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

CASWELL FILE JUL 22 1994
→ #079A

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM: PC CODE 098901

SUBJECT: **PROXEL® Press Paste** - Review of upgrade information and prepare reviews for data package

EPA IDENTIFICATION NUMBERS: P.C. Code: 098901
Caswell No.: 079A
DP Barcode: D194530
Submission No.: S446850

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 21 July 94.
Toxicology Branch II, Section IV
Health Effects Division (7509C)

TO: Tom Myers
Product Manager (51)
Registration Division (7505C)

THRU: Susan L. Makris, M.S. Susan L Makris 7/21/94
Toxicology Branch II, Head Section IV
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 7/21/94
Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Zeneca, Inc. (ICI Americas, Inc.)

Chemical: PROXEL press paste, 1,2-Benzisothiazolin-3-one

Action Requested: Review additional data pertaining to test sample composition and prepare Data Evaluation Records for acute, mutagenicity and developmental toxicity studies.

The Registrant has provided additional information pertaining to the analytical chemistry of the test compound. The additional information was judged to be adequate; Data Evaluation Reports of the submitted studies have been prepared for the following studies:

1. Acute Oral (§81-1)

PROXEL® Press Paste: Acute Oral Toxicity to the Rat (MRID No's: 410221-01, original; 428581-01, addendum)

CLASSIFICATION: Core - Guideline
TOXICITY CATEGORY III

Male and female Wistar rats (5/group/sex) were orally gavaged with test article at doses of 100, 300, 500 or 900 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. At 300 mg/kg and higher doses, piloerection and upward curvature of the spine were observed. Signs of dehydration were present at 500 and 900 mg/kg doses. At 900 mg/kg decreased activity, prostration, decreased abdominal muscle tone, hypothermia, pale appearance, pinched in sides, reduced righting reflex, and decreased depth and rate of breathing were also observed. Animals found dead or sacrificed in moribund condition included 1/5 females at 500 mg/kg and 3/5 females and 5/5 males at 900 mg/kg. No treatment-related gross pathological findings were present. The acute oral LD_{50} in rats is 670 mg/kg in males and 784 mg/kg in females.

2. Acute Dermal (§81-2)

PROXEL® Press Paste: Acute Dermal Toxicity to the Rat (MRID Nos: 410221-02, original; 428581-02, addendum)

CLASSIFICATION: Core - Guideline
TOXICITY CATEGORY II

Wistar rats (5/sex) were dermally exposed to 2000 mg/kg test article for 24 hours and observed for 14 days for signs of toxicity, mortality and moribundity. All animals survived to terminal sacrifice. Clinical signs of toxicity included urine incontinence a nd upward curvature of the spine; these effects were transient and were absent after Day 5. Dermal toxicity included desquamation in all animals, erythema in one male and four females and edema in one female. No treatment-related gross pathological findings were present. The acute dermal LD_{50} was greater than 2000 mg/kg (limit dose) in males and females.

3. Dermal Sensitization study (§81-6)

Benzisothiazolin-3-one: Skin Sensitization to the Guinea Pig (MRID Nos.: 417500-01, original; 428581-03, addendum)

CLASSIFICATION: Core - Guideline

The Maximization test was used to evaluate the dermal sensitization potential of test article in female guinea pigs. A 3% solution of test article produced scattered mild redness in 2/20 animals at 24 hrs and 1/20 at 48 hrs. A 10% solution produced moderate diffuse redness to intense redness and swelling

in 10/20 at 24 hr and 8/20 at 48 hr with a net response of 35 and 45%, respectively. The test article was graded as having moderate dermal sensitization potential.

4. Developmental Toxicity - Teratology Study [§83-3(a)]

PROXEL® Press Paste: Teratology Study in the Rat (MRID Nos: 409612-01, original; 428581-04, addendum)

CLASSIFICATION: Core - Minimum

Alpk:APfSD (Wistar-derived) rats (24/dose group) were orally gavaged with Proxel press paste at dosages of 0 (vehicle only), 10, 40 or 100 mg/kg/day (adjusted for percent purity of active ingredient) during gestation days 7 through 16, inclusive.

