]Only items appropriate to this DER have been included.

3. Animal Maintenance: Animals were quarantined for 1 week and group housed (no more than five/cage) in an environment controlled for temperature (74 to 78°F), relative humidity (32 to 58%), and light (12 hours). Mice were fed Purina Certified Rodent Chow; water was available ad libitum.

4. Assignment to Groups: Thirty-two males and 34 females were randomized, identified by ear punch, and assigned to the treatment groups as outlined below:

Substance	Dose (mg/kg)	Number of Mice/Sacrifice Interval			
		Male		Female	
		16 hours	48 hours	16 hours	48 hours
Vehicle Control					
corn oil	-	3	3	3	3
Positive Control					
Dimethylnitrosamine (UDS assay)	10	3	-	3	-
Carbon tetrachloride (SDS assay)	100	0	3	0	3
Test Materials					
Low dose	300	3	3	3	3
Mid dose	600	3	3	3	5
High dose	1000	4	4	3	5

Note: The disposition of the excess animals obtained for this study was not reported.

5. Compound Administration: The vehicle control, positive controls, and three doses of the test material were administered by a single oral gavage to the appropriate groups of animals. The three test doses or positive control, carbon tetrachloride, (CCl_4) were suspended in corn oil; dimethylnitrosamine (DMN) was prepared in water.

6. UDS/SDS Assay

   a. Perfusion Techniques/Hepatocyte Harvest: The livers of animals in the appropriate sacrifice groups were perfused with an undefined collagenase solution for an unspecified time. The method used to anesthetize the mice was not reported. Hepatocytes were released with a metal comb and inoculated into Linbro six-well culture dishes. Each well contained a coverslip and Williams'

Medium E (WME), supplemented with L-glutamine, fetal bovine serum, and antibiotics. Cultures were placed in a humidified incubator at 37°C and 5% CO_2 for a 1.5- to 2-hour attachment period. Unattached cells were removed; viable cells were refed serum-free WME containing 10 µCi/mL [^{3}H]-thymidine for 4 hours, washed, and reincubated for 13 to 18 hours in serum-free WME containing 0.25 mM unlabeled thymidine.

b. Slide Preparation: Hepatocytes attached to coverslips were washed, swollen with 1% sodium citrate, fixed in glacial acetic acid: ethanol (1:3), washed, and mounted.

c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB-2 emulsion, stored for 1 week at -20°C, stained with 1% methyl-green, and coded.

d. Grain Counting:

1. UDS Determination: Hepatocytes harvested from animals that were sacrificed at 16 hours were used to determine UDS. The nuclear grains of 30 morphologically normal cells/slide/animal/dose group were counted with an automatic colony counter. Net nuclear grain counts were determined by subtracting the nuclear grain counts of each cell from the highest cytoplasmic grain count of two nuclear-sized areas adjacent to each nucleus. Mean net nuclear grain counts, standard error of the mean, and the percentage of cells in repair were calculated.

2. SDS Determination: Hepatocytes harvested from animals sacrificed at 48 hours were used to determine SDS. One thousand cells/slide/animal/dose group were scored manually for replicating (S-phase) and nonreplicating cells; the percentage of cells in S-phase was calculated.

e. Statistical Methods: Data for the UDS evaluation were not analyzed statistically. The Student's t-test modified for unpaired observations was used to analyze the SDS data at p values of 0.05 and 0.02.

7. Evaluation Criteria

a. Assay Validity: The assay was considered acceptable if 1) the vehicle control data were within historical ranges and 2) if the positive control(s) showed an increase in net nuclear grain counts, the percentage of cells undergoing repair, and a significant increase in the number of cells undergoing DNA replication.

b. Positive Response: For the UDS assay, the test material was considered positive if the mean net nuclear grain count for any dose was >5. If mean net nuclear grain counts fell between 0 and 5, the data were assessed relative to dose response, increases in percent cells in repair, reproducibility of the results among animals, and the frequency of the cellular responses. For the SDS assay, the test material was considered positive if the percent cells in S-phase at any dose was significantly higher than the vehicle control value.

B. Protocol: A protocol was not provided; however, historical data on S-phase levels in male and female B6C3F1 mice treated with corn oil was presented in the appendix to the final report (see CBI pp. A2-A3).

12. REPORTED RESULTS:

A. Clinical Observations: The report stated that no signs or evidence of significant clinical disease were noted at any time during the course of the study.

B. UDS/SDS Assay: Hepatocytes recovered from a minimum of three male and three female mice per group were scored for UDS and SDS activities; however, due to a technical error, only hepatocytes from two vehicle control females (SDS assay) were scored. As shown in Table 1, the single gavage administrations of 300, 600, and 1000 mg/kg of the test material did not induce UDS in male and female mouse hepatocytes harvested 16 hours after treatment. By contrast, 6.3- and 15.3-fold increases in the percent cells in S-phase were seen in the males and females, respectively, of the high-dose group; the increase in males was significant ($p < 0.01$). The authors attributed the lack of significant SDS activity in high-dose females to the small number of control animals and to animal-to-animal variation in the DNA replication response for both the

TABLE 1. Results of the *in vivo*/*in vitro* Unscheduled DNA Synthesis (UDS) and Unscheduled DNA Synthesis (SDS) Assay in Mice Dosed with para-Dichlorobenzene

Substance	Dose (mg/kg)	UDS Activity[a] No. of Animals Per Group	UDS Activity[a] No. of Hepatocytes Scored	UDS Activity[a] Mean Net Nuclear Grain Count ± S.E.[c]	UDS Activity[a] % Cells in Repair ± S.E.[c]	SDS Activity[b] No. of Animals Per Group	SDS Activity[b] No. of Hepatocytes Scored	SDS Activity[b] % Cells in S-phase ± S.E.[c]
Vehicle Control								
Corn oil	0	3M	270	-3.4±0.2	2.0±1.0	3M	9000	0.24±0.09
		3F	270	-7.1±2.2	3.0±1.0	2F[d]	6000	0.29±0.22
Positive Controls								
Dimethylnitrosamine	10.0	3M	270	16.9±4.0	59.0±2.0	--	--	--
		3F	270	15.8±2.8	67.0±2.0	--	--	--
Carbon tetrachloride	100.0	3M	--	--	--	3M	9000	10.16±1.28*
		3F	--	--	--	3F	9000	8.10±2.24
Test Material								
para-Dichlorobenzene	1000.0[e]	4M	360	-5.8±0.8	3.0±2.0	3M[d]	9000	1.52±0.07**
		3F	270	-5.3±2.5	2.0±1.0	5F	15,000	4.45±2.78

[a]Animals sacrificed 16 hours posttreatment.

[b]Animals sacrificed 48 hours posttreatment.

[c]SE = Standard error.

[d]One animal lost due to technical error.

[e]UDS assay results for lower dose groups (300 and 600 mg/kg) were comparable to the corresponding control group. SDS assay results for these groups showed nondose-related increases in the % cells in S-phase compared to the corresponding control value.

*Significantly higher than the vehicle control group ($p<0.02$) by Student's t-test.

**Significantly higher than the vehicle control group ($p<0.01$) by Student's t-test.

007021

control and dosed groups. It was noted that the 27.9-fold increase in percent S-phase cells for females administered the positive control (100 mg/kg CCL_4) was also not significant. For the remaining test groups, nondose-related increases in DNA replication were seen for both males and females.

C. Chemical Analysis: Chemical analysis of dosing solutions prepared in corn oil indicated that the concentrations of the test material were 10 to 16% higher than expected. The authors attributed the increases over the target concentrations to changes in corn oil density as the concentration of the test material increased.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that para-dichlorobenzene did not induce UDS in hepatocytes following a single administration by oral gavage to male or female B6C3F1 mice, but did induce an elevated DNA replication response in male and female mouse livers.

B. A quality assurance statement was signed and dated May 6, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We assess that the study was well conducted and the study authors interpreted the data correctly. The test material was assayed to levels that induced hepatotoxicity, but not genotoxicity. The biological relevance of the 15.3-fold increase in SDS for females exposed to the high dose (1000 mg/kg) was not diminished by the lack of statistical significance. Compared to the reporting laboratory's historical background SDS frequency for females (0.31), the increase in SDS for the high-dose group (14.4-fold) also indicated a strong hepatotoxic response.

The ability of the test system to detect both UDS and SDS activities was clearly demonstrated by the response of male and female mice to the positive controls (10 mg/kg DMN for the UDS assay and 100 mg/kg CCl_4 for the SDS assay.)

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3-5.

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APPENDIX A

Materials and Methods

Paradichlorobenzene toxicology review

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___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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007021

EPA: 68D80056
DYNAMAC No. 121-C
December 21, 1988

ITIAL BUSINESS INFORMATION
DOES NOT CONTAIN
SECURITY INFORMATION (EO 12065)

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--in vivo/in vitro Unscheduled and Scheduled DNA Synthesis Assay in Rat Kidney Cells

STUDY IDENTIFICATION: Steinmetz, K. L., and Spanggord, R. J. para-Dichlorobenzene--Evaluation of the potential of p-dichlorobenzene to induce unscheduled DNA synthesis or DNA replication in the in vivo - in vitro rat kidney DNA repair assays. (Unpublished study No. LSC-2603 prepared by SRI International, Menlo Park, CA, for Chemical Manufacturers Association, Washington, DC; dated May 1987.) MRID No. 405210-09.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 12-21-88

1. CHEMICAL: PDCB; para-dichlorobenzene.

2. TEST MATERIAL: para-Dichlorobenzene from lot No. 09086-PA was described as a white crystalline material with a purity of >97%.

3. STUDY/ACTION TYPE: Mutagenicity--in vivo/in vitro Unscheduled and scheduled DNA synthesis assay in rat kidney cells.

4. STUDY IDENTIFICATION: Steinmetz, K. L., and Spanggord, R. J. para-Dichlorobenzene--Evaluation of the potential of p-dichlorobenzene to induce unscheduled DNA synthesis or DNA replication in the in vivo - in vitro rat kidney DNA repair assays. (Unpublished study No. LSC-2603 prepared by SRI International, Menlo Park, CA, for Chemical Manufacturers Association, Washington, DC; dated May 1987.) MRID No. 405210-09.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E McCarroll
Date: 12-21-88

I. Cecil Felkner, Ph.D.
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Genetic Toxicology Studies
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Marion Copley, D.V.M., D.A.B.T.
EPA Acting Section Head,
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Branch I (TS-769C)

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Date: 1/30/89

7. CONCLUSIONS:

A. Three doses (300, 600, or 1000 mg/kg) of para-dichlorobenzene (PDCB) were administered once by gavage to male and female rats. Kidney cells harvested 16 hours posttreatment were analyzed for the induction of unscheduled DNA synthesis (UDS); kidney cells recovered from animals sacrificed 96 hours postexposure were evaluated for scheduled DNA synthesis (SDS). Results for males indicate that 1000 mg/kg PDCB induced a significant increase in SDS; no increase in UDS was observed. In females, the test material was neither cytotoxic to the target organ as indicated by the lack of an effect on SDS nor increased the frequency of UDS. The inability to demonstrate test material interaction with the target organ precludes acceptance of the findings with females as valid evidence that PDCB is not genotoxic in female rat kidney cells.

B. Although the study is acceptable for male rats, as a whole the study is unacceptable.

8. RECOMMENDATIONS: It is recommended that the assay be repeated in females with either the maximum tolerated dose or a level that clearly shows signs of renal toxicity in these animals (if necessary, repeated exposures, 3-5 days, should be employed).

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: para-Dichlorobenzene from lot No. 09086-PA was described as a white crystalline material with a purity of >97%. The test material was stored at room temperature and was suspended in corn oil. Samples of the dosing solutions were analyzed for test material concentration.

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[1]Only items appropriate to this DER have been included.

2. Test Animals: A total of 113 male and 85 female Fisher -344 rats were received in two shipments from Harlan Sprague-Dawley, Inc., Indianapolis, IN. Upon receipt animals weighed between 140 and 200 g.

3. Animal Maintenance: Animals were quarantined for 1 week and group housed (no more than three/cage) in an environment controlled for temperature (64 to 82°F), relative humidity (29 to 77%), and light (12 hours). Rats were fed Purina Certified Rodent Chow; water was available ad libitum.

4. Assignment to Groups: Thirty-two males and 32 females were randomized, identified by ear punch, and assigned to the treatment groups as outlined below:

Substance	Dose (mg/kg)	Number of Rats/Sacrifice Interval			
		Male		Female	
		16 hours	96 hours	16 hours	96hours
Vehicle Control					
Corn oil	--	3	3	3	3
Positive Control					
Streptozocin	250	3	--	3	--
Mercuric chloride	10	0	3	0	0
	1.5	0	0	0	4
Test Material					
Low dose	300	3	3	3	3
Mid dose	600	3	3	3	3
High dose	1000	4	4	4	4

Note: The disposition of the excess animals obtained for this study was not reported.

5. Compound Administration: The vehicle, mercuric chloride ($HgCl_2$) and the three doses of the test material were administered once by oral gavage to the appropriate groups of test animals; the other positive control, streptozocin (STZ), was administered by i.p. injection. The test material was prepared in corn oil, STZ was dissolved in saline, and $HgCl_2$ was prepared in water.

6. UDS/SDS Assay

a. Kidney Cell Harvest: The report did not include the methods used to sacrifice the animal or a description of the kidney perfusion technique. Kidneys were removed, trimmed of extraneous tissue, minced in a collagenase-trypsin solution, and stirred for 45 to 60 minutes to release the cells. Cells were harvested, centrifuged, and resuspended in Williams' Medium E (WME) supplemented with L-glutamine, fetal calf serum, and antibiotics. Cell viability was determined by trypan blue exclusion. Viable cells, at a density of ≈3 x 10^6, were inoculated into half of the wells of six-well culture dishes containing coverslips and WME plus 10 µCi/mL [^{3}H]thymidine. The remaining three wells/dish received 6 x 10^6 cells. Cultures were placed in a humidified, 37°C, 5% CO_2 incubator for 20-22 hours.

b. Slide Preparation: Cells attached to coverslips were washed, swollen with 1% sodium citrate, fixed in glacial acetic acid:ethanol (1:3), washed, and mounted.

c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB-2 emulsion and stored for 1 week at -20°C (SDS slides) or 14 days (UDS slides), stained with 1% methyl-green Pyronin Y, and coded.

d. Grain Counting:

1. UDS Determination: Kidney cells harvested from animals sacrificed at 16 hours were used to determine UDS. The nuclear grains of 30 morphologically normal cells/slide/animal/dose group were counted with an automatic colony counter. Net nuclear grain counts were determined by subtracting the nuclear grain counts of each cell from the highest cytoplasmic grain count of two nuclear-sized areas adjacent to each nucleus. Mean net nuclear grain counts, standard error of the means, and the percentage of cells in repair were calculated.

2. SDS Determination: Kidney cells harvested from animals sacrificed at 96 hours were used to determine SDS. One thousand cells/slide/animal/dose group were scored manually for replicating (S-phase) and nonreplicating cells; the percentage of cells in S-phase was calculated.

   Statistical Methods: Data from the UDS evaluation were not analyzed statistically. The Student's t-test modified for unpaired observations was used to analyze the SDS data at p values of 0.05 and 0.02.

7. Evaluation Criteria

   a. Assay Validity: The assay was considered acceptable if 1) the vehicle control data were within historical ranges and 2) if the positive control(s) showed an increase in net nuclear grain counts, in percentage of cells undergoing repair, and a significant increase in the number of cells undergoing DNA replication.

   b. Positive Response: For the UDS assay, the test material was considered positive if the mean net nuclear grain count for any dose was >3. If mean net nuclear grain counts fell within 0 to 3, the data were assessed relative to dose response, increases in percent cells in repair, reproducibility of the results among animals, and the frequency of the cellular responses. For the SDS assay, the test material was considered positive if the percent cells in S-phase at any dose was significantly higher than the vehicle control value.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Clinical Observations: The report stated that no signs or evidence of significant clinical diseases were noted at any time during the course of the study.

B. UDS/SDS Assay: Kidney cells recovered from a minimum of three male and three female rats per group were scored for UDS and SDS activities; for the high-dose group, four male and four female rats were scored for both endpoints. As shown in Table 1, the single gavage administration of 300, 600, or 1000 mg/kg of the test material did not induce UDS in male or female kidney cells harvested 16 hours after treatment. In high-dose males, however, the percentage of S-phase cells was significantly increased. Nonsignificant and nondose-related elevations in SDS were seen at the remaining levels. In females exposed to the three test material doses and the positive control (1.5 mg/kg $HgCl_2$), the percentage of cells in S-phase was lower than the solvent control value suggesting that neither the test material nor the positive control were renal toxins in female rats. The report stated that further investigations of 5 and 10 mg/kg $HgCl_2$ showed no induction of SDS in female rats.

C. Chemical Analysis: Chemical analysis of dosing solutions prepared in corn oil indicated that the concentrations of the test material were 10 to 16% higher than expected. The authors attributed the increases over the target concentrations to changes in corn oil density as the concentration of the test material increased.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors stated, "We conclude that although PDCB is not a genotoxic agent in the male F-344 rat kidney, it induces a significant S-phase response in male rats receiving 1000 mg/kg by oral gavage. We also conclude that in female F-344 rats PDCB is not a genotoxic agent, and it does not induce S-phase synthesis in the kidney."

B. A quality assurance statement was signed and dated June 3, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study provided acceptable evidence that PDCB was assayed to a level (1000 mg/kg) that induced significant renal toxicity but no genotoxicity in male rats. However, no signs of clinical toxicity, target cell cytotoxicity, or genotoxicity were apparent in the females treated with the three assayed levels. Similarly, the positive control for renal toxicity ($HgCl_2$) did not increase the frequency of SDS in kidney cells recovered from the females. The lack of PDCB-induced toxicity or cytotoxicity to the target organ (kidneys) and the inability to demonstrate that $HgCl_2$ is a renal toxin

TABLE 1. Results of the in Vivo/in Vitro Unscheduled DNA Synthesis Assay in Rats Dosed with para-Dichlorobenzene

Substance	Dose (mg/kg)	No. of Animals Per Group	UDS Activity[a] No. of Kidney Cells Scored	UDS Activity[a] Mean Net Nuclear Grain Count ±S.E.[c]	UDS Activity[a] % Cells in Repair ± S.E.[c]	SDS Activity[b] No. of Animals Per Group	SDS Activity[b] No. of Kidney Cells Scored	SDS Activity[b] % Cells in S-phase ± S.E.[c]
Vehicle Control								
Corn oil	0	3M	270	-4.6±0.9	6.0±2.0	3M	9000	0.38±0.09
		3F	270	-4.5±0.2	2.0±1.0	3F	9000	0.52±0.17
Positive Control								
Streptozocin	250	2M	180	11.4±1.6	79.0±7.0	--	--	--
		3F	270	4.7±1.0	58.0±9.0	--	--	--
Mercuric chloride	10	--	--	--	--	3M	9000	10.81±1.41**
	1.5	--	--	--	--	4F	12,000	0.37±0.08
Test Material								
para-Dichlorobenzene	1000[d]	4M	360	-6.4±0.8	7.0±2.0	4M	12,000	1.01±0.15**
		4F	360	-6.1±0.5	2.0±0.0	3F	9000	0.32±0.04

[a]Animals sacrificed 16 hours posttreatment.

[b]Animals sacrificed 96 hours posttreatment.

[c]S.E. = Standard error.

[d]UDS assay results for lower dose groups (300 and 600 mg/kg) were comparable to the corresponding control group. SDS assay results for male rats exposed to 300 and 600 mg/kg showed nondose-related increases in the % cells in S-phase compared to the corresponding control value. No increase in SDS was seen for the females of the low- and mid-dose groups.

*Significantly higher than the vehicle control group (at $p < 0.05$) using Student's t-test.

**Significantly higher than the vehicle control group (at $p < 0.02$) using Student's t-test.

for female rats precludes acceptance of the findings as valid evidence that PDCB is not genotoxic in kidney tissue. The assay should, therefore be repeated in females (using repeated exposures, if necessary). It is acknowledged, however, that the in vivo/in vitro UDS/SDS assay in kidney cells is not a published requirement to support EPA registration of pesticides.[2] However, it may become a requirement if it is deemed relevant by the agency, based on evidence from other studies (e.g., chronic or acute toxicity studies).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-9.

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[2] EPA FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation--Human and Domestic Animals, dated 11-30-82.

C07021

APPENDIX A

Materials and Methods

Paradichlorobenzene toxicology review

Page _____ is not included in this copy.

Pages 55 through 62 are not included in this copy.

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___ A draft product label

___ The product confidential statement of formula

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EPA: 68D80056
DYNAMAC No. 121-D
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--In Vivo Cytogenetic Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Ira Cecil Felton for

Date: 12-21-88

EPA: 68D80056
DYNAMAC No. 121-D
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--In Vivo Cytogenetic Study in Rats

REVIEWED BY:

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Principal Reviewer
Dynamac Corporation
Signature: Nancy E. McCarroll
Date: 12-21-88

I. Cecil Felkner, Ph.D.
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Mike Ioannou, Ph.D.
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Date: 1-3-89

Marion Copley, D.V.M.,
D.A.B.T.
Acting EPA Section Head,
Section II,
Toxicology Branch I (TS-769C)
Signature: M Copley
Date: 1/30/89

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity--In vivo cytogenetic study in rats.

ACCESSION/MRID NUMBER: 405210-10.

TEST MATERIAL: para-Dichlorobenzene.

SYNONYM(S): PDCB; 1,4-dichlorobenzene.

STUDY NUMBER(S): CTL/P/293.

SPONSOR: Chlorobenzene Producers Association.

TESTING FACILITY: Imperial Chemical Industries, Ltd., Cheshire, England.

TITLE OF REPORT: para-Dichlorobenzene--Cytogenetic Study in The Rat.

AUTHOR(S): Anderson, D., and Richardson, C. R.

REPORT ISSUED: November 22, 1976.

CONCLUSIONS - EXECUTIVE SUMMARY: para-Dichlorobenzene (PDCB) was assayed for the potential to induce chromosome aberrations by inhalation in male rat bone marrow cells following acute exposures (299 and 682 ppm PDCB X 2 hours), subacute exposures (75 and 500 ppm PDCB for 5 hours/day x 5 days) and subchronic exposures (75 and 500 ppm PDCB for 5 hours/day x 5 days/week x 3 months). The test material was not toxic to the animals or cytotoxic to the target cell at the highest generated dose (682 ppm) and was not clastogenic. However, no meaningful conclusions can be drawn for the following reasons:

1. Too few animals were exposed at each experimental dose (three per test group in the acute and two per test group in the subacute and subchronic assays).

2. Females were not evaluated.

3. The effect, if any, of PDCB on different stages of the mitotic cell cycle was not determined for the acute exposures.

4. No analytical data on the test atmospheres were provided.

5. Methods of animal identification and randomization were not reported.

6. A statement of compliance with Good Laboratory Practices (GLP) and Quality Assurance (QA) was not presented.

Classification: The study is unacceptable and should be repeated in accordance with recommended protocols.[1]

Additionally, the authors should provide detailed analytical data, detailed animal information, and a QA statement of compliance with GLPs.

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[1]Preston, R. J., Au, W., Bender, M. A., Brewen, J. G., Carrano, A. V., Heddle, J. A., McFee, A. I., Wolf, S., and Wassom, J. S. Mammalian *in vivo* and *in vitro* cytogenetic assays: a report of the Gene-Tox program. Mutat. Res. 87(1981): 143-188.

A. MATERIALS:

1. Test Compound: PDCB was manufactured by a division of the performing laboratory's company; no other information was supplied.

2. Test Animals: Species: rat; strain: not reported; sex: male; age: 8-10 weeks old; weight: 150-200 g; source: Alderley Park.

Males were housed individually in the exposure chambers. Alderly Park rat cubes and water were available ad libitum. Environmental conditions were not specified.

3. Inhalation Chambers:

a. Description: Animals were exposed to test atmospheres and the negative and positive controls in 60-L "Perspex" exposure chambers;[2] the number of air changes/hour was not reported.

b. Aerosol Generation: Test atmospheres of PDCB were generated by passing metered volumes of dry/clean air through the test material contained in a test tube that was held in a temperature-controlled water bath. The appropriate dilution of PDCB was achieved by altering temperature (not reported), air flow rate (not reported), or the dilution of the generated atmosphere. Achieved PDCB concentrations for the single 2-hour exposure were 299 and 682 ppm (maximum dose successfully generated) for longer exposures (5 hours/day x 5 days and 5 hours/day x 5 days/week x 3 months) the achieved concentrations were 75 and 500 ppm PDCB.

2. Positive Controls:

a. Benzene: The concentrations of benzene (10, 750, and 7500 ppm) were generated by continuously passing a known volume of benzene through a concentric jet atomizer and vaporizing this positive control chemical with a calculated volume of clean dry air. Benzene exposures lasted 2 hours.

b. Vinyl chloride: Atmospheres of 1500 ppm vinyl chloride (VC) were produced by mixing known volumes of VC and air; rotameters were used as the indicator of VC concentration. Animals in these groups were either exposed to VC for 5 hours/day x 5 days or 5 hours/day x 5 days/week x 3 months.

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[2]Gage, J. C. Br. J. Ind. Med. 16(1959):11.

c. Chamber Concentration Analysis: Atmospheres of PDCB, benzene, and VC were monitored with an infrared gas spectrophotometer. The report did not indicate that homogeneity of test atmospheres was determined or the frequency at which atmospheres were monitored. The analytical data to support nominal test material chamber concentrations were not provided. Similarly, the report did not indicate how exhaust from the chambers was handled.

B. STUDY DESIGN:

1. Assignment to Groups: Methods used to identify and randomize the animals were not reported. Animals were assigned to the following test groups:

		Number of Male Rats/Exposure Interval		
Substance	Dose (ppm)	2 hours	5 hours/day x 5 days	5 hours/day x 5 days/week x 3 months
Negative Control				
Air	--	4	2	2
Test Material				
PDCB	299	3	--	--
	682	3	--	--
	75	--	2	2
	500	--	2	2
Positive Control				
Benzene	10	3	--	--
	750	3	--	--
	7500	3	--	--
Vinyl chloride	1500	--	2	2

4. Cytogenetic Assay:

a. Animal Sacrifice/Bone Marrow Harvest: Animals were sacrificed with an overdose of Fluothane 22 hours after completion of the appropriate exposure. Colchicine (dose not reported) was injected intraperitoneally 1 hour prior to the scheduled sacrifice. Bone marrow cells were harvested from both femurs by aspiration

into Hanks' balanced salt solution. Cells were incubated in 0.075 M KCl for 20 minutes and fixed in glacial acetic acid:methanol (1:3). Slides were prepared, stained with Giemsa, and coded.

b. Slide Analysis: Fifty to 100 cells per animal were scored for chromosome aberrations, which were characterized as chromatid or chromosome gaps, chromatid breaks, fragments, and other complex aberrations. Gaps were included in the overall evaluation of the results.

c. Statistical Evaluation: Data were transformed using the Freeman-Tukey transformation and evaluated for significance by an analysis of variance and the Student's t-test at p values of 0.05, 0.01, 0.001.

e. Quality Assurance: A quality assurance statement was not provided.

C. REPORTED RESULTS: No clinical observations were reported for the acute (2-hour), subacute (5 hours/day x 5 days) or subchronic (5 hours/day x 5 days/week x 3 months) exposures. As shown in Table 1, no appreciable increase in the percent aberrant cells was observed in male rats exposed by inhalation to test atmospheres of PDCB. The study authors included chromatid and chromosome gap data in the final analysis; although this is an unacceptable practice, recalculation of the data omitting gaps did not affect the outcome of the study. It was noted following the subchronic exposure that the percent aberrant cells in the 75- and 500-ppm dosed groups were higher than the corresponding control value; however, the test group values were within the range of background aberrations for the entire study. By contrast, acute exposure to 10, 75, and 7500 ppm benzene induced dose-related increases in the percent aberrant cells, total number of aberrations, and number of aberrations per cell. VC (1500 ppm) was detected as a clastogen following the subacute but not the subchronic exposure.

D. STUDY AUTHORS' CONCLUSIONS: The study authors concluded that PDCB was negative in the rat bone marrow cytogenetic assay.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that under the three exposure conditions, PDCB was neither toxic to the animals nor cytotoxic to the target cells. We can only assume, based on the authors' comments stating that 682 ppm was the highest successfully achieved concentration, that

TABLE 1. Representative Results of the in vivo Cytogenetic Study in Male Rats Exposed by Inhalation to para-Dichlorobenzene

Substance	Dose (ppm)	Exposure[a]	No. of Animals Analyzed per Group	No. of Metaphases Examined	% Aberrant Cells per Group[b]	Total No. of Aberrations per Group[c]	No. of Aberrations per Cell[d]
Negative Control							
Air	--	Acute	4	200	5.5	4	0.020
		Subacute	2	200	6.0	1	0.005
		Subchronic	2	200	6.5	0	0.000
Negative Control							
Benzene	10	Acute	3	150	13.0*	10	0.067
	750	Acute	3	150	20.7**	24	0.160
	7500	Acute	3	150	30.0***	46	0.307
Vinyl chloride	1500	Subacute	2	200	12.5*	7	0.035
		Subchronic	2	200	10.0	0	0.000
Test Material							
para-Dichloro-benzene	299	Acute	3	150	4.0	2	0.013
	682	Acute	3	150	4.0	2	0.013
	75	Subacute	2	200	6.5	3	0.015
	500	Subacute	2	200	6.0	1	0.005
	75	Subchronic	2	200	6.5	4	0.020
	500	Subchronic	2	200	6.0	3	0.015

[a]Acute exposure = 2 hours.
Subacute exposure = 5 hours/day x 5 days.
Subchronic exposure = 5 hours/day x 5 days/week x 3 months.

[b]Including gaps as reported.

[c]Excluding gaps; calculated by our reviewers.

[d]No. of aberrations per cell = $\frac{\text{Total No. of Aberrations - Gaps}}{\text{No. of Metaphases Examined}}$; calculated by our reviewers.

*Significantly higher than the control value ($p < 0.05$) by Student's t-test.

**Significantly higher than the control value ($p < 0.01$) by Student's t-test.

***Significantly higher than the control value ($p < 0.001$) by Student's t-test.

the generation of higher test atmospheres was limited by the physical and/or chemical properties of the test material. Nevertheless, we are unable to draw meaningful conclusions for the following reasons:

1. The sample sizes were too small to provide confidence in the findings.

2. Unless justified, the use of a single sex is unacceptable.

3. For the acute exposure, multiple posttreatment sampling times were not performed. Hence, the potential clastogenic effects, if any, of PDCB on cells in different stages of the cell cycle cannot be determined.

Based on the above considerations, we conclude that the study is unacceptable and should be repeated.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 6-9.

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APPENDIX A

Materials and Methods

Paradichlorobenzene toxicology review

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007021

EPA: 68D80056
DYNAMAC No. 121-E
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--Dominant Lethal Assay in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 12-21-88

77

EPA: 68D80056
DYNAMAC No. 121-E
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--Dominant Lethal Assay in Mice

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-21-88

I. Cecil Felkner, Ph.D.
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Marion Copley, D.V.M.,
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Acting EPA Section Head,
Section II
Toxicology Branch I (TS-769C)

Signature: M Copley 1/30/89
Date: 1/30/89

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity--Dominant lethal assay in mice.

ACCESSION/MRID NUMBER: 405210-11.

TEST MATERIAL: para-Dichlorobenzene.

SYNONYM(S): 1,4-Dichlorobenzene; PDCB.

STUDY NUMBER(S): CTL/P/296.

SPONSOR: Chlorobenzene Producers Association, Washington, DC.

TESTING FACILITY: Imperial Chemical Industries, Ltd., Central Toxicology Laboratory, Cheshire, England.

TITLE OF REPORT: para-Dichlorobenzene--Dominant Lethal Study in the Mouse.

AUTHOR(S): Anderson, D., and Hodge, M.C.E.

REPORT ISSUED: November 18, 1976.

CONCLUSIONS - EXECUTIVE SUMMARY: Male mice were continuously exposed by inhalation to 75, 225, and 450 ppm para-dichlorobenzene (PDCB) 6 hours/day for 5 consecutive days and sequentially mated with virgin females for 8 weeks. Although the results did not indicate a PDCB-induced dominant lethal effect in male germinal cells sampled over the entire period of spermatogenesis, no toxicity or definitive target cell cytotoxicity was seen at any dose. We conclude, therefore, that a maximum tolerated dose (MTD) was not achieved. Additionally, no analytical data were presented to support the study authors' claim that 75-, 225-, and 450-ppm test atmospheres were achieved. Monitoring of test atmospheres throughout the daily 6-hour exposures was also not reported.

Classification: The study is unacceptable and should be repeated to include a PDCB level that approximates the MTD as the high dose. Additional recommendations for the repeat assay include providing analytical data to support achieved test material concentrations, assurance of test material homogeneity throughout the exposure chamber, and results of monitoring chamber concentrations during the exposures. It is further recommended that methods of animal identification and randomization and statements for good laboratory practices and quality assurance be furnished with the repeat study.

A. MATERIALS:

1. Test Compound: PDCB was manufactured by a division of the performing laboratory's company; no other information was supplied.

2. Test Animals: Species: mouse; strain: CD-1; age: males (after treatment) were 10-12 weeks old; females (undosed) were 9 to 10 weeks old; source: Charles River Ltd., Kent, U.K. Males were housed individually and females were housed in pairs. Alderly Park mouse cubes and water were available ad libitum. Environmental conditions were not specified.

3. Inhalation Chambers:

   a. Description: Exposure chambers were constructed of stainless steel and glass and had an internal capacity of 3 L. Negative and positive control animals were housed in similar exposure chambers during the dosing period. The number of air changes/hour was not reported.

b. Aerosol Generation: Test atmospheres of PDCB were generated by passing metered volumes of dry/clean air through the test material contained in a test tube that was held in a temperature-controlled water bath. The appropriate dilution of PDCB was achieved by altering temperature (not reported), airflow rate (not reported), or dilution of the generated atmosphere. The report did not indicate how the exhaust from the chambers was handled.

c. Chamber Concentration Analysis: Test atmospheres were analyzed with an infrared gas spectrophotometer. The report did not indicate whether the homogeneity of test atmospheres was determined or the intervals at which test atmospheres were monitored. The analytical data to support achieved test material concentrations were not provided.

B. STUDY DESIGN:

1. Animal Assignment: Methods used to identify and randomize the animals were not reported. Animals were assigned to the following test groups:

Test Group	Dose	Males/ Group	Females/ Mating Interval
Negative Control[a]			
Air	--	35	70
Test Material[a]			
Low dose	75 ppm	16	32
Medium dose	225 ppm	16	32
High dose	450 ppm	16	32
Positive Control[b]			
Cyclophosphamide (CP)	200 mg/kg	13	26
Ethylmethanesulphonate (EMS)	150 mg/kg	5	10
Nitrogen mustard (HN_2)	2.5 mg/kg	12	24

[a] Negative control and test doses were administered by inhalation for 6 hours/day for 5 consecutive days.
[b] CP and HN_2 were administered once by intraperitoneal injection on day 5; EMS was administered orally for 5 consecutive days.

2. Preliminary Toxicity Study: Prior to initiation of the dominant lethal assay, groups of six mice were exposed for 6 hours/day for 5 consecutive days to five test atmospheric concentrations of PDCB (40, 80, 160, 320, and 640 ppm). The report stated that two deaths occurred in the high-dose group on the final exposure day. Surviving animals in the 640-ppm dose group were reported to be subdued, shaking, and in- continent. Based on these findings, 450 ppm of PDCB was selected as the high dose; 75 and 225 ppm were chosen as the low and intermediate doses.

3. Dominant Lethal Assay:

   a. Compound Exposure: Sixteen males in each test group were exposed by inhalation to the appropriate atmospheric concentrations of PDCB for 6 hours/day for 5 consecutive days. Positive control animals either received single i.p. injections of CP or HN_2 or five consecutive daily oral administrations of EMS.

   b. Fertility Test: At the completion of treatment, each of the males was mated with two virgin females for 5 days. Females were sacrificed 15 days after introduction to the males and were examined for pregnancies. The report stated that male mice that survived treatment and were fertile were selected for continuation in the study. Data generated for animals in the fertility test were designated as week 0. It was noted, however, that subsequent discussion in the report and tabular presentation of the data referred to week-0 findings as the period "before treatment."

   c. Subsequent Matings/Animal Sacrifice: Each of the males was mated with two virgin females for 5 days. Females were replaced with two virgin females and the mating sequence was repeated for a total of 8 weeks. Females were sacrificed 13 days after the assumed date of fertilization; uteri were examined for live implants and for early and late deaths. Males were sacrificed at the completion of mating and were not examined.

   d. Statistical Methods: Early deaths/pregnancy and early deaths/total implants/pregnancy were transformed using the Freeman-Tukey Poisson variance or the Freeman-Tukey transformation for proportions. Significant differences for all measured parameters were identified by Chi-square and Dunnett's t-test.

e. Quality Assurance: No quality assurance statement was provided.

C. REPORTED RESULTS:

1. Animal Observations: One male in the low-dose (75 ppm) group died during week 3 of mating; the study authors did not consider this death to be treatment related. Three males dosed with the positive control chemical, CP, died. No other clinical signs were reported.

2. Dominant Lethal Assay: We can only assume, based on the reported results for week 0 (fertility test), that these findings represent a pretreatment mating since no adverse effects on relevant dominant lethal parameters (i.e., total implants, live implants, and/or early deaths) were seen in the positive control groups. Additionally, males were mated through an 8-week mating cycle posttreatment; this is the recommended mating protocol for mice in this assay. We, therefore, do not consider the data from the 0 mating week to be relevant to the overall results of the study.

As shown in Table 1, a nonsignificant decrease in the pregnancy rate for the high-dose group (450 ppm) occurred following the first pretreatment mating. Sporadic decreases in the pregnancy rates of test groups were noted at other mating weeks and were significantly ($p < 0.05$) lower than the corresponding negative control group for the low-dose (75 ppm) group at weeks 6 and 7. These reductions were, however, not dose related. Throughout the course of the study, total implants, live implants, and early deaths for females mated to males exposed to PCDB were generally comparable to the appropriate air-control group mating.

The only exceptions were the significant ($p < 0.05$) but nondose-related decreases in total implants for low- and high-dose groups at week 8. However, the lower-than-control, mean early-fetal deaths for these groups did not indicate a dominant lethal effect.

By contrast, total implants and early postimplantation loss for the three positive control groups (CP, EMS, and HN_2) were significantly (p [illegible] 0.01 or 0.001) different from the negative con [illegible] following the first and second mating inte[illegible]val- [illegible]ant ($p < 0.05$) adverse effects on early dea[illegible] w[illegible]uced by CP at week 3.

83

TABLE 1. Representative Mean Results from Selected Mating Weeks of the Dominant Lethal Assay in Mice Exposed to para-Dichlorobenzene (PDCB)

Mating Week	Substance	Dose	No. of Females Mated	Average Pregnancy Rate[a]	Average Total Implants	Average Live Implants[b]	Average Early Deaths	Average Late Deaths[b]	% Females with at Least One Early Death	Mutation Index[c,d]
1	Air	0 ppm	70	80.0	12.1^{***}	11.3	0.75^{***}	0.04	50.0^{**}	0.06^{***e}
	CP[f]	200 mg/kg	26	65.4	9.7^{**}	8.4	5.53^{***}	0.00	94.1^{*}	0.57^{***e}
	EMS	150 mg/kg	10	70.0	9.5^{***}	4.2	5.29^{*}	0.00	100.0^{*}	0.56^{**e}
	HN_2	2.5 mg/kg	24	79.2	9.7	8.4	1.26^{*}	0.00	78.9	0.13^{**e}
	PDCB	450 ppm[g]	32	68.8	11.8	11.0	0.68	0.09	45.5	0.06
3	Air	0 ppm	70	85.7	11.4	10.6	0.07^{*}	0.78	50.0	0.07^{*e}
	CP[f]	200 mg/kg	26	88.5^{*}	11.9	10.2	1.70^{*}	0.00	65.2	0.14^{*e}
	EMS	150 mg/kg	10	60.0^{*}	10.7	9.7	1.00	0.00	83.3	0.09
	HN_2	2.5 mg/kg	24	79.2	11.4	9.7	1.53	0.21	68.4	0.13
	PDCB	450 ppm	32	90.6	12.8	11.8	0.97	0.07	51.7	0.08
6	Air	0 ppm	70	95.7^{*}	12.5	11.8	0.66	0.06	47.8	0.05
	CP[f]	200 mg/kg	20	80.0	13.0	12.0	1.00	0.00	50.0	0.08
	EMF	150 mg/kg	10	90.0^{*}	11.5	10.9	0.56	0.00	33.3	0.05
	HN_2	2.5 mg/kg	24	79.2^{*}	13.1	13.0	0.10	0.00	10.5	0.01
	PDCB	450 ppm	32	100.0	12.3	11.6	0.69	0.06	50.0	0.06

[a] Pregnancy Rate = $\frac{\text{No. of Females with Implants}}{\text{No. of Mated Females}} \times 100$.

[b] Values calculated by our reviewers.

[c] Mutation Index (Early Deaths) = $\frac{\text{Average No. of Early Deaths}}{\text{Total Implants}}$; calculated by our reviewers.

[d] Percent of early deaths/total implants/pregnancy was reported by the study authors for this parameter. Recalculation of the data as presented by our reviewers indicated slightly different values, but did not affect the outcome of the study.

[e] Study authors indicated statistical significance for this experimental point.

[f] Abbreviations used:
CP = Cyclophosphamide
EMS = Ethylmethanesulphonate
HN_2 = Nitrogen mustard.

(Footnotes cont'd)

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[9] Results for the low- (75 ppm) and mid- (225 ppm) dose groups showed no dose- or time-related differences from the air control groups.

*Significantly different from the control values ($p < 0.05$).
**Significantly different from the control values ($p < 0.01$).
***Significantly different from the control value ($p < 0.001$).

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D. STUDY AUTHORS' CONCLUSIONS: The study authors concluded, "PDCB is not mutagenic in the mouse at the stated exposure levels as measured by the dominant lethal test."

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that PDCB was not adequately tested for the potential to induce dominant lethal mutations in male mouse germinal cells because the highest assayed dose was not toxic to the male mice nor did it induce a clear cytotoxic effect on the target cell tissue. The data, therefore, are insufficient to establish that the MTD, as recommended by the U.S. Environmental Protection Agency Gene-Tox Program[1] was assayed.

The following deficiencies also compromised the study's integrity:

1. Analytical data to support exposure concentrations and homogeneity of the test atmospheres throughout the daily 6-hour inhalation exposures were not provided.

2. No information was furnished on environmental conditions, animal identification, or randomization procedures.

3. The study was not performed under good laboratory practices.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3-6.

[1]Green, S., Auletta, A., Fabricant, J., Kapp, R., Manandhar, M., Shea, C., Springer, J., and Whitfield, B. Current status of bioassays in genetic toxicology--The dominant lethal assay. Mutat. Res. 154(1985):49-67.

APPENDIX A
MATERIALS AND METHODS

Paradichlorobenzene toxicology review

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007021

EPA: 68D80056
DYNAMAC No. 121-F
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--In Vivo Micronucleus Assay in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: for

Date: 12-21-88

92

007021

EPA: 68D80056
DYNAMAC No. 112-F
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--In Vivo Micronucleus Assay in Mice

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
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Signature: Nancy E. McCarroll
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I. Cecil Felkner, Ph.D.
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(TS-769C)

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Date: M Copley

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity--In vivo micronucleus assay in mice.

ACCESSION/MRID NUMBER: 405210-12.

TEST MATERIAL: para-Dichlorobenzene.

SYNONYM(S): PDCB; 1,4-dichlorobenzene.

STUDY NUMBER(S): T.1021071.

SPONSOR: Chlorobenzene Producer's Assocation.

TESTING FACILITY: Bayer AG, Institute of Toxicology, Wuppertal, West Germany.

TITLE OF REPORT: para-Dichlorobenzene--Investigation of p-Dichlorobenzene, 1,4-Dichlorobenzene for Clastogenic Effects in Mice using the Micronucleus Test.

AUTHOR(S): Herbold, B.

REPORT ISSUED: June 10, 1986.

CONCLUSION(S) - EXECUTIVE SUMMARY: Bone marrow cells were harvested from male and female mice at 24, 48, and 72 hours postexposure to a single oral administration of 2500 mg/kg para-dichlorobenzene (PDCB) and were evaluated for the induction of micronuclei in polychromatic erythrocytes (MPE). Results indicate that the test material was toxic to the test animals and cytotoxic to the target cells, but was not genotoxic. It was concluded that PDCB was adequately tested at an appropriate dose level and was not genotoxic in a well-controlled study. Therefore, the requirement to investigate lower doses of the test material is waived.

Study: The study is acceptable.

A. MATERIALS

1. Test Material: para-Dichlorobenzene (PDCB).

   a. Description: white crystalline material.
   b. Purity: 99.9%.
   c. Batch No. 922.
   d. Contaminants: None reported.
   e. Solvent used: Corn oil.

2. Control Materials:

   a. Vehicle control:
   Corn oil was administered orally at a dosing volume of 10 mL/kg.

   b. Positive control:
   20 mg/kg cyclophosphamide (CP) was administered orally.

3. Test Compound:
   a. Route of administration: Oral.
   b. Dose level(s) used: 2500 mg/kg.

4. Test animals:
   a. Species: mouse ; strain: Bor:NMRI (SPF Han); age: 8 to 12 weeks ; source: F. Winkelmann, Borchen .

   b. No. animals used per dose: 5 male; 5 females.
   c. Properly maintained? Yes .

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
   a. Test compound:
   Dosing x once ____ twice (24 hr apart)
   _____ other (describe):
   Sampling (after last dose): _____ 6 hr _____12 hr
   x 24 hr x 48 hr x 72 hr.

b. Positive control:

Dosing: __x__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr _____ 48 hr _____ 72 hr.

2. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 1000.

No. of normochromatic erythrocytes (NCE) examined per animal: Number observed/1000 PCEs.

3. Preliminary Range-Finding Assay:

Groups of five animals (including males and females) received single oral administrations of 1000, 2500, 3000 4000, and 5000 mg/kg PDCB. The author reported that all animals in the 4000-mg/kg dose group died, two died in the 3000-mg/kg dose group, and all animals survived exposure to lower test material doses. No comments were made regarding the high-dose animals. Animals in the 1000- and 2500-mg/kg groups exhibited a wide variety of clinical signs, including apathy, reduced motility, tremors, labored breathing, accelerated respiratory rate, and lung noise. Based on these findings, 2500 mg/kg PDCB was selected as the appropriate dose level for the micronucleus assay.

4. Micronucleus assay:

A. Animal Observations: No deaths occurred following the oral administration of 2500 mg/kg PDCB to five male and five female mice per sacrifice interval. Signs of toxicity, similar to those reported for the preliminary range-finding assay, were seen and were reported to persist up until the final sacrifice.

B. Micronucleus Assay Results: As shown in Table 1, the percentages of MPE scored in bone marrow cells harvested from males and females sacrificed 24, 48, and 72 hours postexposure to 2500 mg/kg were comparable to the vehicle control group. However, the PCE to NCE ratios for females in the 48-hour sacrifice group and both sexes at 72 hours were distorted in favor of the mature erythrocytes. At 72 hours, the combined PCE to NCE ratios for males and females was significant ($p < 0.01$) by Wilcoxon's non-parametric rank sum test.

Based on the above findings, the author concluded that no evidence of clastogenic effect was found after a single oral administration of 2500 mg/kg PDCB.

TABLE 1. Representative Results of the Micronucleus Assay in Mice with para-Dichlorobenzene

Substance	Dose (mg/kg)	Sacrifice Time (hours)[a]	Sex	No. of Animals Analyzed per Group	No. of PCEs[b] Analyzed per Group	Percent MPEs[b] per Group	Group PCE:NCE[b]
Vechicle Control							
Corn oil	0.0	24	M	5	5000	0.18	1:0.85
			F	5	5000	0.06	1:1.23
Positive Control							
Cyclophosphamide	20.0	24	M	5	5000	1.34[c]	1:1.19
			F	5	5000	1.06	1:1.13
Test Material							
para-Dichlorobenze	2500.0[d]	24	M	5	5000	0.14	1:0.84
			F	5	5000	0.18	1:1.28
		48	M	5	5000	0.22	1:1.24
			F	5	5000	0.10	1:1.97
		72	M	5	5000	0.14	1:1.95[c]
			F	5	5000	0.10	1:1.89

[a]Time after compound administration.

[b]Abbreviations used:
PCE - Polychromatic erythrocytes
MPE - Micronucleated polychromatic erythrocytes
NCE - Normochromatic erythrocytes.

[c]Combined results for males and females were significantly higher than the vehicle control value ($p < 0.01$) by Wilcoxon's nonparametric rank sum test.

[d]Toxic signs were reported to persist to the final sacrifice.