Maternal Toxicity: No treatment-related mortalities were observed during the study; one high-dose animal died as a result of gavaging error on GD 17. Body weight gain was significantly decreased at 100 mg/kg/day during GDs 7-16 and 10-13 of the dosing period, and to a lesser, but not significant, extent at 40 mg/kg/day. Food consumption was significantly decreased in high-dose animals during GDs 7-16, 10-13 and 13-16 of the dosing period and GDs 16-19 of the post-dosing period; the decrease in food consumption correlated well with the decrease in mean body weights.

The maternal toxicity LOEL = 100 mg/kg/day and the maternal toxicity NOEL = 40 mg/kg/day, based on decreased body weight gain and decreased food consumption.

Developmental Toxicity: A slight, but statistically significant decrease in the mean fetal body weight was observed in the high-dose group, no other treatment-related differences in cesarean section data were noted. All pregnant dams delivered litters with viable fetuses. The mean numbers of corpora lutea and percent pre- and post-implantation loss were also comparable in all study groups.

Treatment-related increases in the incidence of skeletal, but not external or visceral, abnormalities were noted the in high-dose group, where statistically significant increases in the litter and fetal incidence of nonossified 5th cervical vertebral centrum were observed. Also observed in the high-dose group were statistically significant increases in the number of fetuses, but not litters, with a single nonossified cervical centrum (2nd through 4th vertebrae), nonossified odontoid and calcaneum (unilateral) and partially ossified 5th sternebra. Although statistically significant fetal findings were present in the mid-dose group, there was a lack of a dose-response relationship in several observations. Further, because of the absence of significant litter findings, the lack of correlation to other indicators of inhibited fetal development, and comparisons to historical control data, the observed effects in the mid-dose group do not appear to be related to treatment.

The Developmental Toxicity LOEL = 100 mg/kg/day and the Developmental Toxicity NOEL = 40 mg/kg/day based decreased fetal body weight and increased incidence of delays in skeletal ossification.

6. **Gene Mutation - L5178Y Lymphoma Cells [§84-2(a)]**

PROXEL® Press Paste: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells (MRID No.: 410221-03, original; 428581-05, addendum)

CLASSIFICATION: Core - Acceptable

Cytotoxicity and mutation frequency of Proxel press paste was evaluated in L5178Y (TK +/-) mouse lymphoma cells at 0.03 to 2.00 µg/ml without metabolic activation (-S9) and 1 to 64 µg/ml with activation (+S9). In three separate experiments, survival ranged from 9 to 53% at the highest dose -S9 and from 15 to 75% +S9. Within each experiment, cytotoxicity increased with increasing concentration of test compound; however, inter-experimental comparisons at the same dose levels produced varying results. All of the mutation frequencies without metabolic activation and essentially all with metabolic activation were less than two-times the negative control mutation frequency. There did not appear to be any strong inter-experimental correlation between the observed cytotoxicity and the mutation frequency. No biologically significant increase in mutation frequency was noted at the thymidine kinase locus either with or without metabolic activation at any of the concentrations tested.

7. In vivo **Micronucleus Test - Mice [§84-2(b)]**

PROXEL® Press Paste: An Evaluation in the Mouse Micronucleus Test (MRID Nos: 410221-04, original; 428581-06, addendum)

CLASSIFICATION: Core - Acceptable

The test compound, at 245 or 392 mg/kg (males) and 331 or 529 mg/kg (females), was administered by oral gavage to male and female mice (5/dose/sex). After treatment, bone marrow was harvested 24 hrs for all study groups and at 48 and 72 hrs for the high-dose animals.

Based on the observed mortality, the test compound was evaluated at an adequate top dose. No significant differences were noted in the percentage of PCE/NCE, indicating a lack of cytotoxicity to the bone marrow. No significant increases in the frequency of micronucleated polychromatic erythrocytes were noted in the bone marrow at any of the times tested.

8. **Mutagenicity: UDS Assay in Primary Rat Hepatocytes (§84-4)**

PROXEL® Press Paste: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures (MRID Nos: 410221-05, original; 428581-07, addendum)

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CLASSIFICATION: Core - Acceptable

The test compound was evaluated over a concentration range of 10^{-8} to 10^{-2}M. At 10^{-4}M and higher cytotoxicity was observed. UDS was therefore assessed over a range of 10^{-8} to 10^{-5}M; at 10^{-5} M, some cytotoxicity was observed. The percent of cells in repair and the mean nuclear, cytoplasmic and net grain counts for control and treated cells were comparable to each other. The positive control cells showed a higher percent of cells in repair and marked increases in the mean nuclear, cytoplasmic and net grain counts, compared to the solvent control. The test compound did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 26 Apr 94
Section IV, Tox. Branch II (7509C)

011114

Secondary Reviewer: Susan L. Makris, M.S. Susan L Makris 5/2/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute oral - rat (§81-1)

EPA ID NO's: MRID No.: 410221-01 (Original Submission)
428581-01 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST MATERIAL: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one

STUDY NUMBER: CTL/P/2079

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc, Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: PROXEL® Press Paste: Acute Oral Toxicity to the Rat

AUTHORS: A.M. Leah (Original Submission).
M.E. Burt (Addendum)

REPORT ISSUED: 10 June 1988 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: Male and female Wistar rats (5/group/sex) were orally gavaged with test article at doses of 100, 300, 500 or 900 mg/kg and observed for 14 days for signs of toxicity, mortality and moribundity. At 300 mg/kg and higher doses, piloerection and upward curvature of the spine were observed. Signs of dehydration were present at 500 and 900 mg/kg doses. At 900 mg/kg decreased activity, prostration, decreased abdominal muscle tone, hypothermia, pale appearance, pinched in sides, reduced righting reflex, and decreased depth and rate of breathing were also observed. Animals found dead or sacrificed in moribund condition included 1/5 females at 500 mg/kg and 3/5 females and 5/5 males at 900 mg/kg. No treatment-related gross pathological findings were present. **The acute oral LD_{50} in rats is 670 mg/kg in males and 784 mg/kg in females.**

This study is classified as **Core - Guideline Data (Acceptable)** with a **TOXICITY CATEGORY III** and satisfies the guideline requirements (§81-1) for an acute oral toxicity (LD_{50}) study in rats.

I. MATERIALS and METHODS

A. Test Compound: PROXEL press paste Description: Yellow/brown moist powder Batch No.: ADH374793 BX973 Reference No: Y00180/020/001 Purity: 73.1% (w/w) [redacted]

B. Test Animals: Species: Rat Strain: Alpk:APfSD (Wistar derived) Age: not stated Weight (g): 240-267 (males), 182-206 (females) Source: Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK Housing: Five/cage Feed: Porton Combined Diet, Special Diets Services Ltd., *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature: 15 to 24°C; Humidity: 50 ± 10%; Air changes: 20 to 30/hr; Light cycle: 12 hr light/12 hr dark

C. Study Design: Animals (5/dose/sex) were randomly assigned to treatment groups and, following a 16 to 24 hr fasting, were orally gavaged with test article at doses of 100, 300, 500 or 900 mg/kg. Test article was dissolved in 0.5% (w/v) aqueous polysorbate 80 and administered in a dose volume of 10 ml/kg. Dosing formulations were analyzed by HPLC and determined to be between 95 and 103% of nominal. Animals were observed for signs of toxicity, moribundity and mortality once between 30 and 60 min after dosing, twice between 2.5 and 5 hr, and once daily, thereafter, for 14 days. Animals were weighed on Days -1, 1 (day of dosing), 3, 4, 8, and 15; high-dose females were also weighed on Days 2 and 6, but not on Day 4. At the end of the observation period surviving animals were necropsied for gross pathological examination.

D. Statistics: Means and standard deviations were calculated for animal body weights. The LD_{50} was calculated using either probit analysis (females) or linear log dose interpolation (males).

II. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

III. RESULTS and DISCUSSION: Treatment-related clinical signs are summarized in Appendix 1. The number of signs, the severity and the duration of clinical signs, in general, increased with increasing dose. No toxicity was evident at 100 mg/kg. At 300 mg/kg and higher, piloerection and upward curvature of the spine were observed. Signs of dehydration were present at 500 and 900 mg/kg doses. At 900 mg/kg decreased activity, prostration, decreased abdominal muscle tone, hypothermia, pale appearance, pinched in sides, reduced righting reflex, and decreased depth and rate of breathing were also observed.