5. Reviewers' Discussion:
We assess that the study was properly conducted and that the study author interpreted the data correctly. The single dose of PDCB (2500 mg/kg) was toxic to the animals. The increased frequency of peripheral blood cells (NCEs) in male and female bone marrow tissue further indicates that PDCB induced stem cell cytotoxicity following the 72-hour exposure. It was, therefore, concluded that PDCB was assayed to an appropriate toxic and cytotoxic dose level with no indication of a genotoxic effect; therefore, the requirement to evaluate lower test material concentrations is waived. The ability of the test system to detect genotoxic events was demonstrated by the increased frequencies of MPEs in the male and female mice treated with the positive control (20 mg/kg CP); the combined data were significant.

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

7. CBI Appendix: Appendix A, Materials and Methods, CBI pp. 4-8.

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APPENDIX A

Materials and Methods

Paradichlorobenzene toxicology review

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Pages 102 through 106 are not included in this copy.

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___ 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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007021

PA: 68D80056
DYNAMAC No. 121-G
December 21, 1988

DATA EVALUATION RECORD

PDCB (para-Dichlorobenzene)

Mutagenicity--Chinese Hamster Ovary Cell/HGPRT
Forward Mutation Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 12-21-88

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Signature: M Copley
Date: 1/30/89

[redacted]

007021

DATA EVALUATION RECORD

STUDY TYPE: Chinese hamster ovary cell/HGPRT forward mutation assay.

ACCESSION/MRID NUMBER: 405210-13.

TEST MATERIAL: para-Dichlorobenzene.

SYNONYM(S): PDCB; 1,4-dichlorobenzene.

STUDY NUMBER(S): E-9419.

SPONSOR: Chlorobenzene Procedures Association/Bayer AG Institut fuer Toxicology, West Germany.

TESTING FACILITY: Litton Bionetics, the Netherlands.

TITLE OF REPORT: Mutagenicity Evaluation of p-Dichlorobenzene in the CHO HGPRT Forward Mutation Assay.

AUTHOR(S): de Boer, W. C., and Hoorn, A. J. W.

REPORT ISSUED: May 1986.

CONCLUSIONS - EXECUTIVE SUMMARY: para-Dichlorobenzene (PDCB) was evaluated for the potential to cause forward gene mutations in Chinese hamster ovary (CHO) cells. Over a concentration range of 50 to 400 µg/mL, both with and without S9 activation, the test material did not induce a reproducible or dose-related increase in the frequency of mutation at the HGPRT locus. Severe cytotoxicity was apparent at nonactivated and S9-activated doses ≥ 320 µg/mL. We conclude that PDCB was assayed up to an acceptable level of cytotoxicity with no evidence of a mutagenic effect.

Classification: The study is acceptable.

A. MATERIALS: (See Appendix A for details.)

1. Test Compound:

   a. Description: para-Dichlorobenzene (PDCB) from batch No. 922 was described as a white lumpy material with a purity of > 99.9%. The test material was stored in the dark at 4°C.

   b. Solubility/pH Determination: The test material was soluble in dimethylsulfoxide (DMSO) at a stock concentration of 1000 mg/mL. Subsequent dilution in treatment medium (Ham's F10) indicated that the test material was soluble up to 10 mg/mL (final concentration) and that this level did not adversely affect the pH of the tissue culture medium.

2. Mammalian Cells: CHO cells, subclone CHO-K1, were obtained from Dr. T. T. Puck, University of Colorado, Denver, CO. Stock cultures were held in liquid nitrogen and routinely selected against the HGPRT phenotype; monolayers were maintained in F10 medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics.

3. S9 Activation: The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254; it was supplied by Litton Bionetics, Inc. The concentration of S9 in the S9 cofactor mix was not reported.

4. Preliminary Cytotoxicity Assay: Cytotoxicity was determined by treating 200 cells/dish for 4 hours with 10 nonactivated or S9-activated doses of the test material that spanned a 3-log concentration range. Exposed cells were washed and incubated in media for 7 days. Colonies

were counted and survival in treatment groups relative to growth in the control was determined. Based on these results, six to eight test material doses that caused a 0 to 90% reduction in colony-forming ability were selected for the mutation assay.

5. Mutagenicity Assay: Cultures, seeded at 4 x 10^6 cells, were treated in the presence or absence of S9 activation with appropriate levels of test material, negative control (DMSO), or positive controls [5-bromo-2'-deoxyuridine (BrdU) at 50 µg/mL/-S9; 3-methylcholanthrene (3-MCA) at 5 µg/mL/+S9].

   After a 4-hour exposure, cells were washed, trypsinized, suspended in media, counted, and replated at two cell densities. Triplicate plates containing 200 cells were incubated for 7 to 8 days and were used to determine cytotoxicity as described for the preliminary cytotoxicity assay. Cells were also seeded in duplicate at a density of 1.5 x 10^6 cells/plate to allow expression of mutations. Throughout the 6- to 7-day expression period, cells were subcultured and reseeded at 1.5 x 10^6 cells. Mutant selection was accomplished by plating 2 x 10^5 cells/dish (12 dishes) in medium containing 10 µg/mL 6-thioguanine. Viability at selection was determined by seeding 200 cells/dish (three dishes/treatment) in complete medium. Selection and viability cultures were incubated for 10 days, fixed, stained with Giemsa, and counted. Cloning efficiencies (CEs) and mutation frequencies (MFs) were calculated.

6. Evaluation Criteria:

   a. Assay Validity: For the assay to be considered valid, the following criteria must be satisfied: 1) the CE for the negative control must be between 70 and 115%; 2) the background MF must be between 1 and 15 x 10^{-6}; 3) the MF for the positive controls must be within the historical range of the reporting laboratory and be significantly higher ($p \leq 0.05$) than the concurrent negative control; 4) a minimum of three test doses must be available for analysis; and 5) the test material must be assayed to a dose causing ≈85% cytotoxicity or to a maximum dose of 10 mg/mL.

   b. Positive Response: The test material was considered positive if a dose-related or cytotoxicity-related significant increase ($p < 0.05$) in the MF over at least three test concentrations was observed.

7. Statistical Analysis: The statistical tables of Kastenbaum and Bowman were used to determine significance at p values of 0.05 and 0.01.

B. Protocol: See Appendix B.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Ten doses ranging from 19.5 to 10,000 µg/mL were assayed in the presence or absence of S9 activation. Less than 10% of the cells survived following exposure to the three highest non-activated and S9-activated test doses (2500, 5000, and 10,000 µg/mL). Below doses of 2500 µg/mL, ≥ 80% cell survival was seen under nonactivated and S9-activated conditions. Based on these results, a dose range of 750 to 2500 µg/mL was selected for the mutation assay.

2. Mutation Assay:

a. Nonactivated Test Material: Three nonactivated trials were attempted. The authors stated that the first two trials were aborted due to an "unexpected shift" in cytotoxicity, indicating, in contrast to the preliminary results, that doses ≥ 500 µg/mL were extremely cytotoxic. Accordingly, the third trial was conducted with ten concentrations ranging from 50 to 400 µg/mL.

As shown in Table 1, 53.7% of the cells survived treatment with 240 µg/mL of the test material; doses ≥ 320 µg/mL were reported to be completely cytotoxic. At the lower concentrations, percent survival was dose related and ranged from ≈62.5% at 160 µg/mL to ≈90% at 50 µg/mL. No significantly increased MFs were seen at any nonactivated dose.

b. S9-Activated Test Material:

1. Initial Assay: Eight doses (70 to 350 µg/mL) were assayed in the presence of S9 activation. No cells survived treatment with the two highest concentrations of PDCB (280 and 350 µg/mL). Cell survival following exposure to 210 µg/mL PDCB was ≈36%; for the remaining doses, percent survival ranged from ≈ 80% at 175 µg/mL to >100% at 70 µg/mL. Data presented in Table 2 show that mutant colony counts for the majority of the treatment groups were increased compared to the concurrent negative control values. The increased MFs for

TABLE 1. Representative Results of the Nonactivated CHO Forward Gene Mutation Assay with para-Dichlorobenzene

Substance	Dose (µg/mL)	Relative Percent Survival[a]	Total Mutant Colonies/ 12 Dishes[a]	Percent Cloning Efficiency[a]	Mean Mutation Frequency[a,b] x10^{-6}
Solvent Control					
Dimethylsulfoxide	--	100	17.5	91.3	8.95
Positive Control					
5-Bromo-2'-deoxy-uridine	50	48.5	103	91.5	46.75*
Test Material					
para-Dichlorobenzene	240[c]	53.7	8	97.2	3.4

[a]Average results from duplicate treatment cultures; calculated by our reviewers.

[b]Mutation Frequency = $\frac{\text{Total mutants}}{\text{No. of dishes} \times 2 \times 10^5 \times \text{cloning efficiency}}$

[c]Highest dose cultured for mutant colonies; higher doses (320 and 400 µg/mL) were completely cytotoxic. Results for lower doses (80, 100, 120, 160, and 200 µg/mL) were not significantly different than the control values.

*Significantly higher ($p < 0.05$) than the negative control as determined by the tables of Kastenbaum and Bowman.

TABLE 2. Representative Results of the S9-Activated CHO Forward Gene Mutation Assays with para-Dichlorobenzene

Substance	Dose (μg/mL)	Relative Percent Survival[a]	Total Mutant Colonies/ 12 Dishes[a]	Percent Cloning Efficiency[a]	Mean Mutation Frequency[a,b] x10^{-6}
Solvent Control					
Dimethylsulfoxide	--	100[c]	9	90.8	4.15
	--	100[d]	18	84.7	8.55
Positive Control					
3-Methylcholanthrene	5 μg	91.5[c]	195	89.9	91.05*
		63.5[d]	134	87.1	63.30*
Test Material					
para-Dichlorobenzene	70	119.0[c]	19	95.2	8.55*[e]
	87.5	117.5	11	89.3	4.90
	105	98.6	14	98.3	5.30*[e]
	140	61.0	13	102.8	5.75*[e]
	175	78.8	8	90.0	3.50
	210[f]	36.0	33	80.6	16.60*
	210[g]	78.5[d]	12	85.7	5.90
	280	45.0	10	81.4	4.85
	350	0.4	0	64.2	0.00

[a]Average results from duplicate treatment cultures; calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Total mutants}}{\text{No. of dishes} \times 2 \times 10^{-5} \times \text{cloning efficiency}}$.

[c]Results from the initial assay.

[d]Results from the repeat assay.

[e]MF for one of two replicate cultures was significantly higher than the MF for the negative control.

[f]Higher doses (280 and 350 μg/mL) were completely cytotoxic.

[g]Results for lower doses (70, 87.5, 105 140, and 175 μg/mL) were comparable to the solvent control results.

*Significantly higher ($p < 0.05$) than the negative control as determined by the tables of Kastenbaum and Bowman.

both high-dose replicate cultures were significant ($p < 0.05$) and one of the two duplicate cultures in the 70-, 105-, and 140-μg/mL dose group was also significantly higher than control MFs. It was noted, however, that while percent relative survival was generally dose related, total mutant colony counts and MFs did not show a clear dose-responsive trend. It was further noted that the significantly increased MF for the high-dose group (16.6×10^{-6}) was only slightly higher than the reporting laboratory's acceptable range of background MFs (1×10^{-6} to 15×10^{-6}). Based on these findings, a repeat assay was performed with a range of test material concentrations similar to those used in the initial assay.

2. Repeat Assay: Results of the repeat assay are shown in Table 2. The authors reported the presence of test material precipitate at the two highest dose levels (280 and 350 μg/mL); this observation was not previously mentioned. In contrast to the earlier findings, 0.4 and 45% of the cells survived exposure to 350 and 280 μg/mL PDCB, respectively. In the initial assay, these doses were reported to be completely cytotoxic. Similarly, survival data for the 210-μg/mL dose group did not agree with the earlier results. Below this concentration, survival percentages for both assays were in general agreement and a dose-related response was indicated. Neither total mutant counts nor MFs were increased at any assayed dose level when the S9-activated test was repeated.

Under nonactivated and S9-activated conditions, the positive mutagen controls (50 μg/mL BrdU/-S9 and 5 μg/mL 3-MCA/+S9) induced marked increases in total mutant colonies and significant ($p < 0.05$) increases in the MFs.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

1. The study authors concluded that the test material was nonmutagenic in the CHO/HGPRT forward mutation assay both with and without S9 activation.

2. A quality assurance statement was signed and dated May 1, 1986.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:
We assess that the wide variation in survival data between the preliminary range-finding study and the nonactivated and

S9-activated assays may have been due to inaccurate preparation of dosing solutions. It is possible that concentrations above a critical level (≈200 to 300 μg/mL) or the chemical and/or physical properties of PDCB (e.g., test material insolubility at doses lower than the initially reported 10 mg/mL level) may have made the preparation of accurate dosing solutions difficult. Nevertheless, the test material was assayed up to an acceptable level of cytotoxicity both in the presence and absence of S9 activation. We agree with the authors' conclusion that the significant but not dose-related increases in MFs seen in the initial S9-activated assay probably resulted from normal assay variability rather than a genotoxic effect. In the initial S9-activated assay, the significantly increased MF at 210 μg/mL was accompanied by an increase in total mutant colonies; however, this finding was not reproduced in the repeat assay and the initial MF was within the generally accepted range ($0-2x10^{-5}$)[1] of background MFs for this test system.

In all trials, the sensitivity of the CHO cells to detect a mutagenic response under nonactivated and S9-activated conditions was adequately demonstrated by the significant and marked increase in MFs of cells exposed to either 50 μg/mL BrdU/-S9 or 5 μg/mL 3-MCA/+S9.

We, therefore, concur with the study authors that PDCB is not mutagenic in this test system.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 1.; Appendix B, Protocol, CBI pp. 10-19.

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[1]Hsie, A. W., Casciano, D. A., Couch, D. B., Krahn, D. F., O'Neil, J. P., and Whittenfield, B. L. The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals. Mutat. Res. 86(1981):193-214.

APPENDIX A

Materials and Methods

Paradichlorobenzene toxicology review

Page _____ is not included in this copy.

Pages 118 through 129 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

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EPA: 68D80056
DYNAMAC No. 121-H
December 21, 1988

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PDCB (para-Dichlorobenzene)

Mutagenicity--BALB/3T3 Cell Transformation Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Ira Cecil Felkner for

Date: 12-21-88

130

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Toxicology Branch I (TS-769C)

Signature: M. Ioannou
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Marion Copley, D.V.M.,
D.A.B.T
Acting EPA Section Head,
Section II,
Toxicology Branch I (TS-769C)

Signature: M Copley
Date: 1/30/89

131

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity--BALB/3T3 cell transformation assay.

ACCESSION/MRID NUMBER: 405210-14.

TEST MATERIAL: para-Dichlorobenzene.

SYNONYM(S): PDCB; 1,4-dichlorobenzene.

STUDY NUMBER(S): E-9419.

SPONSOR: Chlorobenzene Producers Association/Bayer AG Institut fuer Toxikologie, West Germany.

TESTING FACILITY: Litton Bionetics, the Netherlands.

TITLE OF REPORT: para-Dichlorobenzene--Evaluation of p-Dichlorobenzene in the *in vitro* Transformation of BALB/3T3 Cell Assay.

AUTHOR(S): den Boer, W. C., and Hoorn, A. J. W.

REPORT ISSUED: June 1986.

CONCLUSIONS - EXECUTIVE SUMMARY: Under the conditions of the BALB/3T3 cell transformation assay, five doses (60, 80, 100, 120, and 140 µg/mL) of para-dichlorobenzene (PDCB) induced a dose-related cytotoxic response ranging from noncytotoxic at the lowest dose to extreme cytotoxicity at the high dose (2.2% cell survival), but did not increase the frequency of morphological transformations at any dose. However the study can not be assessed because of the massive contamination in solvent control cultures; only three were available for comparison.

Classification: The study is unacceptable.

A. MATERIALS:

1. Test Compound: para-Dichlorobenzene (PCDB) from batch No. 922 was described as a white lumpy material with a purity of >99.9%. The test material was stored in the dark at 4°C and a stock solution containing 200 mg/mL was prepared in dimethylsulfoxide (DMSO). The report stated that a 1:200 dilution of the DMSO-stock solution (final concentration = 1 mg/mL) appeared to be soluble in Eagle's Minimum Essential Medium (EMEM) after vigorous sonication and mixing; no change in the pH of the tissue medium was reported.

2. Test Organism: BALB/3T3 (clone I-13) cell line, subclone C-14, obtained from Takeo Kakunaga, National Cancer Institute, Bethesda, MD, was used in this assay. Cells were stored in liquid nitrogen and grown in EMEM supplemented with bovine serum, glutamine, and antibiotics.

3. Cytotoxicity Assay: Cytotoxic effects of the test material on clonal growth were measured by the reduction in the ability of the 3T3 cells to form colonies after treatment with 15 doses of the test material (up to 1500 µg/mL) or the solvent control (DMSO). Triplicate flasks/dose, seeded with 200 cells/flask, were exposed for 3 days to the selected test material doses. Exposed cells were washed, reincubated for 3 to 5 days, stained, and counted. Relative survival was calculated as the ratio of cells surviving treatment to cells in the control group. Based on these results, doses for the transformation assay were chosen to include levels that included a cytotoxic response ranging from 0 to 90% inhibition.

4. Transformation Assay:

   a. Exposure: Prepared cultures, seeded with 3 x 10^4 3T3 cells/flask, were treated with five selected doses of the test material, solvent (DMSO), or the positive control, 2.5 µg/mL 3-methylcholanthrene (3-MCA); 20 replicates were prepared for each treatment group. At

the conclusion of a 72-hour exposure, the cells were washed and refed with growth medium. Throughout the 4-week incubation period, the cells were refed twice weekly with growth medium. Termination of the assay was accomplished by fixing (methanol) and staining (Giemsa) the monolayers and determining the number of transformed foci present in each flask. A parallel cytotoxicity test was included in the assay for the determination of relative survival and the cloning efficiency (CE) of the solvent control.

b. Scoring of Transformed Foci: Foci were scored microscopically as follows:

+++ : Dense mass of cells superimposed on the monolayer that may exhibit random, criss-crossing of fibroblastic cells at the periphery; may also contain a necrotic center and exceed 2 mm in diameter.

++ : As above, maximum diameter < 2 mm.

+ : Focal areas of rounded cells, some overlapping, and no random arrangement of fibroblastic cells.

5. Evaluation Criteria

a. Assay Validity: The assay was considered acceptable if 1) the negative control flasks contained a healthy contiguous monolayer of cells and the background frequency of transformed foci did not exceed 2 to 3 foci/flask; 2) a significant ($p < 0.01$) increase in transformed foci was scored in the positive control cultures; 3) a minimum of 10 flasks from at least 3 dose levels of the test material were available for analysis; and 4) the test material dose range yielded 10 to 100% cell survival.

b. Positive Response: The assay was considered positive if the test material induced a significant ($p < 0.05$) and dose-related increase in transformed foci or if a significant ($p < 0.01$) increase was seen at one or more treatment levels.

6. Statistical Methods: The statistical method used was Bailey's modification of Student's t-test.[1]

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[1] Bailey, N.T.J. 33 Statistical Methods in Biology, Wiley and Sons, Inc., NY, p. 50, 1959.

B. Protocol: See Appendix B.

C. REPORTED RESULTS:

1. Cytotoxicity Assay: Three preliminary cytotoxicity assays were performed. Data from the first two assays were discarded because of questionable results and the study authors' observation that PDCB may have reacted with the plastic culture flasks. The third assay evaluated a series of 9 test material concentrations (125 to 1500 μg/mL) and was performed in glass tissue culture flasks. Compound precipitation was noted in cultures exposed to the five highest test doses (625, 750, 1000, 1250, and 1500 μg/mL). No cells survived exposure to doses ≥ 250 μg/mL; at the lowest assayed dose, 18.4% cell survival was reported. A fourth preliminary cytotoxicity assay was performed with five dose levels of PDCB ranging from 13 to 200 μg/mL. Results from this assay indicated that 200 μg/mL was completely cytotoxic (0% survival); percent survival at the lower levels was ≥ 83%. Based on these findings, five doses (60, 80, 100, 120, and 140 μg/mL) were selected for the transformation assay.

2. Transformation Assay: Three transformations assays were attempted; results from the first two experiments, conducted with plastic culture flasks, were discarded. Results from the third assay, which used glass tissue culture flasks, are presented in Table 1. As shown, survival was dose related and ranged from 2.2% at the highest dose (140 μg/mL) to 96.9% at the lowest dose (60 μg/mL).

   Seventeen of the 20 solvent control flasks were contaminated; test material and positive control results were, therefore, compared to the data derived from three DMSO-treated monolayers. Nondose-related increases in the total number of transformed foci were scored for the 140- and 80-μg/mL treatment groups. However, the total number of foci scored for these groups (23 total foci for 80 μg/mL and 6 total foci for 140 μg/mL) were confined to two of the 20 replicate flasks. Since the number of flasks with foci is considered a more meaningful parameter than the total number of foci, the results did not suggest a positive response.

   Monolayers exposed to the positive control (2.5 μg/mL 3-MCA) had increased frequencies of total foci and average foci/flask; the number of flasks with foci was significantly different from the control ($p < 0.001$).

TABLE 1. Representative Results of the BALB/3T3 Transformation Assay with para-Dichlorobenzene

Substance	Dose (μg/mL)	Percent Relative Survival	Total No. of Foci/ 20 Flasks	Average Foci/Flask	No. of Flasks with Foci[a]
Solvent Control					
Dimethylsulfoxide	--	100	0[b]	0.00	0/3[b]
Positive Control					
3-Methylcholanthrene	2.5	26.9	28	1.40	16/20*
Test Material					
para-Dichlorobenzene	60	96.9	0	0.00	0/20
	80	131.9	23	1.15	2/20
	100	75.9	1	0.05	1/20
	120	44.9	1	0.05	1/20
	140	2.2	6	0.30	2/20

[a]Calculated by our reviewers.

[b]Seventeen of 20 solvent control flasks were contaminated; solvent control results are, therefore, based on the findings from 3 flasks.

*Log_{10} analysis of foci/flask was significantly higher ($p \leq 0.001$) than the solvent control value by Bailey's modification of the Student's t-test.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY CONTROL MEASURES

1. The authors concluded that "The test material, p-dichlorobenzene, batch no. 922, did not induce a statistically significant increase in the number of transformed foci over the concentration range of 60 to 140 μg/mL. Therefore, p-dichlorobenzene, batch no. 922, is considered to be inactive in the BALB/3T3 In Vitro Transformation Assay, according to our evaluation criteria."

2. A quality assurance statement was signed and dated June 5, 1986.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS
We assess that the study cannot be evaluated because of the low number of solvent control flasks (3/20) available for comparison. While we believe that PDCB probably does not induce cell transformation in this cell system, a firm documentation is required with a repeat assay and careful tissue culture practices to avoid such massive contamination.

CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 1; Appendix B, Protocol, CBI p. 10-13.

007021

APPENDIX A

Materials and Methods

Paradichlorobenzene toxicology review

Page _____ is not included in this copy.

Pages 139 through 144 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.

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch I - IRS (TS-769C)

007021

DATA EVALUATION REPORT

I. SUMMARY

TB Project: 8-0774A
(8-0063)
Caswell No.: 632
MRID No.: 405210-07A
Shaugh. No.: 061501

Study Type: Mutagenicity - in vitro cytogenetics (CHO/SCE)

Chemical: P a-dichlorobenzene

Synonyms: PDCB

Sponsor: US EPA; submitted by the Chlorobenzene Producers Association

Testing Facility: Bioassay Systems Corporation (BSC), Woburn, MA

Title of Report: Effects of Para-Dichlorobenzene on the in vitro Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells.

Author: K.S. Loveday

Study Number: BSC Project 11135

Date of Issue: November 9, 1984

TB Conclusions:

Negative for the induction of sister-chromatid exchanges (SCE) in cultured Chinese hamster ovary cells in the presence or absence of metabolic activation at doses into cytotoxic levels.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Para-dichlorobenzene (P-dcb)

Description: Solid
Batch (Lot): 05181-P
Purity (%): 99.7
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)
Source: Standard Chlorine Chemical Company

B. Test Organisms - Mammalian cells in culture (cell line)

Species: Chinese hamster ovary
Strain: (Not stated)
Source: Dr. S. Latt, Children's Hospital Medical Center, Boston, MA

C. Study Design (Protocol) - No formal protocol was presented. A signed and dated quality assurance statement was included in the Final Report, as well as a statement of compliance with FDA GLPs.