No treatment-related changes in body weights or gross pathological findings were observed.

Mortality data are presented in Table 1. On Day 1, the high-dose group had five deaths, four males and one female; one female was sacrificed *in extremis*. On Day 2, one 500 mg/kg female and one 900 mg/kg male were sacrificed *in extremis*. The calculated LD_{50} values are 670 mg/kg for males and 785 mg/kg for females.

Table 1: Summary of mortality data[a]

Dose (mg/kg)	Mortality	
	Males	Females
100	0/5	0/5
300	0/5	0/5
500	0/5	1/5
900	5/5	3/5

[a] Summarized from Table 1 of the report

IV. CONCLUSIONS: Based on the results of this study, the oral LD_{50} values in rats were 670 mg/kg for males and 784 mg/kg for females. Approximate 95% confidence intervals were 500 - 900 mg/kg for males and 475 mg/kg for females.

Toxicity category III

Classification: Core - Guideline

This study satisfies guideline requirements (§81-1) for an acute oral toxicity study in the rat.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 26 Apr 94
Section IV, Tox. Branch II (7509C)

011114

Secondary Reviewer: Susan L. Makris, M.S. Susan L Makris 5/2/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute dermal - rat (§81-2)

EPA ID NO's: MRID No.: 410221-02 (Original Submission)
428581-02 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST ARTICLE: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one

STUDY NUMBER: CTL/P/2065

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc., Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: PROXEL® Press Paste: Acute Dermal Toxicity to the Rat

AUTHORS: A.M. Leah (Original submission)
M.E. Burt (Addendum)

REPORT ISSUED: 10 June 1988 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: Wistar rats (5/sex) were dermally exposed to 2000 mg/kg test article for 24 hours and observed for 14 days for signs of toxicity, mortality and moribundity. All animals survived to terminal sacrifice. Clinical signs of toxicity included urine incontinence and upward curvature of the spine; these effects were transient and were absent after Day 5. Dermal toxicity included desquamation in all animals, erythema in one male and four females and edema in one female. No treatment-related gross pathological findings were present. **The acute dermal LD_{50} was greater than 2000 mg/kg (limit dose) in males and females.**

This study is classified as **Core - Guideline Data (Acceptable)** with a **TOXICITY CATEGORY II** and satisfies the guideline requirements (§81-2) for an acute dermal toxicity (LD_{50}) study in rats.

I. MATERIALS and METHODS

A. Test Compound: PROXEL press paste Description: Yellow/brown moist powder Batch No.: ADH 374793 Bx973 Reference No: Y00180/020/001 Purity: 73.1% (w/w) [redacted]

B. Test Animals: Species: Rat Strain: Alpk:APfSD (Wistar derived) Age: not stated Weight (g): 249-261 (males), 180-189 (females) Source: Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK Housing: Two/cage Feed: Porton Combined Diet, Special Diets Services Ltd., *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature: 15 to 24°C; Humidity: 50 ± 10%; Air changes: 20 to 30/hr; Light cycle: 12 hr light/12 hr dark

C. Study Design: Animals (5/sex) were randomly assigned to a single 2000 mg/kg treatment group. Animals were prepared one day before dosing by clipping a 10 cm x 5 cm area of the dorsal-lumbar region free of hair. A weighed amount of test article was mixed with 0.3 or 0.5 ml olive oil to form a paste, which was spread on the bare skin and covered with a 4 cm x 6 cm gauze pad. The gauze pad was covered with plastic film and held in place with adhesive bandage and two pieces of PVC tape (wrapped around the animal). After the 24-hour exposure, the dressing was removed and the application site cleaned with cotton swabs dipped in water and dried with tissue paper. Animals were observed once between 1 and 4 hours after dosing and daily, thereafter, for 14 days for signs of dermal and systemic toxicity, moribundity and mortality. Animals were weighed on Day 1, immediately before dosing and on Days 3, 4, 8, and 15. At the end of the observation period animals were necropsied for gross pathological examination.