D. Procedures/Methods of Analysis - After preliminary cytotoxicity testing, a series of cell cultures was exposed to 2 x 10^{-5} M bromdeoxyuridine (BrdU), followed immediately by addition of the test article, or 1% DMSO (negative solvent control), or mitomycin C (MMC, positive control); all cultures were then incubated for 27 to 30 hours, the final 2 to 3 hours in the presence of vinblastine sulfate (a mitotic arresting agent). To a second set of cultures were added the selected concentrations of test article, or DMSO or cyclophosphamide (CP, as positive control), together with a metabolic activation system consisting of Aroclor 1254-induced liver microsomal enzyme homogenate prepared from young male Sprague-Dawley rats (S9, purchased from Litton Bionetics) plus cofactors (S9 mix). These cultures were incubated for 2 hours, then rinsed free of test substances, and incubated for a further 26 or 30 hours in fresh medium containing BrdU, during the last 2 hours of which the cells were exposed to vinblastine.

The entire assay (with and without S9 mix) was repeated twice.

Following mitotic arrest, all cell cultures were prepared for microscopic examination of sister-chromatid exchanges (SCE) by conventional techniques (staining with the light-sensitive dye, Hoechst 33258, then ultraviolet irradiation for 30 minutes, rinsing in saline sodium citrate, SSC, and counterstaining with Giemsa). Second

division cells were scored for SCE from enlarged photographic prints, and results analyzed by one-way ANOVA, with the level of significance set at $p < 0.05$.

III. RESULTS

In the preliminary range-finding test, cells were exposed for 30 hours to PDCB at five concentrations ranging from 20 to 100 ug/mL (maximum solubility = 79 ug/mL). Although no significant differences in mitotic index from controls (0.016, 0.049) were found at any test dose (range, 0.026 to 0.037), cytotoxicity was recorded at the two highest doses, 80 and 100 ug/mL, as evidenced by cell cycle delay (70 to 80% of cells in first division vs. 0% in controls) (Report Table 1). Therefore, 100 ug/mL was selected as the top dose for the initial nonactivated assay.

Although three complete SCE experiments were performed (with/without S9), one had to be discarded due to what the author described as "data inconsistencies."

In the first nonactivated assay, concentrations of 20, 40, 60, 80, and 100 ug/mL PDCB had no effect on mitotic index (range of test values was 0.023 to 0.048, vs. the control values, 0.026 and 0.034), cell cycle kinetics (90 to 95% of cells were in second division), or SCE (range of 9.6 to 13.1 SCE in test cells vs. 9.6 and 13.1 in controls) (Report Table 2). In the repeat, cells were exposed to 120, 160, and 200 ug/mL. High-dose cultures were not analyzed for SCE because the vast majority of cells (89%) were in first division; SCE frequencies at the two lower doses were 8.0 and 7.5 per cell, respectively, and in the controls, 6.9 and 7.9 per cell (ibid.). Values in positive control (MMC) cultures were 43.4 and 31.1 SCE/cell in the first and second nonactivated assays, respectively, both induced at nontoxic doses (0.03 and 0.06 ug/mL).

In the presence of S9 activation, 40, 80, and 120 ug/mL PDCB induced 19.3, 17.3, and 17.0 SCE/cell with no associated toxicity in terms of either mitotic index or cell cycle delay, compared to 14.4 and 18.9 in activated negative controls (Report Table 3). In cultures treated at 120 ug/mL but sampled at 30 hours (rather than 26 hours) after treatment, SCE frequency was 17.9/cell. Activated cultures treated at higher doses could not be analyzed for SCE; at 160 ug/mL, the majority of cells (72%) were in first division, which indicated moderately severe cell cycle delay, and 200 and 240 ug/mL were lethal. The repeat assay with activation was run at 160 and 200 ug/mL PDCB. No cells were found at the higher dose, and the SCE frequency was 13.6 per cell at a low degree of mitotic delay (35% first division cells), compared to 10.6 per cell in solvent plus S9 controls.

The positive control, CP, significantly increased SCE frequencies in both activated assays, 61.6 per cell in the first, and 78.4 in the second.

Hence, the authors concluded that PDCB did not induce SCE in CHO cells with or without activation.

TB Evaluation:

ACCEPTABLE. The study was well-conducted under acceptable controlled conditions, and the results correctly interpreted by the investigators. The test article is considered negative for inducing SCE in this cell line.

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch I - IRS (TS-769C)

12-6-88
12/7/88

007021

DATA EVALUATION REPORT

I. SUMMARY

TB Project: 8-0774A
(8-0063)
Caswell No.: 632
MRID No.: 405210-07B
Shaugh. No.: 061501

Study Type: Mutagenicity - in vitro cytogenics (CHO/CA)

Chemical: Para-dichlorobenzene

Synonyms: PDCB

Sponsor: US EPA; submitted by the Chlorobenzene Producers Association

Testing Facility: Bioassay Systems Corporation (BSC), Woburn, MA

Title of Report: Effects of Para-Dichlorobenzene on the in vitro Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells.

Author: K.S. Loveday

Study Number: BSC Project 10506

Date of Issue: November 9, 1984

TB Conclusions:

In repeat assays, PDCB was not clastogenic to CHO cells in vitro in the presence or absence of metabolic activation up to cytotoxic levels (300 ug/mL).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - PDCB

Description: Solid at room temperature
Batch (Lot): 05181-P
Purity (%): 99.7
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)
Source: Standard Chlorine Chemical Company

B. Test Organisms - Mammalian cells in culture

Species: Chinese hamster ovary
Strain: (Not stated)
Source: Dr. S. Latt, Children's Hospital Medical School, Boston, MA

C. Study Design (Protocol) - A formal protocol was not presented.

A signed and dated Quality Assurance Statement was included, as well as a statement of compliance with FDA GLPs.

D. Procedures/Methods of Analysis - No preliminary range-finding assay was performed. The investigators selected concentrations for the initial aberration assay based upon the published solubility limit of the test article in water, 79 ug/mL.* Thus, 50 ug/mL was chosen as the highest dose to be tested in both activated and nonactivated assays. When no toxicity was evident in these initial assays, higher concentrations were employed in subsequent experiments, up to 300 ug/mL.

Without activation, duplicate cultures were exposed for 12 hours to graded concentrations of the test substance, or to solvent (DMSO) or culture-medium alone (negative controls), or to the mutagen mitomycin-C (MMC, 5 or 10 ug/mL, as positive control). It was stated that other assays were initiated but were not reported due to "data inconsistencies" (however, see below, under RESULTS).

A metabolic activation system, consisting of Aroclor 1254-stimulated liver microsomal enzyme homogenate from male Sprague-Dawley rats (S9) plus cofactors, was added to other sets of cultures, which were exposed at the

*Support Document Health Effects Test Rule: Chlorinated Benzenes, US EPA. EPA 560/11-80=014, June, 1980, US EPA Assessment Division, Office of Toxic Substances, Washington, DC 20460.

same time in duplicate for 2 hours (preferably) or 4 hours to test substance. After treatment, the test medium was replaced with fresh medium and the cultures incubated a further 3 to 10 hours. Medium alone, S9, and/or DMSO served as negative controls, while cyclophosphamide (CP - 30, 50, 80, or 100 ug/mL) served as positive control in this activated series.

For the last 2 to 3 hours of incubation, all cultures were treated with vinblastine (0.25 ug/mL) or colcemid (1×10^{-6} M) to arrest cells in metaphase, then expanded in hypotonic salt solution, fixed in Carnoy's Fluid (methanol:acetic acid::3:1), and cells dropped onto microscope slides and stained with Giemsa. Metaphases were analyzed from photographic enlargements for chromosome aberrations (breaks, gaps, and other damage) from at least 100 cells (where possible), and mitotic index determined from 500 to 1000 cells.

Chromosome breakage frequencies per cell* were calculated for test cells, and compared to controls by Chi-Square after normalization (per 100 cells).

III. RESULTS

In the first nonactivated assay, break frequencies ranged from 0.023 to 0.067 in cultures exposed to 20, 30, or 50 ug/mL PDCB, compared to negative control values of 0.045 and 0.056 for medium, and up to 0.103 for 1% DMSO (Report Table 1). In the second nonactivated assay, the same PDCB doses caused break frequencies of 0.009 to 0.089, while control values were 0 to 0.020 for medium and 0 to 0.018 for DMSO (ibid.). Since toxicity was not observed in these first two assays, two additional nonactivated tests were run at higher concentrations. In the first of this second series, at 150 and 200 ug/mL PDCB, break frequencies in duplicate cultures were 0.027 to 0.038 and 0 to 0.027, respectively, compared to a background range of 0 to 0.072; while in the second (at 150, 200, 250, and 300 ug/mL), breakage in test cultures ranged from 0.01 to 0.02, compared to control values of 0 to 0.05 (Report Table 2). In neither of these last two tests were mitotic indices affected, although few to no cells survived 300 ug/mL and these cultures could not be analyzed.

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*Chromatid breaks and (terminal) deletions were counted as single breaks, chromosome breaks and interstitial (more complex) deletions as double breaks.

Other than simple breaks (and nonstaining "gaps"), a few more complex aberrations were found equally distributed between PDCB-treated and control cultures (e.g., quadriradials, triradials, double-minutes).

The positive control, mitomycin C, produced significant amounts of damage in all four nonactivated assays, breakage frequencies ranged from 5 to 10 times background values. In addition, MMC-treated cultures had significant increases in cells with quadriradials, triradials and other complex rearrangements, as well as cells with more than 10 aberrations.

When the breakage data were analyzed by Chi-Square (Report Table 5, attached to this DER), the second and fourth assays were significant ($p < 0.05$). The author discounted the importance of these statistically significant results, suggesting that the first (Assay A22), was due to a single high value, while in the second, variation in control frequencies contributed to inflated test values. Further, there was no consistency in response between assays, nor reproducible dose-relationship, and the highest concentrations of PDCB were negative.

PDCB was assayed in three experiments in the presence of rat S9 activation. In the first, concentrations of 20, 30, and 50 ug/mL produced break frequencies ranging from 0.012 to 0.076, compared to negative control (S9-medium) values of 0.051 and 0.073 (Report Table 3). The second activated assay (also Table 3), performed at the same doses, resulted in frequencies ranging from 0.020 (at the HDT, 50 ug/mL) to 0.102 (at the LDT, 20 ug/mL); control values were 0.025 and 0.057 (medium without S9), and 0.016 and 0.080 (medium plus S9). The third assay was conducted at higher PDCB doses (150, 200, 250, and 300 ug/mL), and breakage frequencies ranging from 0 to 0.02 were recorded (Report Table 4), compared to 0 to 0.01 for controls (DMSO, medium plus S9). Substantially decreased mitotic indices (evidence of cytotoxicity) occurred at the HDT (300 ug/mL), 0.008 to 0.010, compared to control values, 0.045 to 0.056. Statistical analysis revealed no significant increases in chromosome aberrations in any ot the three activated assays (Table 5, attached).

In addition to gaps, isolated and nondose-related instances of complex aberrations (triradials and double minutes) were observed in PDCB cultures, comparable to negative controls. Several concentrations of the activated positive control, cyclophospnamide caused consistent increases in breakage: 0.393 and 0.520 in duplicate cultures at 30 ug/mL; 0.151 and 0.122 at 50 ug/mL. At 100 ug/mL CP, a higher incidence of complex aberrations (quadriradials, pulverization, etc.) were found, indicating more severe

damage at the apparent expense of simple breakage (frequency of 0.02 and 0.06).

The author concluded PDCB did not induce increased chromosomal damage in CHO cells, under either nonactivated or activated conditions.

TB Evaluation:

ACCEPTABLE. This study showed a larger variability in control cytogenetic values than generally reported for this cell line* (e.g., for simple breakage per 100 cells, 0.94 to 2.65 without activation and 0.96 to 3.10 with S9 versus, in this study, 0 to 10.3 and 0 to 8.0, respectively). Nevertheless, repeat assays demonstrated clearly that the test compound was not clastogenic in this cell line with or without activation up to cytotoxic levels.

Attachment

*B.H. Margolin, M.A. Resnick, J.Y. Rimpo, P. Archer, S.M. Galloway, A.D. Bloom, E. Zeiger (1986) "Statistical Analyses for In Vitro Cytogenetic Assays Using Chinese Hamster Ovary Cells," Environmental Mutagenesis 8:183-204.

B (CHO/CA)

007021

TABLE 5
CHI-SQUARE TESTS

Nonactivated Assays

Assay A9		Assay A22		Assay 2R		Assay A37	
Conc.[a]	Breaks[b]	Conc.[a]	Breaks[b]	Conc.[a]	Breaks[b]	Conc.[a]	Breaks[b]
50	5.0	50	4.2	250	1	200	0.0
50	6.7	50	8.9	250	2	200	2.7
30	2.8	30	1.0	200	2	150	3.8
30	5.8	30	0.9	200	1	150	4.2
20	4.7	20	4.9	150	1	DMSO	1.9
20	2.2	20	2.7	150	1	DMSO	7.2
DMSO	1.7	DMSO	0.0	DMSO	5	Medium	0.0
DMSO	10.3	DMSO	1.8	DMSO	2	Medium	1.9
Medium	5.6	Medium	0.0	Medium	0		
Medium	4.5	Medium	2.0	Medium	2		
Mean	4.9		2.6		1.7		2.7
Chi^2	11.4		26.0		9.5		14.7
Chi^2 critical (α= 0.05)	15.5		15.5		15.5		12.6
df[c]	8		8		8		6

Activated Assays

Assay A14		Assay A25		Assay 3R	
Conc.[a]	Breaks[b]	Conc.[a]	Breaks[b]	Conc.[a]	Breaks[b]
50	7.6	50	2.0	300	1
50	3.9	50	8.8	300	2
30	7.6	30	6.5	250	0
30	5.9	30	7.3	250	1
20	3.6	20	4.2	200	0
20	1.2	20	10.2	200	1
S9	7.3	S9	1.6	150	0
S9	5.1	S9	8.0	150	0
				DMSO	1
				DMSO	0
				S9	0
				S9	0
Mean	5.3		6.1		0.5
Chi^2	6.9		11.5		10.0
Chi^2 critical (α= 0.05)	12.6		12.6		18.3
df[c]	5		6		10

[a] in ug/ml
[b] per 100 cells
[c] degrees of freedom

Bioassay Systems Corp.
Project No. 10506

154

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch I - IRS (TS-769C)

007021

Judith W. Hauswirth 12/7/88

DATA EVALUATION REPORT

I. SUMMARY

TB Project: 8-0774A
(8-0063)
Caswell No.: 632
MRID No.: 405210-07C
Shaugh. No.: 061501

Study Type: Mutagenicity - in vivo cytogenetics (rat BM/CA)

Chemical: Para-dichlorobenzene

Synonyms: PDCB

Sponsor: US EPA; submitted by the Chlorobenzene Producers Association

Testing Facility: Bioassay Systems Corporation (BSC), Woburn, MA

Title of Report: Effect of Para-Dichlorbenzene on the in vivo Induction of Chromosomal Aberrations in Rat Bone Marrow.

Author: K.S. Loveday

Study Number: BSC Project 10506

Date of Issue: November 9, 1984

TB Conclusions:

Intraperitoneal administration of test material did not cause increased chromosomal aberrations at acute doses up to 800 mg/kg.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Para-dichlorobenzene

Description: Solid at room temperature
Batch (Lot): 05181-P
Purity (%): 99.7
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)
Source: Standard Chlorine Chemical Company

B. Test Organisms - Rodent

Species: Rat
Strain: Sprague-Dawley
Age: 8 to 10 weeks
Weights - Males and Females Combined: 205 to 358 g
Source: Charles River Labs, Wilmington, MA

C. Study Design (Protocol) - No protocol was presented.

A Quality Assurance statement was included in the Final Report, but no statement of compliance with GLPs.

D. Procedures/Methods of Analysis - A preliminary range-finding test was conducted, in which three animals per sex per group were injected once ip with 500, 1000, 1500, 2000, or 2500 mg/kg, and observed for 5 days. For the main assay, single ip doses of PDCB were administered ip once to groups of 3 males and 3 females, which were sacrificed by CO_2 asphyxiation 6, 12, or 24 hours later. Two hours prior to sacrifice, animals were given colchicine (mitotic-arresting agent). Bone marrow was rinsed from femurs, cells expanded with hypotonic saline, and prepared for microscopic examination by routine cytological techniques.

Chromosome aberrations were scored from photographic enlargements of approximately 100 cells in test and control groups. Frequencies of simple breaks as well as more complex aberrations (triradials, quadriradials, translocations, rings, etc.) were recorded, and the former analyzed by Chi-Square as well as the Willcoxon pairwise ("U") Test.

Three groups of 3 males and 3 females each received only an equal volume (5 to 8 mL/kg) of solvent (DMSO) and served as negative control (sacrificed at the same times as PDCB groups), while a positive control group received cyclophosphamide (CP, 3 mg/kg) and was sacrificed 12 hours later.

III. RESULTS

In the preliminary cytotoxicity test, deaths occurred at all doses except the LDT, as follows:

Dose (mg/kg)	Number Died		
	Male	Female	Total
500	0	0	0
1000	1	0	1
1500	0	1	1
2000	3	0	3
2500	0	1	1

From these data, the MTD was estimated to be 1600 mg/kg and a dose schedule of 200, 400, 800, and 1600 mg/kg selected for the cytogenetic assay. However, animals treated at 1600 mg/kg were moribund at the 6-hour harvest, and no cytogenetic data were recorded at this dose, which was eliminated from the remainder of the study.

Frequencies of chromosome breakage in individual animals ranged between 0 and 0.011 per cell at 6 hours (Report Table 2), 0 and 0.009 at 12 hours (Report Table 3), and 0 to 0.017 at 24 hours (Report Table 3), averaging 0.006, 0.003, and 0.002, respectively, for the three sampling times (Report Table 5, attached to this DER). There was no apparent effect of PDBC treatment on mitotic index, no relationship to dose or harvest time, no differences between treated males and females, and statistical analyses (ChiSquare, Willcoxon) revealed no differences in break distribution between test values and DMSO controls, the latter ranged between 0 and 0.018/cell (mean, 0.005).

Gaps were equally distributed between PDCB-treated and DMSO groups, and more complex chromosome aberrations (such as tri- and quadriradials) were not observed in either test animals or controls.

Breakage in bone marrow cells of CP-treated animals sampled 12 hours after treatment ranged between 0.056 and 0.333 (mean, 0.186; i.e., > 90X the mean DMSO value), and a number of triradials and quadriradials were observed.

The authors concluded that PDCB was not a clastogen in male or female Sprague-Dawley rats.

TB Evaluation:

The study was well conducted and adequately controlled according to expert practitioners of this type of study,* and generally conforms to current Agency Test Guidelines.

1. Although the highest dose of PDCB sampled for aberrations (800 mg/kg) showed no evidence of clinical toxicity (none reported), or cytotoxicity (mitotic indices were not altered to any great extent), it was a sufficiently high enough dose close to the limit for that type of study at which death was recorded (1000 mg/kg).

2. Five animals per sex per group per time period are routinely recommended. However, an average of over 100 cells per animal (600/group) were analyzed in PDCB-treated groups, and the incidence of breakage was comparable to DMSO controls at all time periods and treatment levels. In contrast, less than 50 cells per animal were scored in the positive control, which registered a breakage rate 90X the DMSO value.

Attachment

*R.J. Preston et al. (1981): Mammalian in vivo and in vitro cytogenetic assays. A report of the US EPA's GENE-TOX Program. Mutation Res. 87:143-188.

007021

TABLE 5

Summary of Break Frequencies*

Compound	Dose (mg/kg)	Sex	Exposure Time 6	12	24	Avg.
Para-dichloro benzene	800	M	0.000	0.000	0.000	0.000
		F	0.003	0.003	0.003	0.003
		Avg.	0.002	0.002	0.002	0.002
	400	M	0.003	0.003	0.012	0.006
		F	0.000	0.000	0.000	0.000
		Avg.	0.002	0.001	0.007	0.003
	200	M	0.009	0.000	0.009	0.006
		F	0.003	0.003	0.010	0.005
		Avg.	0.006	0.002	0.009	0.006
DMSO		M	0.003	0.009	0.003	0.005
		F	0.009	0.000	0.006	0.006
		Avg.	0.006	0.006	0.004	0.005

* The frequency of breaks per cell is rounded for each time of sacrifice

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Section VI, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch I - IRS (TS-769C)

12-6-88

007021

Judith W. Hauswirth
12/7/88

DATA EVALUATION REPORT

I. SUMMARY

TB Project: 8-0774A
(8-0063)
Caswell No.: 632
MRID No.: 405210-07D
Shaugh. No.: 061501

Study Type: Mutagenicity - forward gene mutation in vitro (CHO/HGPRT)

Chemical: Para-dichlorobenzene

Synonyms: PDCB

Sponsor: US EPA; submitted by the Chlorobenzene Producers Association

Testing Facility: Bioassay Systems Corporation (BSC), Woburn, MA

Title of Report: In Vitro Gene Mutation Assay (HGPRT Locus) in Cultured Chinese Hamster Ovary (CHO) Cells on Para-Dichlorobenzene.

Author: K.S. Loveday

Study Number: BSC Project 10506

Date of Issue: November 9, 1984

TB Conclusions:

The test article did not induce a significant increase in HGPRT mutants in CHO cells exposed in the presence and absence of metabolic activation (and with and without serum) at concentrations up to severely cytotoxic levels (200 to 250 ug/mL).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - Para-dichlorobenzene (PDCB)

Description: Solid at room temperature
Batch (Lot): 05181-P
Purity (%): 99.7
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)
Source: Standard Chlorine Chemical Company

B. Test Organisms - Mammalian Cell Cultures

Species: Chinese hamster ovary
Strain: (Not stated)
Source: Dr. S. Latt, Children's Hospital Medical School, Boston, MA

C. Study Design (Protocol) - No formal protocol was presented.

A Quality Assurance Statement was included in the Final Report, but no statement indicating compliance with GLPs.

D. Procedures/Methods of Analysis - A preliminary range-finding study was performed in both activated (rat S9 from livers of rats treated with Aroclor 1254) and nonactivated cultures. Parallel toxicity assessments were also conducted with the mutation studies.

For the main study, cultures were exposed to test article for 4 hours (medium without serum) and 16 hours (with serum) in nonactivated assays, or for 4 hours in serum-free S9-supplemented medium. After the treatment period, cells were replated to determine toxicity and to allow for mutant expression. After 1 week, the cells were exposed to 6-thioguanine (TG, 2 ug/mL) to select for mutants; as well cloning efficiency was determined in medium without TG. After at least 1 week's selection, the colonies were fixed and stained according to standard cytological techniques.

The culture medium and solvent (DMSO) served as negative controls, and the mutagens ethylmethanesulfonate (EMS, 234 ug/mL) and benzo(a)pyrene (BaP, 10 ug/mL) as positive controls. Each portion of the assay was repeated at least once.

The following parameters were calculated for each treatment:

$$\text{Mutation Frequency (MF)} = \frac{\text{Number of Mutants}}{\text{Number of Selected}}$$

$$\text{Plating Efficiency (PE)} = \frac{\text{Cells x PE}}{\text{Average Number of Colonies}} \Big/ \text{Number of Plated Cells}$$

$$\text{Percent Survivors (PS)} = 100 \times \frac{\text{PE of Sample}}{\text{PE of Negative Control}}$$

Mutation data were statistically analyzed by ANOVA of transformed frequencies, according to methods developed of Snee and Irr (1981) and Snedecor and Cochran (1980).*

III. RESULTS

In range-finding tests, a sharp increase in severe toxicity (< 10% survivors) was found at 200 ug/mL, whereas toxicity was only moderate (25 to 50%) at 150 ug/mL and mild (> 60%) below this concentration (Report Tables 1, 5, and 8). The presence of serum in the cell culture medium (in this case 10% fetal calf serum (FCS) in F10 medium) appeared to inhibit the toxicity of PDCB. In the presence of FCS, 9.2 percent of nonactivated cells survived at 200 ug/mL for 16 hours, whereas without serum, only 0.1 percent survived and only for 4 hours. A comparable difference in toxicity was seen at the HDT, 250 ug/mL, where cell survival was 2.1 percent with serum, and < 0.03 percent without. In activated cultures (without serum, 4-hour exposure), survival was 1.3 percent at 200 ug/mL and < 0.04 percent at the HDT. Since both of these values were less than those observed when serum was present in nonactivated cultures exposed to the same concentrations, the author concluded that unlike the inhibiting effect of FCS, S9 had no effect on survival of CHO cells.

Based on the results of these preliminary tests, up to nine concentrations of PDCB were selected, ranging from 25 to 250 ug/mL, for the three test conditions.