D. Statistics: Means and standard deviations were calculated for animal body weights.

II. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

III. RESULTS and DISCUSSION: All animals survived to terminal sacrifice; no treatment-related changes in body weights or gross pathological findings were observed. Systemic effects consisted of urine incontinence and upward curvature of the spine (Table 1). Although the study states that these effects were caused by the occlusive dressing, the lack of a control group makes this conclusion difficult to confirm. After removal of the dressing, however, the effects persisted through Day 5 for upward curvature of the spine and Day 4 for urine incontinence.

Dermal effects are summarized in Table 2. Desquamation generally appeared at about Day 6 and lasted through Day 13; this finding was still present in one male and one female at terminal sacrifice. On Day 2, erythema appeared in four females and one male; on Days 3 and 4 only one male and one female were affected. Edema was present in one female on Day 2.

Table 1: Incidence of Systemic Effects[a]

Observation		Males	Females
Upward curvature of the spine	Day 2	5/5	5/5
	Day 3	3/5	1/5
	Day 4	3/5	0/5
	Day 5	1/5	0/5
Urine Incontinence	Day 2	3/5	5/5
	Day 3	3/5	2/5
	Day 4	1/5	0/5

[a] Summarized from Table 2 of the report

Table 2: Incidence of Dermal Effects[a]

Observation	Male	Female
Desquamation	5/5	5/5
Erythema	1/5	4/5
Edema	0/5	1/5

[a] Data summarized from Table 2 of the report

CONCLUSIONS: Based on the results of this study, the dermal LD_{50} value was greater than 2000 mg/kg (limit dose) for male and female rats.

Toxicity category II

Classification: core - Guideline

This study satisfies guideline requirements (§81-2) for an acute dermal toxicity study in the rat.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 28apr94.
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Susan L. Makris, M.S. Susan L Makris 5/2/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - Guinea Pig (81-6)

EPA ID NO's: MRID No.: 417500-01 (Original Submission)
428581-03 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST MATERIAL: 1,2-Benzisothiazolin-3-one

SYNONYMS: PROXEL press paste

STUDY NUMBER: CTL/P/3101

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc., Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: Benzisothiazolin-3-one: Skin Sensitization to the Guinea Pig

AUTHORS: P. Botham and P. Robinson (Original Submission)
M.E. Burt (Addendum)

REPORT ISSUED: 19 October 1990 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: The Maximization test was used to evaluate the dermal sensitization potential of test article in female guinea pigs. A 3% solution of test article produced scattered mild redness in 2/20 animals at 24 hrs and 1/20 at 48 hrs. A 10% solution produced moderate diffuse redness to intense redness and swelling in 10/20 at 24 hr and 8/20 at 48 hr with a net response of 35 and 45%, respectively. The test article was graded as having moderate dermal sensitization potential.

This study is classified as **Core - Guideline Data (Acceptable)** and satisfies the guideline requirements (§81-6) for a dermal sensitization study in guinea pigs.

B

I. MATERIALS

A. Test Compound: 1,2-Benzisothiazolin-3-one Description: off-white solid Batch No.: NBW 8008/71 and NBW 7015/16 Reference No: Y00180/017 Purity: 99.5% Contaminants: not given

B. Test Animals: Species: Guinea Pig, female Strain: Alpk: Dunkin Hartley Age: not stated Weight (g): 313-393 (females) Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK Housing: one/cage Feed: Labsure RGP Guinea Pig Diet, *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature: 18 to 22°C; Humidity: 50 ± 10%; Air changes: 20 to 30/hr; Light cycle: 12 hr light/12 hr dark

II. STUDY DESIGN

A. Preliminary dermal irritation study: A preliminary dermal irritation study was carried out to determine the doses of test compound to be used in the induction and challenge phases of the main study. For the intradermal induction phase, the criteria for dose selection was the highest concentration, up to 0.01% (w/v), which was well tolerated and free of systemic and dermal effects. For the topical induction phase, the dose selected would be the highest dose which did not produce greater than a mild to moderate irritation response. For the challenge phase the highest, non-irritating dose was selected. Animals used for dose selection in the topical induction and challenge phases were administered Freund's Complete Adjuvant 14 days before use. A dose of 0.01% solution of test article in 3% (w/v) dimethylformamide (DMF) in corn oil was selected for the intradermal induction phase; 30% (w/v) in DMF, for the induction phase; and 3% and 10% (w/v) in DMF, for the challenge phase.