Five experiments were performed without metabolic activation at doses ranging from 25 to 250 ug/mL, three of them in the presence of FCS and with exposure to test

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*Snee, R.D. and J.D. Irr (1981) Design of a statistical method for the analysis of mutagenesis at the hypoxanthine-guanine phosphoribosyl transferase locus of cultured Chinese hamster ovary cells. Mutation Res. 85:77-93.

Snedecor, G.G. and W.G. Cochran (1980) Statistical Methods, 7th Edition, Ames: Iowa State University Press.

compound for 16 hours (+ FCS, 16 hours), and two without serum and with 4 hours exposure (-FCS, 4 hours). As anticipated from range-finding tests, PDCB concentrations ≥ 200 ug/mL were severely toxic and resulted in marginal mutation frequencies calculated from a diminished number of colonies (most often less than one) [see summary tabulation on following page of this DER, extracted from Report Tables 2, 3, 4, 6, and 7]). At lower doses, there was large variability in response between replicates.

None of the test concentrations in the first experiment of the nonactivated series with serum (+ FCS, 16 hours) induced MFs different from negative controls (< 1.3 to 5.0 in duplicate test cultures, vs. background values of < 1.1 and 14.9). In the second experiment of this series, MFs at several test doses were higher than background (namely at 100, 150, 160, and 180 ug/mL--see summary table in this DER); however, the DMSO control values in duplicate flasks were very low (< 1.0 and 1.0), and further, all test values were within the acceptable background range of 0 to 20 mutants per 10^{-6} plated cells.* Of the seven concentrations tested which survived the expression period in the last experiment of this series, several induced MFs slightly higher than the average frequencies of negative controls (25 and 34 mutants/ 10^{-6}), namely 50, 120, and 180 ug/mL, and one dose significantly so, namely 100 ug/mL. In this experiment, the concurrent control values were also slightly higher than the published background, attributed by the investigators to random fluctuation. The author did not consider these increases in some test cultures treatment-related, since no dose response was found and statistical analysis of the combined data of all three experiments in this series revealed no significant difference between test and negative control results (Statistical Analysis, from the Final Report, attached to this DER).

Moderate to severe toxicity was found at PDCB concentrations ≥ 160 ug/mL applied to nonactivated cultures without serum (-FCS, 4 hours, in summary table). While no test dose induced MFs significantly different from negative controls in the first trial of this series, several flasks displayed values higher than DMSO and medium-S9 controls. However, no concentration-dependent response was observed, and analysis of variance on the combined data from both of these experiments yielded a nonsignificant difference ($p > 0.05$).

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*Hsie, A.W, D.A. Casiano, D.B. Couch, D.F. Krahn, J.P. O'Neill, and B.L. Whitfield (1981) The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals. A report of the Gene-Tox program. Mutation Res. 86:193-224.

Effect of PDCB on Induction of HGPRT Mutants in CHO Cells

Dose (ug/mL)	Nonactivated (+FCS, 16 Hours) Rel. Surv. (%)	Nonactivated (+FCS, 16 Hours) MF (x 10^{-6})	Nonactivated (- FCS, 4 Hours) Rel. Surv. (%)	Nonactivated (- FCS, 4 Hours) MF (x 10^{-6})	Activated (-FCS, 4 Hours) Rel. Surv. (%)	Activated (-FCS, 4 Hours) MF (x 10^{-6})
PDCB: 25	68, -, -	2, -, -	-, -	-, -	109, 77	19, < 2
50	86, 106, 106	< 2, 2, 42	104, 87	< 1, 33	95, 22	19, 18
100	43, 119, 112	5, 8, 83	74, 83	2, 38	83, 26	24, 2
120	66, 108, 118	< 1, 2, 37	78, 94	1, 9	71, 42	22, < 2
150	54, 105, 115	< 2, 5, 21	57, 72	3, 61	56, 21	29, 7
160	64, 114, 124	< 2, 6, 25	26, 17	3, 5	69, 7	26, < 2
180	54, 99, 85	5, 6, 64	8, 22	< 1, < 1	24, 2	72, 9
200	53, 46, 19	2, < 1, < 2	(T), 4	(T), < 2	25, 2	38, 12
225	-, 1, (T)	-, < 8, (T)	(T), (T)	(T), (T)	-, -	-, -
250	< 1, (T), (T)	< 2, (T), (T)	(T), (T)	(T), (T)	14, < 2	78, (T)
DMSO (1%)	100, 100, 100	8, 1, 25	100, 100	4, 9	100, 100	34, 3
F10 Media (100%)	89, 133, 147	4, 1, 34	121, 93	8, 31	66, 61	18, 2
EMS: 234	5, 28, 13	746, 822, 2320	68, 66	153, 218	-, -	-, -
B(a)P: 10	-, -, -	-, -, -	-, -	-, -	18, 16	182, 423

Extracted from Tables 2, 3, 4, 6, 7, 9, and 10 of the Final Report (mean values in replicate experiments, calculated by the reviewer, and rounded to the nearest unit).
Rel. Surv., percent survivors relative to DMSO (= 100%).
MF, mutation frequency = Number of mutant colonies per 1 x 10^{-6} plated cells.
-, Not done.
(T), Culture lost to toxicity.

007021

Positive control (EMS) cultures had MFs 24 to 400X DMSO values.

Two trials were conducted with PDCB at the same dose range but in the presence of the S9 activation system. Toxicity was severe at the highest dose tested, 250 ug/mL, and moderate at the two lower doses, 200 and 180 ug/mL. In both activated trials, isolated data points had MFs significantly higher than concurrent DMSO control values (see summary table). These elevations in test cultures were not concentration-related and, together with the higher than historical value in the first activation experiment (34 mutants/10^{-6}), were attributable to random fluctuations inherent in the test system. Statistical analysis of the combined data from both S9 experiments revealed no significant differences (see attachment).

Activated cultures treated with the indirect mutagen, B(a)P, had MFs 5 to 140 times DMSO controls.

The authors concluded that PDCB did not induce concentration-related increases in HGPRT mutations in CHO cells treated under different conditions, namely, long (16-hour) vs. short (4-hour) duration of exposure to compound, with vs. without FCS, and in the absence vs. the presence of metabolic activation. Thus the test article was not mutagenic in this test system under a variety of conditions.

TB Evaluation:

ACCEPTABLE. The CHO/HGPRT test system is known for its large variability in background/spontaneous toxicity and mutability, as well as responses to test agents. The investigators have conducted a comprehensive series of experiments under adequately controlled conditions and we concur with their conclusion that PDCB was not mutagenic at the HGPRT locus.

Attachment

D (HGPRT)

Statistical analysis

Statistical analysis of the mutagenesis assays is based on methods published by Snee and Irr (Mutation Research, 85, 77-93, 1981) and Snedecor and Cochran (Statistical Methods 7th edition, 1980, The Iowa State University Press). The analysis consists of two steps:

1) Data transformation using the power formula:

$$y = (X + 1)^{\lambda}$$

X = observed mutation frequency
y = transformed frequency
λ = 0.15 (constant)

2) Analysis of variance of transformed data using the following formulas:

$$y = \frac{n_i(y_i - y)2}{n_i}$$

y - the pooled mean
y_i - average of transformed frequencies in replicate flasks
n_i - no. of replicate flasks per concentration

$$Var = \frac{n_i(y_i - Y)2}{p-1}$$

Var_B = variance between groups
p = number of concentrations tested including negative controls

$$Var_w = \frac{(n_i - 1)s.d.2}{(n_i - 1)}$$

Var_w = variance within groups
$s.d._w$ = standard deviation of transformed mutation frequencies within each concentration group

Calculated Values

		Nonactivated (+serum)	Nonactivated (-serum)	Activated
F	=	1.11	0.63	0.55
df_1	=	10	7	9
df_2	=	43	18	27
c.v.($a + \alpha = 0.05$)	=	2.055	2.58	2.25

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch I - IRS (TS-769C)

007021

Judith W. Hauswirth
12/11/84

DATA EVALUATION REPORT

I. SUMMARY

TB Project: 8-0774A
(8-0063)
Caswell No.: 632
MRID No.: 405210-07E
Shaugh. No.: 061501

Study Type: Mutagenicity - gene mutations in insects (Drosophila/SLRL)

Chemical: Para-dichlorobenzene

Synonyms: PDCB

Sponsor: US EPA; submitted by the Chlorobenzene Producers Association

Testing Facility: Department of Zoology, University of Wisconsin, Madison, WI [Prepared for Bioassay Systems Corporation (BSC), Woburn, MA, as subcontract No. 416-81 of BSC Project No. 10506]

Title of Report: Drosophila Sex-Linked Recessive Lethal Test on Para-Dichlorobenzene

Author: Ruby Valencia

Study Number: BSC Project 10506

Date of Issue: (Not stated)

TB Conclusions:

Negative for the induction of sex-linked recessive lethals following exposure of Drosophila melanogaster adult males to concentrations of test article up to toxic levels (13,300 ppm/hour).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - PDCB

Description: Crystalline Solid
Batch (Lot): (Not stated; on file with sponsor)
Purity (%): (Not stated; on file with sponsor)

B. Test Organisms - Insect

Species: Drosophila melanogaster
Strain: Canton-S (wild-type) males plus Laboratory stock of mutant females carrying "Basc" (a balanced series of mutations on both X-chromosomes)
Age: Adult
Source: Department of Zoology, University of Wisconsin

C. Study Design (Protocol) - No formal protocol was presented, but a reference list of published literature on this type of study was provided. No statements affirming quality assurance measures or adherence to GLPs were included the Final Report.

D. Procedures/Methods of Analysis - Following range-finding inhalation tests, wild-type male flies were exposed in sealed vials to air only (negative control), or to air containing test article at concentrations of 1000 ppm for 6 hours (total exposure = 6000 ppm.hour) or in two tests approximately 3300 ppm for 4 hours (13,300 ppm.hour). After recovery from the effects of treatment (narcosis, mortality), surviving males were mated singly for 3 days to virgin Basc females (Brood 1), then transferred to new mutant females for 2 days (Brood 2), and to a third group of females for 2 days (Brood 3). F_1 females (carrying one treated and one Basc X-chromosome) were mated to brothers, and the resulting F_2 populations examined for the presence (nonlethal) or absence (lethal) of wild-type flies. 1,2-Dibromethane (DBE, 60 and 125 ppm.hour) served as positive control.

The criteria for assessing an acceptable assay as well as a positive result were as follows: In order to detect an induced frequency of 0.2 percent or less given the control frequencies previously observed by the testing laboratory (which was a mean of 0.107 in 9 experiments, ranging from 0.058 to 0.154), at least 7000 chromosomes must be tested. Recessive lethal data are then analyzed by applying the Kastenbaum and Bowman

tables.* The probability of differentiating multiple mutations due to independent (nonidentical) events in postmeiotic germ cells, from clusters derived from a spermatogonial (premeiotic) mutation giving rise to identical recessives, is based upon methods developed by Owen (1962) and Margolin (1984).** When identified, clusters are subtracted from the recessive lethal data by this investigator (none were found in this study).

III. RESULTS

The effect of PDCB on the induction of sex-linked recessive lethals is summarized on the following page (derived from Report Tables 1 through 6).

More P_1 males treated at the higher dosages fell into a narcotic state lasting 4 to 5 hours, and/or died. No indication was found for increased mutation due to PDCB treatment at either dose level in any postmeiotic germ cell stage. In contrast, even low exposure to DBE produced elevated mutation frequencies 10- to 15-fold higher than concurrent controls, values consistent with previous rates found in this laboratory (mean of seven experiments, 1.80, with a range of 0.821 to 2.658).

The author concluded that PDCB did not induce sex-linked recessive lethal mutations in Drosophila postmeiotic germ cells when given to adult males by inhalation up to toxic levels.

TB Evaluations:

ACCEPTABLE. This assay was conducted according to metnodology dating back to the classical mutagenicity experiments of Hans Muller (1927, 1929) and Charlotte Auerbach (1940, 1942). Sufficient numbers of chromosomes to detect a positive result were tested for lethals, and appropriate controls were run in parallel, to render as valid the negative result for the test material, PDCB.

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*Kastenbaum, A. M. and K.O. Bowman (1970) Tables for determining the statistical significance of mutation frequencies. Mut. Res. 9:527-549.

**Owen, D.B. (1962) Handbook of Statistical Tables (Addison-Wesley Publishing Company) pages 259-261.

Margolin, B. (Personal communication) Manuscript in preparation outlining the statistical methods used in the Mutagenesis Testing Program (National Toxicology Program).

007021

Effect of Inhaled PDCB in Drosophila melanogaster
(Sex-Linked Recessive Lethal Test)*

Exposure (ppm.hr) (Initial Concentration x Duration)	Number Males Treated	Percent Mortality (Premating)	Number Males Mated	Percent Sterile Males (Total)	Number Lethals: Per Brood 1	2	3	Total Tests	Percent Lethals
0 (air)	134	0	105	18	7	2	2	9190	0.120
PDCB: 6000 (1000 x 6)	58	3.4	35	15	1	0	1	3184	0.063
13,300 (3320 x 4)	73	23.0	56	6	1	1	0	5177	0.039
13,200 (3300 x 4)	45	4.0	43	5	1	0	0	4113	0.024
DBE: 125 (240 x 1/2)	30	0	30	30	0	28	9	2273	1.628
60 (240 x 1/2)	30	0	20	0	1	10	13	1836	1.307

*Extracted from Tables 1 through 6 of the Final Report.

IMD

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Reviewed by: Y.M. Ioannou 2/3/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M.P. Copley 2/3/89
Section II, Toxicology Branch I (IRS)

007021

DATA EVALUATION REPORT

Study Type: Mutagenicity (Salmonella/Mammalian Microsome Assay)

TOX Chem No.: 632
MRID No.: 405680-02

Test Material: Paradichlorobenzene

Synonyms: 1,4-Dichlorobenzene

Study Nos.: Unknown

Testing Facility: Case Western University
Department of Pediatrics
Cleveland, OH

Title of Report: Salmonella Mutagenicity Test Results for 250 Chemicals.

Author(s): S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, and E. Zeiger

Report Issued: 1983

Conclusions:

The mutagenic activity of p-DCB (and 249 other chemicals) were investigated in Salmonella strains TA100, TA98, TA 1535, and TA1537 at dose levels ranging from 1 to 100 ug/plate, with or without metabolic activation.

Results presented by the authors indicate that p-DCB is negative for mutagenicity under the conditions employed.

This study (published in "Environmental Mutagenesis Supplement" 1, 1983) appears to be adequate and can be used as supportive data in evaluating p-DCB mutagenicity.

171

007021

Reviewed by: Y.M. Ioannou YMI 2/2/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M.P. Copley MPC 2/3/89
Section II, Toxicology Branch I (IRS)

DATA EVALUATION REPORT

Study Type: Mutagenicity (Salmonella/Mammalian Microsome Assay)

TOX Chem No.: 632
MRID No.: 405680-03

Test Material: Para-Dichlorobenzene

Synonyms: 1,4-Dichlorobenzene

Testing Facility: University of Kyorin
Department of Environmental Health
Tokyo, Japan

Title of Report: Structural Specificity of Aromatic Compounds with Special Reference to Mutagenic Activity in *Salmonella typhimurium* - A Series of Chloro- or Fluoronitrobenzene derivatives.

Author(s): M. Shimizu, Y. Yasui, and N. Matsumoto

Report Issued: August 1982

Conclusions:

The mutagenic activity of para-dichlorobenzene (p-DCB) (along with another 29 structurally related chemicals) was investigated in Salmonella strains TA98, TA100, TA1538, TA1537, and TA1535 with or without metabolic activation, at dose levels ranging from 51.2 to 13015.2 ug/plate.

Results indicated that p-DCB was negative for mutagenicity under the conditions of the study.

This study (published in "Mutation Research, 116, 1983)" appears to be adequate and can be used as supportive data in evaluating p-DCB mutagenicity.

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Reviewed By: Y.M. Ioannou Y.M.I. 1/19/89
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: A.B. Kocialski for ABK 1/30/89
Section VII, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Teratology (Rat) (83-3) TOX Chem No.: 632

Test Material: Paradichlorobenzene MRID No.: 405210-02

Synonyms: 1,4-Dichlorobenzene

Study Number: CTL/P/340

Sponsor: Chlorobenze Producers Association

Testing Facility: Imperial Chemical Industries
Alderly Park, Cheshire, UK

Title of Report: Paradichlorobenzene - Teratogenicity Study in Rats

Author(s): M.C.E. Hodge; S. Palmer; I.P. Bennett

Report Issued: July 27, 1977

Conclusions:

Based on the available data, paradichlorobenzene (pDCB) technical is not considered teratogenic in specific pathogen-free (SPF) rats when administered via the inhalation route at dose levels of 75, 200, or 500 ppm on days 6 through 15 of gestation. The NOEL for maternal toxicity (or developmental toxicity) was not established and is considered to be greater than 500 ppm, the HDT.

Classification: Core-Supplementary

Materials and Methods:

Virgin female rats, of the SPF strain (from Alderley Park), weighing approximately 230 g were used in this study. The animals were housed in cages divided into two compartments, two rats per compartment and each rat weighed and ear-marked. The rats were mated overnight to males of the same strain. A successful mating was determined the following morning by the presence of sperm in vaginal smears. Four batches of 32 animals each, were mated, randomly allocated to four experimental groups and subsequently the pregnant females were exposed through the inhalation route to pDCB (with purity of greater than 99%) at dose levels shown below (the authors did not specify the basis for the selection of the dose levels tested in this study).

Group	Atmospheric Concentration of pDCB (ppm) Theoretical	Actual	Number of Pregnant Rats	Mean Body Weight (g) at Day 0
1. Control	0	0	24	227 ± 40.3
2. pDCB	75	74.7	20	240 ± 45.6
3. pDCB	200	198.6	20	225 ± 39.4
4. pDCB	500	508.4	22	223 ± 38.7

Food (Alderley Park rat cubes) and water were available to all animals ad libitum except during dosing.

Generation of pDCB inhalable particles was achieved by blowing warm air through a jacketed vessel containing warmed pDCB crystals. The air (from the crystals) was then passed (with the main airstream) into the inhalation chamber. Chamber pDCB concentrations were regulated by varying the temperature of the crystals (between room temperature and 55 °C) and/or the airflow over the crystals. Concentrations in the exposure chamber were sampled and analyzed twice daily (on 5 of 10 exposure days/group) using a Miran 1A infrared gas analyzer.

All groups of animals were exposed to their respective pDCB concentrations for 6 hours per day from days 6 through 15 of pregnancy, inclusive, by suspending the cages over trays lined with absorbent paper inside the inhalation chamber. Body weights were recorded on days 0, 6, 10, 16, and 21 of pregnancy. All animals were observed daily throughout the study for abnormalities.

All animals were killed by cervical dislocation on day 21 of study, their abdomen dissected and the intact uterus removed and examined for the number of live and dead fetuses and early and late resorptions. Corpora lutea were identified and counted in each ovary. For any abnormal fetus, the maternal tissues, including heart, lung, liver, kidney, adrenal, ovary, and uterus, were preserved in formol corrosive and examined histologically. The lung and liver from 10 animals from each treatment group were also subjected to histological examination. Macroscopic examination of maternal tissues was done upon removal of the fetuses. Only 20 pregnant animals were dissected from each group. Fetuses from each litter were labeled (based on their position in the uterus), their viability determined, examined for sex identification, weighed, and examined for external abnormalities. Half of the fetuses from each litter were fixed in 70% methanol and examined for visceral abnormalities. The same fetuses were stained with Alizarin Red and subsequently examined for skeletal malformations and degree of ossification. The remaining fetuses from each litter were decalcified in Bouin's fixative (for at least 10 days) prior to examination.

Statistical Analysis (Abstracted from the Original Report):

When assessing results, data from the test groups were compared statistically with data from the control group. Student's t-test was used for analyzing maternal body weight and litter data. Mean fetal weight per litter was used in the calculation of the group mean. Incidences of skeletal variations and soft tissue abnormalities were calculated from total numbers of appropriately processed fetuses in each group. The results were analyzed using 2 x 2 Contingency Tables if there were indications of adverse effects compared with controls (Finney et al. 1963). Litter distribution was considered in cases of difficulty of interpretation.

Results:

The authors presented data showing that the actual pDCB concentrations in the inhalation chamber were closely comparable to the target concentrations (target concentrations: 75, 200, and 500 ppm for the low-, mid-, and high-dose levels, respectively; actual concentrations: 75, 199, and 508 ppm for the low-, mid-, and high-dose levels, respectively).

Mortality and Clinical Observations:

According to the authors, no mortality or adverse reactions were observed throughout this study that could be attributed to the test chemical. A number of females (2 from the low-dose, 2

from the mid-dose, and 5 from the high-dose groups) littered on day 21 (just prior to terminal sacrifice); these animals were excluded from the results.

Maternal Body Weights:

Mean body weights were comparable between the four groups throughout the study. Mean body weight gains were also approximately similar between groups between day 0 to day 21. Feed consumption was not measured in this study.

Pathology:

No histological lessions that could be attributed to the test substance were seen in any of the females on test. Similarly, macroscopical lessions were limited and did not appear to be of any significance for this study.

Reproductive Parameters and Litter Data:

As shown in Table 1, all the measured maternal reproductive parameters and litter data appear to be comparable between the control and the treated groups.

Fetal Alteration Data:

The authors reported that the only external abnormalities seen included: one fetus of the low-dose group with gastroschisis and malrotation of the left hindlimb; one fetus of the mid-dose group with gastroschisis and malrotation of the right hindlimb; and one fetus of the high-dose group with agnathia and cleft palate. The low incidence of these abnormalities indicates random rather than compound-related effects. Similarly, there was no statistically significant difference between pDCB-treated and control groups as far as soft tissue anomalies or skeletal anomalies (skeletal variations and/or malformations) were concerned.

Table 1

Summary of Maternal Reproductive Parameters and Litter Data

Parameter	Dose (ppm)			
	0	75	200	500
. of Female Rats Mated	32	32	32	32
. of Pregnant Rats	24	20	20	22
. of Females with Viable Fetuses	24	20	20	22
. of Corpora Lutea/Litter	11.5 ± 2.0	12.7 ± 2.0	11.4 ± 2.2	12.3 ± 2.0
. of Implantations/Litter	10.9 ± 3.0	11.6 ± 3.3	10.7 ± 2.8	11.8 ± 2.6
. of Live Fetuses/Litter	10.2 ± 3.0	10.6 ± 4.0	10.2 ± 2.7	10.7 ± 2.3
tal No. of Viable Fetuses	245	212	203	236
. of Early Resorptions	16	8	11	22
. of Late Resportions	1	11	0	1
. of Male Fetuses	110	116	105	117
. of Female Fetuses	135	96	98	119
x Ratio (Males/Females)	0.81	1.21	1.09	0.98
an Fetal Body Weight (g)	5.2 ± 0.5	5.2 ± 0.3	5.2 ± 0.3	5.1 ± 0.3
an Litter Weight (g)	51.7 ± 14.2	54.2 ± 20.4	52.3 ± 13.3	54.8 ± 11.0

007021

Discussion:

The present study has investigated the possible teratogenic potential of pDCB technical in rats, via the inhalation route. Results presented here indicated that the actual pDCB concentrations in the inhalation chamber were approximately the same as the target concentrations of 75, 200, and 500 ppm for the low-, mid-, and high-dose groups, respectively.

Based on the available data, it can be stated that:

1. No mortality was observed in any of the treated female rats.
2. No clinical signs of toxicity were reported for any treatment group.
3. No differences in mean body weight and/or mean body weight gains were seen between the treated and control groups.
4. No macroscropical or microscopical changes attributable to the test chemical were observed.
5. Maternal reproductive parameters were comparable between the treated and control groups.
6. Litter data did not reveal significant differences between the treated and control groups.
7. No differences were observed between the treated and control groups as far as fetal alterations (external anomalies, soft tissue anomalies, or skeletal anomalies) were concerned.

These findings suggest that the high dose of pDCB tested (500 ppm) was not high enough to be considered as the MTD for this study. As a result, the LEL and the NOEL were not established for this study and are considered to be greater than 500 ppm, the highest dose level tested.

Conclusions:

Based on the available data reported here, pDCB technical is not teratogenic when administered to SPF rats, via the inhalation route, on days 6 through 15 of gestation at the dose levels of 75, 200, or 500 ppm. The NOEL for maternal toxicity was found to be greater than the high dose tested (500 ppm). Similarly, pDCB technical did not cause any developmental toxicity indicating that the NOEL for developmental toxicity was higher than 500 ppm, the HDT.

Classification: Core-Supplementary

50822:I:Ioannou:HED-04:KENCO:7/15/88:rw:VO:EK:CS:EK:CB

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007021

DATA EVALUATION REPORT

Study Type: Teratology (Rabbit) (83-3) TOX Chem No.: 632

Test Material: Paradichlorobenzene MRID No.: 405680-01

Synonyms: 1,4-Dichlorobenzene

Study Number: K-1323-(12)

Sponsor: Chlorobenzene Producers Association

Testing Facility: Dow Chemical U.S.A., Midland, MI

Title of Report: Paradichlorobenzene - Inhalation Teratology Study in Rabbits

Author(s): Hayes, W.C.; Gushow, T.S.; John, J.A.

Report Issued: September 15, 1982

Conclusions:

Exposure of New Zealand White rabbits via the inhalation route to paradichlorobenzene (pDCB) Technical at dose levels of 0, 100, 300, or 800 ppm (days 6 through 18 of gestation) does not result in any teratogenic effect. The LEL for maternal toxicity (depression of body weight gain) was 800 ppm (HDT) while the NOEL was 300 ppm. The LEL for developmental toxicity (retroesophageal right subclavian artery) was 800 ppm and the NOEL 300 ppm.

Classification: Core-Minimum

Materials and Methods:

New Zealand White virgin female rabbits (obtained from Longshaws Rabbitry, Augusta, MI), weighing between 3.5 and 4.5 kg, were used for this study. The animals were housed in wire bottomed cages and kept in a room where the temperature was maintained at 22 °C, the relative humidity at 50 percent, and a light/dark cycle of 12 hours. The animals were uniquely identified by metal ear tags, and provided with food (Certified Laboratory Animal Chow) and water ad libitum except during the inhalation exposure. Prior to the initiation of the study the animals were acclimated to laboratory conditions for at least 3 weeks. The rabbits were artificially inseminated with spermatozoa from males of the same strain and source as the females. The day of insemination was considered as day 0 of gestation.

Four groups of 29 to 30 animals each were artificially inseminated and subsequently the pregnant rabbits were exposed to the test chemical pDCB, with a purity of 99.9 percent, through the inhalation route at dose levels shown below (Table 1):

Table 1

Group	Atmospheric Concentration of pDCB (ppm)		Number of Mated Rabbits	Number of Pregnant Rabbits	Mean Body Weight (g) on Gestation Day 6
	Theoretical	Analytical			
	0	0	30	29	3736 ± 277
	100	103	30	24	3690 ± 337
	300	306	29	25	3882 ± 321
	800	791	30	28	3841 ± 323

Exposure of pregnant rabbits to pDCB was carried out in a 4.3 cubic meter Rochester-type stainless steel and glass chambers under a dynamic airflow of approximately 700L air/minute. The temperature and humidity of the chambers were maintained at 21 °C and [illegible] percent, respectively, and were recorded daily. pDCB was vaporized by passing warm air through glass tubes packed with small pieces of pDCB (solid at ambient temperature). The vaporized pDCB was swept from the tubes into the air inlet ducts of the chambers where dilution to the derived concentration could be achieved. Concentrations in the exposure chambers were determined hourly using a Varian Aerograph gas chromatograph with a hydrogen flame ionization detector at 192 °C. Analytical concentration of pDCB in the exposure chamber was obtained by extrapolation from a standard curve derived from vapor standards of known concentrations. Standard curves were prepared prior to the first exposure and

biweekly thereafter. Nominal concentrations (ratio of pDCB amount to total amount of air through the chamber) were calculated daily.

Pregnant rabbits of each group were exposed to their pDCB concentrations for 6 hours daily from days 6 through 18 of gestation. Control animals were exposed to filtered air in an identical chamber. Body weights for all animals on study were recorded on days 6, 9, 12, 15, 19, and 29 of gestation. All animals were sacrificed by carbon dioxide asphyxiation and the maternal livers and kidneys were removed, weighed, and sections were preserved in buffered 10% formalin for histologic examination.

Fetal Observations - At sacrifice, the uterine horns were exteriorized and the following data recorded: 1) number and position of fetuses in utero; 2) number of live and dead fetuses; 3) number and position of resorption sites; 4) number of corpora lutea; 5) sex, body weight, and crown-rump length of each fetus; and 6) any gross external alterations. One-half of the fetuses of each litter was dissected immediately and examined for soft tissue alterations. All fetuses were preserved in alcohol, eviscerated, cleared and stained with alizarin red-S and examined for skeletal alterations.

Statistical Evaluation - (Abstracted From the Original Report)

"Statistical evaluation of the frequency of alterations and of resportions among litters and the fetal population was made by the Wilcoxon test as modified by Haseman and Hoel (1974). Analysis of the percentage of pregnancy and other incidence data was made by the Fisher exact probability test (Siegel, 1956). The fetal sex ratio was analyzed by a binomial distribution test (Steel and Torrie, 1960). Analysis of other data was done by parametric or nonparametric analysis of variance followed by either Dunnett's test or Wilcoxon's test with a Bonferroni correction (Miller, 1966). The reported level of statistical significance was $\alpha = 0.05$."

Results:

As shown in Table 2, the mean daily time-weighted average (TWA) analytical chamber concentrations of pDCB were approximately similar to the target concentrations of 100, 300, and 800 ppm. However, the range of individual values varied greatly especially with the mid- and high-dose levels as shown in Table 2. Also, although the mean daily TWA analytical concentrations and the mean daily nominal concentrations appear to be comparable, the range for individual values (nominal concentration) again is considerably variable for all dose levels.

Table 2

Target Concentration (ppm)	Analytical Concentration			Nominal Concentration	
	Mean ± SD[1] (ppm)	Range (ppm)	Coef. of Variation (%)	Mean + SD (ppm)	Range (ppm)
0	--	--	--	--	--
100	103 ± 5	92 - 116	5	110 ± 21	76 - 153
300	306 ± 25	237 - 349	8	322 ± 44	211 - 400
800	791 + 71	537 - 949	9	751 + 69	552 - 839

[1]Mean ± SD of daily TWA values for N = 29 days.

Maternal Observations - The only mortality reported throughout this study was a single pregnant rabbit of the mid-dose exposure group (300 ppm) which died on day 10 of gestation (after 4 daily exposures to pDCB). Based on the pathological examination, the authors concluded that the death of this dam was not compound-related. No clinical signs of toxicity were reported in any of the other animals on study. One dam of the control group delivered on day 24 of gestation. Body weight measurements revealed that rabbits exposed to the high dose level (800 ppm) between days 6 and 8 on gestation had a statistically significantly lower body weight gain than the corresponding control group. The high-dose group animals gained significantly less weight than controls (185 versus 28 g for controls and high-dose group, respectively) between days 6 and 18 of gestation (during the exposure period). However, once exposure was terminated the dams of the high-dose group had statistically significantly higher body weight gains than controls. Body weights and body weight gains for the low- and mid-dose groups were comparable to controls throughout the study. The absolute and relative mean weights of the liver or kidneys in the pDCB-treated groups were comparable to those of the control group.

Reproductive Parameters and Litter Data - As shown in Table 3 (abstracted in its entirety from the original report - page 19) most of the major parameters measured appear to be comparable between the pDCB-exposed groups and the concurrent control group. The percent implantations resorbed and the percent litters with resorptions were found to be statistically significantly higher in the 300 ppm exposure group compared to the control group. The percent litters with resorptions was also higher than controls in the 100 and 800 ppm dose groups but not statistically significantly higher (Table 3). The authors cited historical control data (22 studies conducted at the same laboratory) for the same strain of rabbits indicating that the percent litters with resorptions ranged from 0 to 70 percent with a mean of 40 percent.

Fetal Alteration Data - A number of major external alterations were observed in all treated groups and the control group. These alterations included: arthrogryposis, spina bifida, acephaly, and omphalocele. However, as shown in Table 4, the incidence of these alterations was not significantly different between the pDCB exposed and the control groups. Similarly, the incidence of major soft tissue alterations was comparable between the treated and control groups. Two minor alterations, pale liver, and retroesophageal right subclavian were found to be of statistically significantly higher incidence in the 300 and the 800 ppm groups, respectively, compared to the controls. These alterations are shown in Table 4 for the record. Skeletal alterations were also variable but in general, there was no significant difference between the incidence of these alterations in the treated as compared to the control groups. Some of these alterations appear in Table 4 (for the record).

Discussion:

The present study has investigated the possible teratogenic potential of pDCB in rabbits, via the inhalation route. The selection of the three exposure levels, 100, 300, and 800 ppm was based on a range-finding study whereby groups of 7 pregnant rabbits were exposed to pDCB concentrations of 300, 600, or 1000 ppm for 6 hours daily on gestation days 6 through 18. The results of this preliminary study, according to the authors, indicated a dose-related decrease in body weight gain, and a slight decrease in absolute and relative liver weights in the 1000 ppm group, compared to controls.

Although individual values for pDCB analytical concentrations in the exposure chamber showed a great variation, mean analytical concentrations were identical to the target concentrations of 100, 300, or 800 ppm. These results suggest that the overall exposure of pregnant rabbits to pDCB during days 6 through 18 of gestation was at acceptable concentration levels.

Data presented here indicate that the high dose tested can be considered as the MTD based on the statistically significant depression of maternal body weight gain on days 6 through 8 of gestation (after only two 6-hour exposure periods). Throughout the exposure period (days 6 to 18 of gestation) body weight gain of the high-dose group was considerably lower than the control group (28 versus 185 g for the high dose and control groups, respectively). The lack of data on food consumption does not allow us to determine whether the lower body weight gain was due to the effect of the chemical on curbing the appetite of the animals or was due to some other systemic effect. Based on the reported evidence however, we consider the HDT (800 ppm) as the LEL for maternal toxicity (body weight gain depression) and the MDT (300 ppm) as the NOEL.

TABLE 3

007021

PARADICHLOROBENZENE - INHALATION TERATOLOGY STUDY IN RABBITS

Observations Made at the Time of Cesarean Section of Rabbits[a]

	PARADICHLOROBENZENE, ppm			
	0	100	300	800
Number of bred females	30	30	29	30
Number of maternal deaths	0	0	1	0
Percent pregnant[b]	97(29/30)	80(24/30)	86(25/29)	93(28/30)
Pregnancies detected by stain[c]	0/1	1/6	1/4	0/2
Percent pregnant, total[d]	97(29/30)	83(25/30)	90(26/29)	93(28/30)
Number of litters	28	24	24	28
Litters totally resorbed[e]	0	1	2	0
Corpora lutea/dam[f]	9±2	10±2	10±2	9±2
Implantation sites/dam[f]	8±2	8±3	9±2	8±3
% Pre-implantation loss[g]	14±20	14±23	12±12	11±18
Fetuses/litter[f]	8±2	7±3	7±3	8±2
Resorptions/litter[f]	0.5±1.0	0.8±0.9	1.4±1.8	0.5±0.7
% Implantations resorbed[e]	7(15/225)	10(19/195)	16(33/208)*	6(15/233)
% Litters with resorptions[e]	29(8/28)	54(13/24)	63(15/24)*	39(11/28)
Resorptions/litters[e] with resorptions	1.9(15/8)	1.5(19/13)	2.2(33/15)	1.4(15/11)
% Dead fetuses	0/210	0/176	0/175	0/218
Sex ratio, M:F	56:44	56:44	56:44	54:46
Fetal body weight, g[h]	37.94±6.56	37.06±7.48	38.57±5.59	37.01±4.39
Fetal crown-rump length, mm[h]	93.73±4.81	92.90±5.84	94.27±4.77	92.80±4.49

[a]Inseminated rabbits were exposed to 0, 100, 300, or 800 ppm of paradichlorobenzene for 6 hours/day on days 6 through 18 of gestation.

[b]Number of females with visible implantation sites at the time of cesarean section or necropsy/total number bred females.

[c]Number of females with implantation sites detected only after staining the uterus with sodium sulfide/total number stained.

[d]Number of females with implantation sites as observed either visually at the time of cesarean section or necropsy, or after staining the uterus with sodium sulfide/total number of bred females.

[e]Resorptions which were detected only after sodium sulfide staining were not included in these calculations.

[f]Mean ± S.D.

[g]Percent per litter, mean ± S.D.

[h]Mean of litter means ± S.D.

*Value was significantly different from the control value by a modified Wilcoxon test.

See Table A-3 for individual animal data.

185

Table 4
Incidence of Fetal Alterations

Alterations	Litters With Specific Alteration (%) Dose (ppm)			
	0	100	300	800
External Alterations				
Arthrogryposis	0	4	5	7
Spina bifida	4	0	0	0
Acephaly	0	0	0	4
Omphalocele	0	0	0	7
Soft Tissue Alterations				
Liver - pale	0	0	18*	4
Right subclavian - retroesophageal	4	0	5	18*
Dilated cerebral ventricles	0	0	0	4
Skeletal Alterations				
Ribs - fused	0	4	5	4
- calloused	4	9	5	4
- extra rib fused to tenth rib	0	0	0	4
- shortened	0	0	0	4
Sternebrae - shortened long bones	0	0	0	4

*Statistically significantly different from the control value by a modified Wilcoxon test $p \leq 0.05$.

Litter data suggest that the exposure of pregnant rabbits to pDCB results in a higher percentage of litters with resorptions in all dose groups compared to controls. However, the lack of statistical significance in the high-dose group coupled with the lack of an obvious dose-response, make these findings of no apparent toxicological importance. Historical control data cited by the authors (but not included in this report) also suggest that the values for the percent litters with resorptions in the dose groups are within the historical control range (0 to 70%).

As far as it could be determined from the reported data, there were no major differences between the pDCB exposed groups and the control group as far as the incidence of major malformations

(i.e., external alterations, soft-tissue alterations or skeletal anomalies) is concerned. Although, a statistically significant increase in the incidence of retroesophageal right subclavian artery was observed in the high dose group litters, this finding is not considered to be a teratogenic effect because it is a minor variant and is not life threatening. However, this alteration probably represents a developmental effect and thus, the high dose tested (800 ppm) is considered to be the LEL for developmental toxicity.

Conclusions:

Based on the evaluation of the available data, pDCB Technical is not considered to be teratogenic to New Zealand White rabbits when administered via the inhalation route on days 6 through 18 of gestation at exposure levels of 0, 100, 300, or 800 ppm. The LEL for maternal toxicity (depression in body weight gain) was considered to be the HDT (800 ppm) while the NOEL was the MDT (300 ppm). The LEL for developmental toxicity (retroesophageal right subclavian artery) was 800 ppm and the NOEL 300 ppm.

Classification: Core-Minimum.

50824:I/Draft:Ioannou:C.Disk:KENCO:7/26/88:SG:vo:rw

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DATA EVALUATION REPORT

Study Type: Long-Term Inhalation (Rat) (83-1) TOX Chem No.: 632

Test Material: Paradichlorobenzene (pDCB) MRID No.: 405680-08

Synonym: 1,4-Dichlorobenzene

Study No : CTL/P/447

Sponsor: Chlorobenzene Producers Association

Testing Facility: Imperial Chemical Industries
Alderly Park
Cheshire, UK

Title of Report: Paradichlorobenzene: Long-Term Inhalation Study in the Rat

Authors: R.A. Riley, I.S. Chart, A. Doss, C.W. Gore, D. Patton, T.M. Weight

Report Issued: August 8, 1980

Conclusions: The present study has investigated the chronic toxicity and oncogenicity of pDCB to male and female Wistar rats via the inhalation route at dose levels of 0, , and 500 ppm. Data presented here are insufficient for a complete evaluation of the study. Additional data are required.

Classification: Core-Supplementary

Materials and Methods:

pDCB, a white crystalline solid with a reported purity of greater than 99.8 percent, was used in this study. Male and female SPF albino rats of the Alderly Park Wister strain (supplied by the Animal Breeding Unit, Alderly Park, Cheshire, UK) weighing between 90 and 110 g were used throughout this study. The animals were housed in stainless steel cages with a removable food hopper and water bottle. Food and water were available ad libitum but withheld during the 5-hour exposure period each day.

Study Design:

A total of 234 male and 234 female rats were assigned to 3 groups/sex and exposed to the following pDCB concentrations:

Test Group	Dose (ppm)	Number of Rats/Group Male	Female
1. Control	0	78	79
2. pDCB	75	79	79
3. pDCB	500	77	76

Animal cages (containing 9 to 10 animals/cage) were placed in stainless steel exposure chambers (8 cages/chamber) and kept there until termination of the study. The animals were acclimated to the chamber conditions for at least 5 weeks prior to the initiation of the study. The chamber temperature and relative humidity were recorded daily. Animals were exposed to their respective pDCB concentrations 5 hours a day, 5 days a week for a total of 76 weeks. All surviving animals were put into a recovery phase (no more exposure) until either an 80 percent mortality was attained or 2 years elapsed from the initiation of the study. Five animals/group/sex were autopsied on weeks 26-17, 52-53, and 76-77 of the study.

Generation of Test Atmospheres (Abstracted from the Original Report):

"The air supply was introduced at the top of each chamber at a constant rate (170 litres/min) and was extracted at the bottom. The extract air duct was fitted with a wire mesh gauze which filtered out large particles such as animal hair.

"Valves on the chamber input and extract lines were adjusted so that the pressure within the chambers was slightly negative with respect to the laboratory. This minimised the possibility of contamination of the laboratory area.

"pDCB vapor was generated by passing clean dry air through pDCB crystals in a water-jacketed glass vessel. The temperature of the jacket was maintained at 55°C by means of a thermostatically controlled water pump (Churchill). The pDCB vapour was introduced into the chamber air supply line. The required exposure concentrations were obtained by adjusting the air flow rate through the pDCB crystals.

" Analysis of Test Atmospheres

"The chamber concentrations of pDCB were analysed using an infra-red analyser (Wilks Miran 1A) set to the following conditions: Wavelength 12.0 um, Path-length 9.75 m, Slit-width 1.0 mm, Time-constant 10 sec.

"Calibration was achieved using pDCB dissolved in acetone with the analyser in the closed-loop mode. The concentration of pDCB after volatilization was then calculated assuming ideal gas laws.

"Atmosphere samples were taken from a central point in each of the pDCB chambers. The samples were drawn through the analyser cell using the analyser pump and returned directly to the chamber. The concentration of pDCB was then determined from a standard curve."

Experimental Observations:

All animals were observed daily for clinical signs of toxicity and mortality. Body weights were recorded individually prior to study initiation and every 2 weeks on study until week 10. On week 13 and every 4 weeks thereafter the rats were weighed by cage. Food and water consumption were recorded weekly prior to exposure, at 2-week intervals after study initiation until week 15, and at 6-week intervals thereafter.

For clinical chemistry measurements, blood samples were taken from the tail vein of 5 animals/sex/group preexperimentally and at weeks 5, 14, 27, 40, and 52 for determination of blood urea, glucose, and plasma alanine transaminase and aspartate transaminase activities. For hematology measurements blood samples were taken from the tail vein of 10 animals/sex/group preexperimentally and at weeks 5 and 14, and from 5 rats/sex/group at weeks 26-27 (cardiac puncture), 40, and 52-53 (cardiac puncture) on study. The following parameters were measured.

Preexperimentally to week 14 - Hemoglobin, packed cell volume, white blood cells, platelets, and leukocyte differential count.

Weeks 26 to 52 - Hemoglobin, white blood cells, red blood cells, hematocrit, platelet count, leukocyte differential count, and mean corpuscular hemoglobin concentration.

Bone marrow smears were prepared from bone marrow taken from the femur of rats at the 26-27 and 52-53 week interim sacrifice.

For urinalysis, urine samples were collected over an 18-hour period from 5 rats/sex/group preexperimentally, and at weeks 5, 14, 27, 40, and 52 on study. The following parameters were

measured: pH, glucose, bilirubin, specific gravity, protein, and coproporphyrin.

Hepatic aminopterin demethylase activity was determined in liver samples of five rats/sex/group at the 52-53 week interim sacrifice.

Sacrifice and Pathology:

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X			
	Digestive system		Cardiovasc./Hemat.		Neurologic		
	Tongue	X	Aorta*	XX	Brain*		
X	Salivary glands*	XX	Heart*	X	Periph. nerve*		
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 level)		
X	Stomach*	X	Lymph nodes*	XX	Pituitary*		
X	Duodenum*	XX	Spleen*	X	Eyes (optic nerve)*		
X	Jejunum*	X	Thymus*		Glandular		
X	Ileum*		Urogenital	XX	Adrenals*		
X	Cecum*	XX	Kidneys*		Lacrimal gland		
X	Colon*	X	Urinary bladder*	X	Mammary gland*		
	Rectum*	XX	Testes*		Parathyroids*		
XX	Liver*	X	Epididymides	X	Thyroids*		
	Gallbladder*	X	Prostate		Other		
X	Pancreas*	X	Seminal vesicle		Bone*		
	Respiratory	XX	Ovaries	X	Skeletal muscle*		
X	Trachea*	X	Uterus*		Skin		
XX	Lung*				All gross lesions and masses		

*Recommended by Subdivision F (October 1982) Guidelines for Chronic Studies.

Statistical Methods (Abstracted from the Original Report):

"Bodyweight, food consumption and water consumption per rat were calculated and these values were considered by analysis of variance separately for males and females. For bodyweight the analysis was carried out for a selection of weeks covering the whole study. For food and water consumption the data for each available week have been analysed, although this has sometimes involved comparing data from different weeks for different groups.

"Hematological and blood biochemical measurements have been considered separately for each period when measurements were made by analysis of variance. Male and female data were considered together but the results were examined for evidence that any treatment effects were not the same in males and females.

"Organ weights were considered, separately for males and females and separately for each time, by analysis of variance and analysis of covariance on bodyweight. Where there was evidence of a linear relationship between organ weight and bodyweight, group means were compared before and after adjustment for bodyweight.

"In all analyses the treated group means were compared with the control group mean using Student's t-test (two-sided) based on the variance estimate from the analysis of variance. Where male and female data were considered together these comparisons were made on the overall means and the male and female means.

"The mortality data were analysed using the Logrank test (Peto and Pike, 1973). Mortality estimates at different times throughout the study were obtained using the method of Kaplan and Meier (1958)."

Results:

The authors reported that the mean chamber pDCB concentrations were approximately the same as the target concentrations. Deviations from the target concentrations were within ± 10 percent for the high dose tested (500 ppm) and up to ± 20 percent for the low dose tested (75 ppm). The temperature in all chambers was approximately 25 °C throughout the study while relative humidity was approximately 70 percent the first 9 months on study and 50 percent the rest of the study (Note: Only mean values were reported).

The rate of mortality was comparable between control and treated groups in both sexes at week 76 (end of exposure) as well as at week 108. The percent mortality at week 108 was 53.4, 46.2, and 53.2 for the control, low-, and high-dose groups, respectively, for males and 46.2, 45.8, and 48.8 for the control, low-, and high-dose groups, respectively, for females. Although the study was terminated for male rats on week 109 and for female rats on week 112, mortality data were not reported beyond week 108 for either sex.

Mean body weights for male rats were statistically significantly lower than controls in both treated groups at the 6-week measurement and only in the low-dose group at the 8-week measurement. For the remainder of the study, mean body weights were comparable between the control and treated groups. At study termination (week 109) the mean body weights of pDCB-treated groups were numerically higher than controls. For female rats, the mean body weight was statistically significantly lower than controls in the low-dose group on week 8 of measurement and comparable to controls for the rest of the study (Tables 5 and 6; from the original report). Females of the high dose group had mean body weights statistically significantly higher than controls for most of the study. Food consumption was for the most part comparable between

the treated and control groups in both sexes throughout the study. Similarly, water consumption was for the most part comparable between treated and control groups. (Note:individual animal data for body weight, food and water consumption were not reported).

Clinical chemistry values for several parameters were found to be statistically significantly different between the treated and control groups in both sexes at different time intervals. However, it does not appear that these findings are of biological significance since there was no dose response and in most cases these changes were not persistent throughout the study. Representative clinical chemistry values are given in Table 1 for the record. Similarly, hematology values for several parameters were found to be statistically significantly different between treated and control groups at different time intervals in both sexes. As shown in Table 1, these changes are not doserelated and they were not consistent throughout the study. Thus the biological significance of these changes is not evident. From the urinalysis parameters measured, protein excretion in the urine was significantly higher in the pDCB-treated groups (with a dose-related trend) as compared to the control group in male rats, at different time points (weeks 27, 40, and 52) on study. (Note: No statistical analysis was performed on urinalysis data.) Some parameters measured had values that were comparable between treated and control groups (pH, specific gravity) while values for other parameters (volume, coproporphyrin) varied tremendously throughout the study so that no conclusions can be drawn on the biological significance of these changes. (Note: individual hematology, clinical chemistry and urinalysis values were not reported.)