B. Main Study

1. Dermal sensitization was assessed using the Maximization test, in which animals were first induced with intradermal and topical applications of test article, followed by a topical challenge phase. The test solutions and application sites for the induction and challenge phases are summarized in Table 1. Main study animals were weighed at the start of the study only; positive control animals were weighed at the start and end of the study.

2. Induction phase

a. Intradermal Injections: Female guinea pigs were randomly assigned to negative control (10 animals), positive control (30 animals) and treatment (20 animals) groups. A 5 cm x 5 cm area

Table 1: Solutions and Application Sites used for the Induction and Challenge Phases of the Study

GROUP	INDUCTION PHASE Application Sites: Top, Middle, Bottom	CHALLENGE PHASE Application Sites: Right, Left
Treatment	Intradermal Injections T: Freund's Complete Adjuvant diluted 1:1 with 3% (w/v) DMF[a] in corn oil M: 0.01% (w/v) test article in 3% DMF in corn oil B: 0.01% (w/v) test article in a 1:1 dilution of Freund's Complete Adjuvant and 3% (w/v) DMF in corn oil Topical Applications 30% (w/v) test article in DMF	R: 3% (w/v) test article in DMF L: 10% (w/v) test article in DMF
Negative Control	Intradermal Injections T: Freund's Complete Adjuvant diluted 1:1 with 3% (w/v) DMF in corn oil M: 3% (w/v) DMF in corn oil B: Freund's Complete Adjuvant diluted 1:1 with 3% (w/v) DMF in corn oil Topical Application DMF only	R: 3% (w/v) test article in DMF L: 10% (w/v) test article in DMF
Positive Control	Intradermal Injection 0.1% (w/v) of 40% (w/v) formaldehyde in water Topical Application 50% (w/v) of 40% (w/v) formaldehyde in water	R: 50% (w/v) of 40% (w/v) formaldehyde in water L: None

a DMF = Dimethylformamide

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of the scapular region on each animal was clipped free of hair. For negative control and treatment groups, the animals received three (top, middle, and bottom) intradermal injections of 50 to 100 µl each on either side of the mid-line (a total of six injections). Positive controls received a single intradermal injection.

b. Topical Application: One week after the intradermal injections, the control and treatment group animals were reclipped. A 200 to 300 µl aliquot of the appropriate solution (Table 1) was pipetted onto a piece of filter paper (4 cm x 2 cm). The saturated filter paper was then applied to the skin and held in place with an occlusive dressing. After a 48-hour exposure period, the dressing was removed.

2. Challenge Phase: Two-weeks after the topical induction phase, the right and left flank areas (15 cm x 5 cm) of all the treatment and control animals were clipped free of hair. An occlusive dressing was prepared by stitching two pieces of filter paper to a backing of rubber sheeting. Fifty to 100 µl aliquots each of the appropriate solutions (Table 1) were applied to the two pieces of filter paper. The pieces of filter paper were placed on the appropriate application sites, held in place with adhesive bandage and secured with plastic tape.

3. Scoring: After 24 hrs, the patches were removed and the challenge sites were evaluated and graded for erythema 24 and 48 hrs later. The percent net response, defined as the % response of treated animals with scores ≥ 1 minus the % response of negative control animals with scores ≥ 1, was determined and graded using the following scale:

1-8%	Weak sensitizer
9-28%	Mild sensitizer
29-64%	Moderate sensitizer
65-80%	Strong sensitizer
81-100%	Extreme sensitizer

II. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

III. RESULTS: The results of the study are summarized in Table 2. A 3% solution of test article produced scattered mild redness in 2/20 animals at 24 hrs and 1/20 at 48 hrs; the net % responses were 10% at 24 hrs and 5% at 48 hrs. A 10% solution produced scattered mild redness in 3/20 animals at 24 hrs and 5/20 animals at 48 hrs; moderate diffuse redness to intense redness and swelling were observed in 10/20 animals at 24 and 8/20 animals at

48 hrs; the net % response was 35% at 24 and 45% at 48 hrs. The positive control material produced scattered mild redness to moderate diffuse redness in 14/17 animals, with a net % response of 82% and was considered to adequately validate the test procedures.