Mean absolute organ weights for liver, kidney, heart, and lung were statistically significantly higher than controls in the high-dose group (500 ppm) in both sexes mainly at the terminal sacrifice (Table 2). Brain mean weights for male rats were statistically significantly lower than controls in the high-dose group at terminal sacrifice. Mean absolute organ weights were comparable between the low-dose groups and the controls for both sexes at the interim sacrifice (76 to 77 weeks) and at study termination.

No Gross pathology was reported for this study.

Table 1
Effect of pDCB on Clinical Chemistry and Hematology Parameters[1]

Parameter	Day of Test	Dose (ppm)					
		Males			Females		
		0	75	500	0	75	500
Clinical Chemistry							
Alanine Transaminase (mu/mL)	5	9	8	12*	7	12**	7
	14				19	6**	7**
Aspartate Transaminase (mu/mL)	5	56	45 (3)	38*			
	14				55	31**	30**
	27				77	45*	75
	52	40	88*	44	56	35	48
Blood Urea (mg/100 mL)	52	34	44*	37	42	35	44
Blood Glucose (mg/100 mL)	52	72	90**	78			
Hematology							
Hemoglobin (g/dL)	5	15.6(9)	15.7(10)	16.3*(10)	15.1(10)	15.4(10)	14.8(10)
	52	15.2	14.9	14.2**	14.5	15.2*	15.6**
Hematocrit (%)	52	0.40	0.39	0.38*	0.39	0.40	0.42**
Red Blood Cells ($\times 10^{12}$/L)	52	8.1	8.1	7.7	7.4	7.9	7.9*
Mean Cell Hemog. Conc. (g/dL)	5				34.7(10)	34.5(10)	33.7**(10)
	14	36.4(10)	34.9**(4)	35.1**(4)	36.0(10)	35.4*(10)	36.6*(10)
	52	37.3	37.8	37.5	36.8	37.9**	37.1
White Blood Cells ($\times 10^{9}$/L)	14	8.8(10)	9.7(10)	7.0*(10)			
	27	4.4	4.3	6.3*			
	52	7.5	8.2	8.5	5.2	5.9	6.9*

[1]Means of 5 observations unless otherwise indicated in parentheses

*,**Statistically significantly different from control values at p = 0.05, p = 0.01, respectively.

Table 2

Effect of Paradichlorobenzene on Absolute Organ Weights[1]

Organ	Sex	Week of Sample	Dose (ppm) 0	75	500
Liver	M	76-77	14.2 (5)[2]	15.4 (5)	16.1 (5)
		109-112	18.2 (27)	19.5 (34)	18.9 (27)
	F	109-112	13.9 (32)	14.3 (32)	16.5* (29)
Kidney	M	76-77	3.36 (5)	3.68 (5)	4.46** (5)
		109-112	4.24 (26)	4.44 (34)	4.67 (27)
	F	109-112	3.08 (32)	3.43 (33)	3.72** (29)
Heart	M	109-112	1.72 (27)	1.82 (34)	1.98* (27)
	F	109-112	1.32 (32)	1.46 (33)	1.54** (29)
Lung	M	109-112	1.99 (26)	2.09 (34)	2.32* (27)
	F	109-112	1.51 (32)	1.57 (33)	1.79** (29)
Brain	M	109-112	2.37 (27)	2.34 (34)	2.29** (27)

[1]Organ weights are in grams.
[2]Numbers in parentheses indicate number of animals sacrificed.
*,**Statistically significantly different from control values at p = 0.05, p = 0.01, respectively.

Histopathological Lesions

1. Interim Sacrifice- Histopathological examination of all animals sacrificed at weeks 26, 52 and 76 revealed a number of neoplastic and non-neoplastic lesions that were, in some cases, of higher incidence in the treated as compared to the control groups. However, the incidence of these lesions was not statistically significantly different between treated and control groups. Some of these lesions appear in Table 3 for the record.

2. Terminal Sacrifice- Neoplastic and non-neoplastic lesions were present in several tissues of male and female rats. As can be seen in Table 4, the incidence of neoplastic and non-neoplastic lesions in both sexes was for the most part very low and only numerically higher in the treated groups, as compared to controls. However, no conclusions can be drawn about the importance of these lesions since the authors failed to specify the total number of animals examined for each particular tissue lesion.

Discussion:

The present study has investigated the chronic toxicity and oncogenicity of pDCB to male and female Wistar rats through the inhalation route. The authors provided data to show that the chamber concentrations of pDCB were within 10 to 20 percent of the target concentrations.

Table 3

Incidence of Neoplastic and Non-neoplastic Lesions -Interim Sacrifices

Organ/Lesion	Week of Sacrifice[1]	Dose (ppm)					
		Males			Females		
		0	75	500	0	75	500
Kidney - Focal sclerosed cortical tubules	26				0	2	3
Liver - Bile duct proliferation							
- Minimal	52	0	0	1	1	3	2
- Slight		1	1	3	0	0	3
- Moderate		2	2	1	1	2	0
Kidney - Calcification of pelvic epithelium	52	0	0	1	2	3	4
Nephropathy							
- Minimal		2	4	0	2	3	2
- Slight		3	1	5	1	0	0
Adrenal - Hypertrophy/ hyperplasia of the glomerulosa	76	1	4	3	2	3	5
Kidney - Pelvic/ papillary calcium mineralization					1	4	4

[1]Five animals/sex were examined at each time point.

Table 4

Summary of Histopathological Lesions[1]

Organ/Lesion	Week of Observation	Dose (ppm)					
		Males			Females		
		0	75	500	0	75	500
Neoplastic Lesions	0-112						
Uterus/Cervix - Stromal cell Sarcoma					1	1	4
Hematopoietic/ Lymphoreticular System Thymic Lymthosarcoma		0	1	1	0	1	1
Ovaries - Granulosa/ Thecal Cell Tumor					1	4	4
Subcutaneous Tissue - Fibrosarcoma		1	3	3			
Non-neoplastic Lesions							
Stomach - Glandular Dilatation in the Fundic Mucosa	0-112	7	21	11			
Pancreas - Chronic Inflammatory Infiltration		1	2	4			
Liver - Focal Chronic Hepatitis/Chronic Infiltration		11	14	19	7	14	13
Hemosiderosis/ hemorrhage		2	10	6	2	3	5
Sciatic Nerve - Segmental demyelination		3	18	17			
Lungs - Foamy Histiocytes					11	18	20
- Neutrophilia		0	3	5			
Kidney - Nephropathy Marked					7	12	14
- Focal Suppurative Nephritis		7	25	14			
Prostate - Atrophy		3	10	9			
Eye - Keratitis					1	4	5

[1]Number of rats with specified observations. The total number of tissues examined was not specified.

198

Although, based on the available data the high dose level of pDCB tested (500 ppm) appears to induce variable toxic effects to male or female rats, due to a number of major deficiencies associated with this study (mainly in reporting the data) it is difficult to establish the chronic toxicity and oncogenicity potential of pDCB in male and female rats via the inhalation route. For a final evaluation of this study the sponsor is required to provide the following additional information and/or clarifications:

1. Report the range of temperature and relative humidity in the exposure chamber.

2. Report all clinical symptoms of toxicity for all animals throughout the study.

3. Report all gross pathology findings in summary tables and as individual animal data.

4. Report individual animal data concerning:

   a. Body weight;
   b. Food Consumtion;
   c. Clinical chemistry values;
   d. Hematology values;
   e. Urinalysis values;
   f. Organ weights (absolute and relative); and
   g. Histopathology.

5. For histopathology summary tables specify the total number of tissues examined for each observation.

6. Evaluate additional clinical chemistry, hematology, and urinalysis parameters as specified in the EPA guidelines.

7. Supply Table 22 (mean blood glucose values in females).

8. Clarify Tables 33 and 34 (no values are given).

9. Perform statistical analyses for all urinalysis data.

10. Report the standard deviation ($\pm$ SD) for all mean values.

Conclusions:

Data presented here were not sufficient for a complete evaluation of this stydy. The MTD, LEL or NOEL could not be established. Additional data are required.

Classification: Core-Supplementary

pDCB: LONG TERM INHALATION STUDY IN THE RAT

TABLE 5

GROUP MEAN BODYWEIGHT (g)

MALES

Period (weeks)	Dose Level (ppm) 0 (control)	75	500	Approximate 95% Confidence Limits
Pre-experimentally	295	291	289	-
2	345	337	335	±9
4	379	377	386	±11
6	409	389**	391**	±8
8	431	409**	426	±9
10	446	438	449[436]	±18 [±14]
13	464	458	452	±17
19	485	488	485	±17
25	509	508	506	±14
38	541	545	540	±11
50	561	568	562	±15
76	577	584	556	±24
109	504	549	520	±25

[] After correcting a probable recording error.

The means are based on the mean weight/rat/cage for each group (8 observations per group).

** Statistically significantly different to the control value at the 1% level (t-test, two-sided).

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200

007021

pDCB: LONG TERM INHALATION STUDY IN THE RAT

TABLE 6

GROUP MEAN BODYWEIGHT (g)

FEMALES

Period (weeks)	Dose Level (ppm) 0 (control)	75	500	Approximate 95% Confidence Limits
Pre-experimentally	205	205	209	-
2	224	225	231	±5
4	242	247	264**	±7
6	255	248	259	±7
8	256	253*	281*	±8
10	276	277	284	±6
13	281	283	293**	±6
19	302	301	315*	±8
25	310	309	324*	±8
38	323	329	343*	±12
50	360	359	372	±14
76	404	390	430	±24
109	371	365	387	±31

The means are based on the mean weight/rat/cage for each group.
(8 observations per group)

* Statistically significantly different to the control value at the 5% level (t-test, two-sided).

** Statistically significantly different to the control value at the 1% level (t-test, two-sided).

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201

Reviewed By: Y.M. Ioannou YMI 1/19/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M. Copley MCopley 1/31/89
Section II, Toxicology Branch I (IRS)

007021

DATA EVALUATION REPORT

Study Type: Subchronic Toxicity - Rat (82-1)

TOX CHEM. No.: 632
MRID No.: 405680-07

Test Material: Para-Dichlorobenzene (pDCB)

Synonyms: 1,4-Dichlorobenzene

Study Number(s): 76-34-106002

Sponsor: Chlorobenzene Producers Association

Testing Facility: Battelle's Columbus Laboratories
Columbus, OH

Title of Report: Para-Dichlorobenzene - Subchronic Toxicity Study: Para-Dichlorobenzene (C54955), Fischer 344 Rats.

Author: G.S. Dill and A.C. Peters

Report Issued: March 23, 1979

Conclusions:

The present study has investigated the toxicity of pDCB when administered by gavage to male and female Fischer 344 rats at dose levels of 0, 300, 600, 900, 1200, and 1500 mg/kg/day for 90 days. Data presented here indicate that the LEL for systemic toxicity (body weight gain depression) was found to be 900 mg/kg/day in female rats and 600 mg/kg/day in male rats. The NOEL was found to be 600 mg/kg/day in females and 300 mg/kg/day in males.

Classification: Core-Supplementary

Materials and Methods:

Male and female Fischer 344 rats (obtained from Harlan Industries, Inc.) weighing 140 to 143 g (males) or 90 to 119 g (females) were used in this study. Upon arrival, the animals were quarantined for 17 days and observed daily for general health status. Two animals/sex were necropsied, prior to study initiation for detection of observable disease. Animals were housed in polycarbonate cages, 5 rats/cage, individually marked for identification, and provided food and water ad libitum. Animal feed was checked daily for each cage and fresh feed and feeders were provided weekly. Animal rooms were maintained at a temperature of 21 to 23 °C, a relative humidity of 40 to 60 percent, 15 air changes/hour and a light/dark cycle of 12 hours.

The test material, pDCB, manufactured by Dover Chemical Company, had a reported purity of 100 percent. The selected dose levels of 300, 600, 900, 1200, and 1500 mg/kg, were prepared by diluting pDCB with corn oil and administered to the animals by gavage at a volume of 5 mL/kg body weight. The control group received only corn oil.

Study Design:

Sixty male and 60 female rats were randomly divided into 6 groups/sex based on body weights and administered pDCB at the following dose levels:

Test Group	Dose (mg/kg/day)	Number of Rats/Group	
		Male	Female
1. Control - Corn oil	0	10	10
2. pDCB	300	10	10
3. pDCB	600	10	10
4. pDCB	900	10	10
5. pDCB	1200	10	10
6. pDCB	1500	10	10

Animals were gavaged 5 days a week (Monday through Friday) for 13 weeks. Clinical observation were made twice daily and animal body weights and food consumptions were recorded weekly.

Hematology parameters were measured in all animals from blood samples (obtained by the orbital bleeding method) taken 1 days prior to study termination. Clinical chemistry measurements were performed in all animals on blood samples obtained by cardiac puncture on the day of necropsy. For urinalysis, urine was collected (in metabolism cages) from male and female animals of the control group and the surviving animals of the 1200 and

1500 mg/kg/day dose groups, approximately 1 week before study termination. The following checked (X) hematology, clinical chemistry and urinalysis parameters were measured:

1. Hematology

X	Hematocrit (HCT)*
X	Hemoglobin (HGB)*
X	Leukocyte count (WBC)*
X	Erythrocyte count (RBC)*
X	Platelet count*

	Total plasma protein (TP)
X	Leukocyte differential count
	Mean corpuscular HGB (MCH)
	Mean corpuscular HGC conc. (MCHC)
X	Mean corpuscular volume (MCV)
X	Reticulocyte count

2. Clinical Chemistry

	Electrolytes:
	Calcium*
	Chloride*
	Magnesium
	Phosphorous*
	Potassium*
	Sodium*
	Enzymes
X	Alkaline phosphatase
	Cholinesterase
	Creatinine phosphokinase*
	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (also SGPT)*
	Serum aspartate aminotransferase (SGOT)*
X	Gamma glutamyltranspeptidase (GGTP)

	Other:
	Albumin*
	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total Bilirubin
X	Total Protein
X	Triglycerides

3. Urinalysis

	Appearance*
	Volume*
X	Specific gravity*
X	pH
	Sediment (microscopic)*
X	Protein*

X	Glucose*
X	Ketones*
X	Bilirubin*
X	Blood*
	Nitrate
	Urobilinogen
X	Creatinine

*Recommended by Subdivision F (October 1982) guidelines for chronic studies.

Sacrifice and Pathology - All animals that died, sacrificed moribund or sacrificed at study termination were subject to gross pathological examination. All tissues (the authors did not name the tissues) from the control groups and the three highest dose groups (900, 1200, or 1500 mg/kg/day) were collected for histological examination. The following organs were weighed: lung, heart, liver, spleen, thymus, right kidney, brain, right testicle, right ovary, and uterus. The organ weight to body weight ratios were calculated for the weighed organs.

No statistical analysis was performed on any of the data obtained.

Results:

Mortality and Clinical Observations - As shown below (Table 1) the incidence of mortality in the highest dose groups (1500 mg/kg/day) for both sexes were very high; in males 8 of 10 rats died during the study while in females 9 of 10 rats died. In males most of the deaths (6 of 8) occurred during the first week of dosing. In females 6 of 9 deaths occurred by week 5 on study. High mortality was also observed in the males of the 1200 mg/kg/day group; 5 of 10 animals died, most of them after week 7 on study.

Table 1

Incidence of Mortality

Test Group (mg/kg/day)	Mortality	
	Males	Females
Control - 0	1/10 (10)[1/]	0/10 (0)
pDCB - 300	0/10 (0)	0/10 (0)
pDCB - 600	0/10 (0)	0/10 (0)
pDCB - 900	1/10 (10)	2/10 (20)
pDCB - 1200	5/10 (50)	1/10 (10)
pDCB - 1500	8/10 (80)	9/10 (90)

1/Number in parentheses denote percent mortality.

The authors reported that the main clinical signs of toxicity were: tremors, poor motor response, ocular discharges, and hypothermy. All symptoms were associated with the males and females of the highest dose group (1500 mg/kg/day) and males of the 1200 mg/kg/day dose group. The authors did not present any summary tables on individual animal data concerning clinical signs of toxicity.

Body Weights - As shown in Table 2, the mean body weights in male rats at study termination (week 13) were significantly lower than controls in all treated groups. Similarly, the percent body weight gain decrement was significantly higher in the 600, 900, 1200, and 1500 mg/kg/day groups compared controls throughout the study and at study termination (Table 2). In female rats, significant changes in body weight gain decrement compared to controls ware seen only in the three high dose groups (900, 1200, and 1500 mg/kg/day).

Food Consumption - In general, mean daily food consumption was comparable between the control and the pDCB-treated groups in both sexes. Although mean weekly consumption varied in all groups throughout the study, the greatest variation was reported in the highest dose group of female rats. In this group, food consumption ranged from 53 percent lower than controls (week 9) to 79 percent higher than controls (week 13). Also, the weekly consumption in the same group (1500 mg/kg/day) females ranged from 31.5 g (week 9) to 116 g (week 13).

From the hematology parameters measured (at study termination), a significant decrease in some values was seen with the 1500 mg/kg/day dose group males compared to controls. The depressed values included: hemoglobin (17.6 vs. 15.3 g/dL for the control and highest dose groups, respectively); hematocrit (50 vs. 43% for the control and highest dose groups, respectively); and red blood cells (10.03 vs. 3.85 x 10^6/cu mm for the control and highest dose groups, respectively) Table 3. The values were the mean of nine animals for the control and only two animals for the highest dose tested. Mean values for other hematology parameters were, in general, comparable between treated and control groups. (Note: No statistical analysis of the hematology data was performed.)

A number of clinical chemistry parameters were found to be significantly different between treated groups and controls. The major changes in clinical chemistry values are shown in Table 3 and included a significant decrease (26 to 50%) in alkaline phosphatase in all treated groups of male rats and a significant increase (29 to 72%) in female rats of two highest dose groups; an increase in cholesterol values in both sexes with an apparent dose-related trend. Changes in other clinical chemistry values do not appear to be of biological significance, but are shown in Table 3 for the record. (Note: No statistical analysis on clinical chemistry data was performed.)

00702

Table 2

Mean Body Weights and Percent Body Weight Gains
at Selected Time Intervals

	Week on Study	Dose (mg/kg/day)					
		0	300	600	900	1200	1500
Males - Mean body weight (g)	0	126.8	118.3	120.8	121.3	121.9	118.2
	3	208.7	187.0	194.3	171.8	172.7	163.0
	6	256.2	228.1	227.4	221.0	199.3	190.0
	9	279.6	245.9	229.8	225.1	212.0	187.3
	13	313.9	281.4	263.4	258.9	238.0	212.5
- Mean body weight gain (%)	3	64.6	58.1	60.8	41.6	41.7	37.9
	6	102.1	92.8	88.2	82.2	63.5	60.7
	9	120.5	107.9	90.2	85.6	73.9	58.5
	13	147.6	137.9	118.0	113.4	95.2	79.8
- Body weight gain change compared to controls (%)	3	--	-10.1	-5.9	-35.6	-35.4	-41.3
	6	--	-9.1	-13.6	-19.5	-37.8	-40.5
	9	--	-10.5	-25.1	-29.0	-38.7	-51.5
	13	--	-6.6	-20.1	-23.2	-35.5	-45.9
Females - Mean body weight (g)	0	103.7	105.9	101.7	103.3	103.0	102.8
	3	138.1	140.2	134.4	125.1	129.6	123.1
	6	162.6	160.8	153.6	158.9	147.9	138.3
	9	168.5	159.4	148.9	153.9	141.3	129.0
	13	174.0	177.1	168.0	163.5	154.8	140.0
- Mean body weight gain (%)	3	33.2	32.4	32.2	21.1	25.8	19.7
	6	56.8	51.8	51.0	53.8	43.6	35.5
	9	62.5	50.5	46.4	49.0	37.2	25.5
	13	67.8	67.2	65.2	58.3	50.3	36.2
- Body weight gain change compared to controls (%)	3	--	-2.4	-3.0	-36.4	-22.3	-40.7
	6	--	-8.8	-10.2	-5.3	-23.2	-37.5
	9	--	-19.2	-25.8	-21.6	-40.5	-59.2
	13	--	-0.9	-3.8	-14.0	-25.8	-46.6

Table 3

Effect of pDCB on Selected Hematology, Clinical Chemistry, and Urinalysis Parameters

Parameter	Sex	Dose (mg/kg/day) 0	300	600	900	1200	1500
Hematology							
HgB (G/dL)	M	17.6[1/] ± 0.6	16.4 ± 0.6	16.5 ± 0.6	16.5 ± 0.5	16.6 ± 0.8	15.3 ± 0.
HCT (%)	M	50 ± 2	48 ± 3	47 ± 2	47 ± 1	48 ± 2	43 ± 1
RBC (10^6/cu mm)	M	10.0 ± 0.4	9.4 ± 0.3	9.5 ± 0.4	9.7 ± 0.3	9.8 ± 0.4	8.9 ± 0.
Clinical Chemistry							
Alkal. Phosph. (IU)	M	164 ± 18	114 ± 14	122 ± 31	116 ± 36	121 ± 11	82 ± 0
	F	112 ± 28	98 ± 33	101 ± 17	123 ± 44	144 ± 30	193 ± 0
Cholesterol (mg/dL)	M	54 ± 12	59 ± 9	72 ± 8	80 ± 12	86 ± 7	91 ± 0
	F	71 ± 8	84 ± 5	69 ± 8	89 ± 11	89 ± 10	92 ± 0
SGPT (IU)	M	84 ± 65	63 ± 46	42 ± 8	46 ± 9	75 ± 49	49 ± 0
	F	58 ± 50	51 ± 27	70 ± 45	90 ± 45	61 ± 23	50 ± 0
Triglycerides (mg/dL)	M	209 ± 48	121 ± 37	158 ± 42	86 ± 23	114 ± 36	38 ± 0
	F	113 ± 46	98 ± 20	118 ± 23	107 ± 10	101 ± 11	142 ± 0
Urinalysis							
Coproporphyrin (ng/3 mL)	M	203 ± 177	-	-	-	816 ± 411	764 ± 23
	F	130 ± 120	-	-	-	-	316 ± 252
Uroporphyrin (ng/3 mL)	M	381 ± 203	-	-	-	806 ± 632	714 ± 76
	F	592 ± 298	-	-	-	-	833 ± 374

[1/] Mean ± SD.

Urinalysis measurements indicated, according to the authors (no data were presented in this report), that urine volume in male and female animals of all treated groups was higher than controls. Considerably higher urine volume was observed in animals of the 1200 and 1500 mg/kg/day groups in both sexes. The authors presented data showing that urine coproporphyrin and uroporphyrin increased two to fourfold in the two highest dose level groups in both sexes compared to controls (Table 3). Other urinalysis measurements were comparable between treated and control groups, according to the authors.

Absolute organ weights were in general significantly lower in the highest dose groups as compared to controls, reflecting the significantly lower body weights in these groups compared to controls, in both sexes. However, liver weights were for the most part higher than controls in all female treated groups and significantly higher in the highest dose group. Tables 4 and 5, abstracted from the original report, are attached for the record. Relative organ weights were higher than controls for some organs of the 600, 900, 1200, and 1500 mg/kg/day dose groups for both sexes (Note: the authors did not present any data on relative organ weights).

Gross pathology, performed on all animals, did not reveal, according to the authors, any treatment-related lesions (Note: No gross pathology summary tables or individual animal data were reported in the study).

Histopathological examinations revealed several lesions which were of higher incidence and/or severity in the treated as compared to control groups. Major lesions considered to be treatment-related were as follows (Note: The authors did not present summary tables for histopathological lesions; they presented only individual animal data, in handwritten form in most cases not legible):

1. Bilateral retinal atrophy; severity increase with time on study (in all treated groups).

2. Degeneration and necrosis of hepatocytes (in the two highest dose groups in males and females).

3. Hypoplasia of bone marrow (in the two highest dose groups in males and females).

4. Lymphoid depletion of the spleen and thymus (in the two highest dose groups in males and females).

5. Epithelial necrosis of the small intestinal mucosa and turbinates (in the two highest dose groups in males and females).

00702

6. Multifocal degeneration or necrosis of renal cortical tubular epithelial cells (in most male rats that survived 45 days or longer on study).

Discussion:

The present study has investigated the toxicity of pDCB to male and female Fischer 344 rats after repeated exposure for 90 days. Mortality data presented here indicate that the highest dose tested (1500 mg/kg/day) was considerably higher than the MTD resulting in the death of 80 percent of the male and 90 percent of the female animals. The 1200 mg/kg/day dose level was also too high resulting in 50 percent mortality in male rats. Data on mean body weights and mean body weight gains at study termination indicate that for male rats the percent body weight decrement was significantly higher than controls in the 600, 900, 1200, and 1500 mg/kg/day groups. Thus, the NOEL for systemic toxicity in male rats is considered to be the LDT (300 mg/kg/day) and the LEL the dose of 600 mg/kg/day. For females, the percent body weight decrements were comparable between the controls and the two lowest dose groups (300 and 600 mg/kg/day) but considerably higher than controls for the rest of the dose levels. Thus, the LEL (for systemic toxicity) was the 900 mg/kg/day dose level and the NOEL was the 600 mg/kg/day.

The depression of the hematology values in male rats of the highest dose group compared to controls suggests that pDCB at high dose levels produces anemia. However, these values were derived from only two animals compared to nine animals for the control values. Some clinical chemistry values were found to be different between control and treated groups. However, a dose-related effect was seen in the increase of cholesterol in both sexes and the increase of alkaline phosphatase activity in female rats. Both of these effects suggest liver and/or renal dysfunction. The high urine volume excreted by the males and females of the two highest dose groups (compared to controls) is also indicative of kidney damage.

Although absolute organ weights were, in general, lower in most treated groups compared to controls reflecting the lower body weight, relative organ weights for liver, kidney and brain were higher than controls in all treated groups of male and female rats with an apparent dose-related trend. The higher relative weights for liver and kidney indicate systemic toxicity to pDCB in both sexes and correlate to some extent with the reported clinical chemistry changes and the histopathologic lesions reported in these organs.

The authors reported a number of microscopic lesions associated especially with the 1200 and 1500 mg/kg/day dose groups in both sexes. However, the toxicological importance of these lesions cannot be fully evaluated since the authors did not provide summary tables with the incidence and severity of each lesion for each treatment group in both sexes.

Conclusions:

Data presented here indicate that the dose levels of 1200 and 1500 mg/kg tested in male rats have exceeded the MTD, resulting in high mortality in these groups. For female rats, the highest dose tested (1500 mg/kg) was higher than the MTD. The LEL for systemic toxicity (body weight gain depression) was found to be the dose of 600 mg/kg/day for male rats and 900 mg/kg/day for female rats. The NOEL for male rats was 300 mg/kg/day and for females was found to be 600 mg/kg/day. However, this study could not be fully evaluated due to the following deficiencies:

1. No statistical analysis was performed on any data reported in this study.

2. No summary tables on the clinical signs of toxicity were reported.

3. No summary tables of urinalysis measurements were reported.

4. No individual animal body weights were reported.

5. No individual animal organ weights were reported.

6. Organ to body weight ratios (relative organ weights) and organ to brain weight ratios were not reported.

7. No individual animal data or summary tables for gross pathology were reported.

8. The individual animal histopathology data were presented in a handwritten (mostly nonlegible) form; no summary tables for the histopathological lesions were reported.

Classification: Core-Supplementary

211

TABLE 4 ORGAN WEIGHT ANALYSIS—MALE RATS

Dose (mg/kg)			BW	LUNG	HEART	LIVER	SPLEEN	THYMUS	RT KIDNEY	BRAIN	RT TESTICLE
0	UM	$\bar{X}$	293	1.65	0.96	9.55	0.58	0.33	0.96	1.82	2.21
	(N=10)	SD	± 21	0.54	0.17	0.84	0.08	0.08	0.14	0.09	0.78
		OW/BW	-	.006	.003	.033	.002	.001	.003	.006	.003
300	AM	$\bar{X}$	261	1.46	0.88	8.64	0.59	0.29	1.01	1.77	2.19
	(N=10)	SD	± 28	0.15	0.12	1.06	0.09	0.07	0.07	0.07	0.34
		OW/BW	-	.006	.003	.033	.002	.001	.004	.007	.008
600	BM	$\bar{X}$	248	1.35	0.92	9.70	0.54	0.31	1.04	1.77	2.18
	(N=10)	SD	± 18	0.17	0.12	1.10	0.05	0.08	0.08	0.08	0.34
		OW/BW	-	.005	.004	.039	.002	.001	.004	.007	.009
900	CM	$\bar{X}$	220	1.49	0.89	10.83	0.58	0.25	1.10	1.78	2.06
	(N=9)	SD	± 43	0.22	0.16	1.13	0.04	0.05	0.10	0.05	0.17
		OW/BW	-	.007	.004	.049	.003	.001	.005	.008	.009
1200	DM	$\bar{X}$	212	1.37	0.83	10.95	0.54	0.27	1.09	1.81	2.11
	(N=5)	SD	± 36	0.32	0.08	1.21	0.07	0.06	0.11	0.08	0.28
		OW/BW	-	.007	.004	.052	.003	.001	.005	.009	.010
1500	CM	$\bar{X}$	143	.90	.60	8.03	.321	.191	.825	1.672	1.321
	(N=6)	SD	± 46	.25	.11	1.30	.134	.050	.141	.081	0.45
		OW/BW	-	.006	.004	.056	.002	.001	.006	.012	.009

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Table 5

ORGAN WEIGHT ANALYSIS--FEMALE RATS

Dose (mg/kg)			BW	LUNG	HEART	LIVER	SPLEEN	THYMUS	RT KIDNEY	BRAIN	RT OVARY	UTERUS
0	UF	$\bar{X}$	168	1.11	0.59	4.98	0.41	0.23	0.66	1.66	0.08	0.86
	(N=10)	SD	± 15	0.18	0.08	0.49	0.05	0.04	0.07	0.08	0.04	0.45
		OW/BW	-	.007	.004	.030	.002	.001	.004	.010	.001	.005
300	AF	$\bar{X}$	169	1.26	0.60	5.44	0.41	0.24	0.66	1.64	0.06	0.41
	(N=10)	SD	± 16	0.36	0.06	0.48	0.05	0.07	0.04	0.11	0.02	0.11
		OW/BW	-	.008	.004	.032	.002	.001	.004	.010	.0004	.002
600	BF	$\bar{X}$	162	1.08	0.61	5.37	0.39	0.22	0.63	1.65	0.05	0.37
	(N=10)	SD	± 8	0.14	0.10	0.56	0.03	0.03	0.03	0.05	0.01	0.10
		OW/BW	-	.007	.004	.033	.002	.001	.004	.010	.0003	.002
900	CF	$\bar{X}$	158	0.98	0.58	5.99	0.38	0.22	0.64	1.65	0.08	0.47
	(N=8)	SD	± 10	0.14	0.05	0.46	0.02	0.05	0.05	0.05	0.05	0.19
		OW/BW	-	.006	.004	.038	.002	.001	.004	.010	.001	.003
1200	DF	$\bar{X}$	151	0.99	0.60	6.15	0.34	0.23	0.66	1.61	0.07	0.40
	(N=9)	SD	± 17	0.16	0.10	0.50	0.04	0.04	0.06	0.08	0.03	0.16
		OW/BW	-	.007	.004	.041	.002	.002	.004	.011	.001	.003
1500	EF	$\bar{X}$	107	0.86	0.51	8.31	0.17	0.13	0.71	1.65	0.04	0.21
	(N=7)	SD	± 18	0.35	0.05	1.83	0.07	0.04	0.08	0.04	0.02	0.17
		OW/BW	-	.008	.005	.078	.002	.001	.007	.015	.004	.002

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Reviewed By: Y.M. Ioannou YMI. 1/19/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M. Copley
Section II, Toxicology Branch I (IRS) 1/31/89

DATA EVALUATION REPORT

Study Type: Subchronic (Mouse) (82-1)

TOX Chem No.: 632
MRID No.: 405680-06

Test Material: Paradichlorobenzene (pDCB)

Synonyms: 1,4-Dichlorobenzene

Study Number(s): 76-34-106002

Sponsor: Chlorobenzene Producers Association

Testing Facility: Battelle's Columbus Laboratories
Columbus, Ohio

Title of Report: Paradichlorobenzene - Subchronic Toxicity Study: Paradichlorobenzene (C54955), B6C3F1 Mice (Rerun)

Author: A.C. Peters

Report Issued: February 12, 1980

Conclusions:

The present study has investigated the toxicity of pDCB when administered (by gavage) to male and female B6C3F1 mice at dose levels of 0, 84.4, 168.8, 337.5, 675, and 900 mg/kg/day for 90 days. The available data were insufficient to allow full evaluation of this study. Additional data are required.

Classification: Core-Supplementary

Materials and Methods:

The test chemical, pDCB, manufactured by Dover Chemical Company, with a reported purity of greater than 99.9 percent was used in this study. Male and female B6C3F1 mice (obtained from Harlan Industries, Inc.), weighing 24 to 29 g (males) and 19 to 24 g (females) were examined for health status upon arrival and quarantined for 17 days prior to use. Two mice/sex were necropsied prior to randomization to detect observable disease. Animals were individually marked and housed in polycarbonate cages (5 mice/cage) which were kept in animal rooms with a temperature of 21 to 23 °C, a relative humidity of 40 to 60 percent, a 12-hour photocycle and 15 air changes/hour. Food and water were available to all animals ad libitum.

Study Design:

A total of 60 male and 60 female mice were used for this study. The animals were randomly assigned to six groups/sex and administered pDCB at the following dose levels:

Test Group	Dose (mg/kg)	Number of Mice/Group	
		Male	Female
1. Control	0	10	10
2. pDCB	84.4	10	10
3. pDCB	168.8	10	10
4. pDCB	337.5	10	10
5. pDCB	675.0	10	10
6. pDCB	900.0	10	10

The different dose levels were prepared by diluting pDCB with corn oil and administered to the animals by gavage at a volume of 5 mL/kg body weight. Control animals received only corn oil. Dose levels were prepared weekly. Animals were gavaged 5 days a week for 13 weeks. Clinical observations were conducted twice daily and animal weights and food consumption were recorded on weekly intervals.

Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta*	X	Brain*
	Salivary glands*	X	Heart*		Periph. nerve*
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)
X	Stomach*	X	Lymph nodes*		Pituitary*
	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	X	Adrenals*
	Cecum*	X	Kidneys*		Lacrimal gland
	Colon*	X	Urinary bladder*	X	Mammary gland*
	Rectum*	X	Testes*		Parathyroids*
X	Liver*		Epididymides		Thyroids*
	Gallbladder*		Prostate		Other
X	Pancreas*		Seminal vesicle	X	Bone*
	Respiratory	X	Ovaries		Skeletal muscle*
	Trachea*	X	Uterus*	X	Skin
X	Lung*				All gross lesions and masses

*Recommended by Subdivision F (October 1982) Guidelines for Chronic Studies.

Detailed microscopic examinations were performed on tissues from all mice of the control group and all animals of the highest dose group (900 mg/kg) only. Organ weights were not reported.

The authors did not report any hematology, clinical chemistry or urinalysis measurements.

No statistical analysis was performed on the reported data.

Results - The authors reported that no clinical signs of toxicity were observed in male or female mice throughout the study. Mortality was reported for male and female mice as follows:

Dose mg/kg	Mortality	
	Males	Females
0	0/10	2/10
84.4	0/10	2/10
168.8	1/10	1/10
337.5	0/10	1/10
675.0	2/10	1/10
900.0	0/10	0/10

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All deaths occurred by week 6 on study and they were apparently due to gavage error rather than to pDCB toxicity.

Mean body weights at study termination were comparable between the control and treated groups in both sexes. Mean body weight gains were higher in all treated groups compared to control in male mice. In females, body weight gains were significantly lower in the dose groups of 168.8, 337.5, and 900 mg/kg/day (Table 1).

Table 1

Dose (mg/kg)	Sex	Initial Body Weight (g)	Terminal Body Weight (g)	Body Weight Gain (%)	Body Weight Gain Change Compared to Controls (%)
			MALES		
0	M	26.4	33.5	26.9	--
84.4		26.1	34.4	31.8	18.2
168.8		24.5	34.0	38.8	44.2
337.5		25.3	33.2	31.2	16.0
675.0		25.9	35.5	37.1	37.9
900.0		26.8	34.5	28.7	6.7
			FEMALES		
0	F	22.3	27.5	23.3	--
84.4		21.4	26.9	25.7	10.3
168.8		22.6	26.1	15.5	-33.5
337.5		22.5	26.1	16.0	-31.3
675.0		22.6	28.2	24.8	6.4
900.0		23.9	28.5	19.2	-17.6

Food consumption was for the most part comparable between the control and treated groups. In the two highest dose groups (675 and 900 mg/kg) the average daily food consumption was slightly higher than the control groups for both sexes. In males there were great fluctuations in food consumption from week to week.

Gross pathology, performed on all male and female mice on study, did not reveal (according to the authors) any consistent differences in gross lesions between the treated and control groups.

Histopathological examimations were performed on tissues from all mice that died during the study, all mice of the control and highest dose groups and on liver sections from all groups except the lowest dose group (84.4 mg/kg). The only microscopic lesion reported in this study that appeared to be

treatment-related was the higher incidence of liver centrilobular or midzonal hepatocytomegaly (multifocal or diffuse) observed in the two highest dose groups (675 and 900 mg/kg) as compared to controls, in both sexes. The incidence of this lesion was 0/10, 8/10, and 9/10 for the control, 675 and 900 mg/kg dose groups, respectively, in males and 0/10, 4/10, and 10/10 for the control, 675 and 900 mg/kg dose groups, respectively, in females.

Discussion:

The present study has investigated the toxicity of pDCB to male and female B6C3F1 mice. Based on the available data, it appears that pDCB when administered to male or female mice for 13 weeks at dose levels of 84.4 to 900 mg/kg/day, does not cause toxicity to these animals. Thus, clinical signs of toxicity were comparable between treated and control groups and the reported mortality was not treatment-related but rather due to gavage technique error; mean body weights were comparable between treated and control groups throughout the study. The percent body weight gains was higher in all treated groups compared to controls in males and significantly lower in females of the dose groups 168.8, 337.5 and 900 mg/kg/day compared to controls with no apparent dose-related trend. Thus, it is not clear if such changes in body weight gains can be used to establish the MTD or the NOEL and LEL for systemic toxicity in this study.

The rest of the data available in this study included gross pathology and histopathology. The incidence of macroscopical lesions was reportedly similar in all groups. The histopathology which was performed on a limited number of animals (i.e., only animals that died on study and animals of the control and the 900 mg/kg groups at terminal sacrifice) also showed that the incidence of microscopical lesions was for the most part similar between the control and the highest dose groups with the exception of liver lesions.

In the liver (the only organ examined histopatologically in all but the lowest dose group, 84.4 mg/kg) the incidence of centrilobular to midzonal hepatocytomegaly was higher in the 675 and 900 mg/kg dose groups in both sexes, compared to controls. However, in the absence of additional supportive liver data, this nonneoplastic lesion cannot be considered for establishing the LEL and NOEL for this study.

Thus, for a final evaluation of this study, additional data are requested from the Registrant as follows:

1. Provide the Agency with hematology, clinical chemistry, and urinalysis data in summary tables as well as individual animal data.

2. Provide the Agency with mean organ weights and individual animal organ weights for those organs required by EPA Guidelines. Calculate organ-to-body weight ratios.

3. Provide individual animal histopathology data as well as summary tables for all tissues (for all treatment groups) in male and female mice.

4. Carry out statistical analyses on all data.

Conclusions:

Data presented here were insufficient for a complete evaluation of this study. The MTD, LEL, and NOEL cannot be established until the Registrant supplies the Agency with additional data.

Classification: Core-Supplementary (for deficiencies listed above).

Reviewed By: Y.M. Ioannou 2/3/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M.P. Copley 2/3/89
Section II, Toxicology Branch I (IRS)

007021

DATA EVALUATION REPORT

Study Type: Oncogenicity - Rat (Gavage) (83-2)

TOX Chem No.: 632
MRID No.: 405210-05

Test Material: Paradichlorobenzene

Synonyms: 1,4-Dichlorobenzene

Study Nos: NTP TR 319

Sponsor: National Toxicology Program

Testing Facility: National Toxicology Program

Title of Report: Toxicology and Carcinogenesis Studies of 1,4-Dichlorobenzene (CAS No. 106-46-7) In F344/N Rats and B6C3F$_1$ Mice (Gavage Studies).

Author(s): National Toxicology Program

Report Issued: January 1987

Conclusions:

The review as presented by the National Toxicology Program (NTP TR 319; NIH Publication No. 87-2575), appears to be acceptable and the interpretation of the data accurate (Summary Discussion and Conclusions attached).

Based on the NTP report, paradichlorobenzene was found to be oncogenic in male rats inducing tubular cell adenocarcinomas of the kidney. Systemic toxicity LEL (body weight depression) was 300 mg/kg/day for males and 600 mg/kg/day for females.The NOEL was 150 mg/kg/day for males and 300 mg/kg/day for females. Dose levels tested: 0,150, and 300 mg/kg/day in males, and 0,300, and 600 mg/kg/day in females.

Classification: Core-Reserved (complete evaluation of this study will be conducted prior to peer review)

Reviewed By: Y.M. Ioannou Y.M.I 2/3/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M.P. Copley MCopley 2/3/89
Section II, Toxicology Branch I (IRS)

007021

DATA EVALUATION REPORT

Study Type: Oncogenicity - Mouse (Gavage) (83-2)

TOX Chem No.: 632
MRID No.: 405210-05

Test Material: Paradichlorobenzene

Synonyms: 1,4-Dichlorobenzene

Study Nos.: NTP TR 319

Sponsor: National Toxicology Program

Testing Facility: National Toxicology Program

Title of Report: Toxicology and Carcinogenesis Studies of 1,4-Dichlorobenzene (CAS No. 106-46-7) In F344/N Rats and B6C3F_1 Mice (Gavage Studies).

Author(s): National Toxicology Program

Report Issued: January 1987

Conclusions:

The review as presented by the National Toxicology Program (NTP TR 319; NIH Publication No. 87-2575), appears to be acceptable and the interpretation of the data accurate (Abstract, Summary of Peer Review Comments, Discussion and Conclusions attached).

Based on the NTP report, paradichlorobenzene was found to be oncogenic in male and female mice inducing the formation of hepatocellular adenomas and carcinomas. Systemic toxicity LEL and NOEL (nephropathy and liver cell necrosis, cytomegaly and karyomegaly) were lower than 300 mg/kg/day (LDT).

Classification: Core-Reserved (complete evaluation of this study will be conducted prior to peer review)

Reviewed by: Y.M. Ioannou Y.M.I. 1/19/89
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: M. Copley MCopley 3/1/89
Section II, Toxicology Branch I - IRS (TS-769C)

007021

DATA EVALUATION REPORT

Study Type: Acute Oral, Dermal, Eye Irritation, and Skin Irritation

TOX Chem No.: 632
MRID No.: 405680-04

Test Material: Paradichlorobenzene (p-DCB)

Synonyms: 1,4-Dichlorobenzene

Study Nos.: Y-75-300

Sponsor: Monsanto Company
St. Louis, MO

Testing Facility: Younger Laboratories, Inc.
St. Louis, MO

Title of Report: Toxicologic Investigations of Paradichlorobenzene.

Author(s): M.D. Birch

Report Issued: December 1975

The following four acute toxicity studies with p-DCB were submitted by the registrant in a summary form. Based on the available information, the following conclusions can be drawn from each study.

1. Acute Oral LD_{50} - Rats - p-DCB (20% solution in corn oil) was used at dose levels of 1000, 1260, 1580, 2000 or 2510 mg/kg. Two or three rats (Sprague-Dawley) from each sex were treated/dose level, for a combined total number of five rats/dose. The LD_{50} was found to be 1860 mg/kg (95% CL of 1690 to 2050 mg/kg) for males and females combined.

   Toxicity Category: III

   Classification - Core-Supplementary (for the following deficiencies: The purity of p-DCB used was not specified; the number of animals used was very low; not enough description and/or data on this study were reported).

2. Acute Dermal LD_{50} - Rabbit - p-DCB (40% solution in corn oil) was applied to the skin of New Zealand Albino rabbits at dose levels of 3160, 5010 or 7940 mg/kg for 24 hours.

   Only one rabbit/dose/sex was used. The acute dermal LD_{50} was found to be greater than 5010 mg/kg for male and female rabbits combined.

   Toxicity Category: III

   Classification - Core-Supplementary (for the following deficiencies: Insufficient number of animals used; the purity of the test article was not specified; very limited data and description of the study were reported).

3. Acute Eye Irritation - Rabbit - p-DCB was applied at 0.1 mL volume (50 mg) to the eye of six New Zealand Albino rabbits. Moderate to severe erythema and slight edema were seen in the cornea and conjunctivae of all animals at 24 hours with gradual improvement at 48 and 72 hours. Irritation was completely absent by 7 days. The primary irritation score (24, 48, and 72 hours) was found to be 13.7. Thus, the test article was considered a slight eye irritant.

   Toxicity Category: III

   Classification - Core-Supplementary (for the following deficiencies: The purity of the test article was not specified; not enough data and/or description of the study were reported).

4. Acute Dermal Irritation - Rabbit - p-DCB was applied to the skin of each of six New Zealand Albino rabbits at the dose of 0.5 g/animal. Skin irritation was seen in all treated animals as erythema at 4, 24 and 48 hours posttreatment, and as edema only at 24 hours after treatment. The primary irritation score was reported to be 1.0, indicating that p-DCB is a mild skin irritant.

   Toxicity Category: IV

   Classification - Core-Supplementary (for the following deficiencies: The purity of the test article was not specified; inadequate description of the study and/or data presented).

007021

Reviewed by: Y.M. Ioannou 2/3/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M.P. Copley 2/3/89
Section II, Toxicology Branch I (IRS)

DATA EVALUATION REPORT

Study Type: Teratology - Rat (83-3)

TOX Chem No.: 632
MRID No.: 405210-03

Test Material: Paradichlorobenzene

Synonyms: 1,4-Dichlorobenzene

Testing Facility: University of Milan
Milan, Italy

Title of Report: Teratologic Evaluation of p-Dichlorobenzene in the Rat.

Author(s): E. Giann, M.L. Broccia, M. Prati, and C. Vismara

Report Issued: 1986

Conclusions:

p-DCB was administered by gavage to female CD rats between days 6 and 15 of gestation at dose levels of 0, 250, 500, 750, or 1000 mg/kg/day. The maternal LEL was 500 mg/kg/day (retardation of body weight gain) and the NOEL was 250 mg/kg/day. The developmental LEL was 1000 mg/kg/day (body weight depression) and the NOEL 750 mg/kg/day. The teratogenic NOEL was greater than 1000 mg/kg/day, the HDT.

This study (published in "Bulletin of Environmental Contamination and Toxicology" 37, 1986) is not acceptable due to the limited data provided.

Classification: Core-Supplementary

Reviewed by: Y.M. Ioannou 2/23/89
Section II, Toxicology Branch I (IRS)
Secondary Reviewer: M.P. Copley 2/3/89
Section II, Toxicology Branch I (IRS)

DATA EVALUATION REPORT 007021

Study Type: Acute Oral and Dermal Toxicity

TOX Chem No.: 632
MRID No.: 405210-01

Test Material: Paradichlorobenzene

Synonyms: 1,4-Dichlorobenzene

Study Nos.: Unknown

Sponsor: U.S. Environmental Protection Agency

Testing Facility: Health Effects Research Lab
U.S. Environmental Protection Agency
Research Triangle Park, NC

Title of Report: Acute Toxicity of Pesticides in Adult and Weanling Rats Attached

Author(s): T.B. Gaines and R.E. Linder

Report Issued: 1986

The acute oral and acute dermal LD_{50} values for paradichlorobenzene (p-DCB) (and another 56 pesticides) were evaluated in male and female Sherman (SPF) rats, by the authors in a study published in "Fundamental and Applied Toxicology" 7, 1986.

Acute Oral LD_{50}: p-DCB (technical grade,>99% purity) was dissolved in peanut oil at 4 different concentrations and administered orally (gavage) to 4 groups of male and 4 groups female (10 animals/group) SPF Sherman rats. Animals were observed for 14 days after dosing. The LD_{50} values were calculated using Finney's maximum likelihood probit technique.

The acute oral LD_{50} value for male rats was found to be 3863 mg/kg (95% CL of 3561-4153 mg/kg), and for female rats was 3790 mg/kg (95% CL of 3425-4277 mg/kg).

Toxicity Category: III

Classification: Core-Minimum

Acute Dermal LD_50: p-DCB (technical grade, >99% purity) was dissolved in xylene and applied to the skin of 10 male and 10 female SPF Sherman rats at a concentration of 6000 mg/kg. The animals were observed for 14 days after dosing for toxicity and mortality.

No mortality was observed throughout this study. Thus, the acute dermal LD_50 value in male and female rats was found to be greater than 6000 mg/kg.

Toxicity Category: III

Classification: Core-Minimum