Table 2: Results (Data summarized from Tables 1 and 3 of the report)

GROUP	Incidence of Dermal Effects by Grade[a]							
	24 Hours				48 Hours			
	0	1	2	3	0	1	2	3
Treatment 3%	18/20	2/20	0/20	0/20	19/20	1/20	0/20	0/20
Treatment 10%	7/20	3/20	8/20	2/20	7/20	5/20	7/20	1/20
Negative Control 3%	10/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10
Negative Control 10%	7/10	3/10	0/10	0/10	8/10	2/10	0/10	0/10
Positive Control[b] 50%	4/17	12/17	1/17	0/17	4/17	11/17	2/17	0/17
Positive Control Vehicle 50%	10/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10

a 0 - No reaction
1 - Scattered mild redness
2 - Moderate diffuse redness,
3 - Intense redness and swelling

b As a result of one death and two bandage failures, the number of animals in the positive control group was reduced from 20 to 17.

IV. CONCLUSIONS: Based on the results of the study, the test compound was found to produce moderate dermal sensitization.

Classification: Core - Guideline

This study satisfies guideline requirements (§81-6) for a dermal sensitization study in guinea pigs.

011114

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 21 July 94
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Susan L. Makris, M.S. Susan L Makris 7/21/94
Section IV, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Teratology, Rat, 83-3(a)

EPA ID NO's: MRID No.: 409612-01 (Original Submission)
428581-04 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST MATERIAL: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one
1,2-Benzisothiazol-3(2H)-one
BIT

STUDY NUMBER: CTL/P/2297

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc., Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: PROXEL® Press Paste: Teratology Study in the Rat

AUTHOR: G.H. Pigott (Original Submission)
M.E. Burt (Addendum)

REPORT ISSUED: November 1988 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: In a developmental toxicity (teratology) study (MRID No.: 409612-01 and 428581-04), Alpk:APfSD (Wistar-derived) rats (24/dose group) were orally gavaged with Proxel press paste at dosages of 0 (vehicle only), 10, 40 or 100 mg/kg/day (adjusted for percent purity of active ingredient) during gestation days (GD) 7 through 16, inclusive, with the day of mating designated as GD 1. Dams were cesarean sectioned on GD 22.

Maternal Toxicity: No treatment-related mortalities were observed during the study; one high-dose animal died as a result of gavaging error on GD 17. Body weight gain was significantly

A

decreased at 100 mg/kg/day during GDs 7-16 and 10-13 of the dosing period, and to a lesser, but not significant, extent at 40 mg/kg/day. Food consumption was significantly decreased in high-dose animals during GDs 7-16, 10-13 and 13-16 of the dosing period and GDs 16-19 of the post-dosing period; the decrease in food consumption correlated well with the decrease in mean body weights.

The **Maternal Toxicity LOEL = 100 mg/kg/day and the Maternal Toxicity NOEL = 40 mg/kg/day,** based on decreased body weight gain and decreased food consumption.

B. Developmental Toxicity: A slight, but statistically significant decrease in the mean fetal body weight was observed in the high-dose group, no other treatment-related differences in cesarean section data were noted. All pregnant dams delivered litters with viable fetuses. The mean numbers of corpora lutea and percent pre- and post-implantation loss were also comparable in all study groups.

Treatment-related increases in the incidence of skeletal, but not external or visceral, abnormalities were noted the in high-dose group, where statistically significant increases in the litter and fetal incidence of nonossified 5th cervical vertebral centrum were observed. Also observed in the high-dose group were statistically significant increases in the number of fetuses, but not litters, with a single nonossified cervical centrum (2nd through 4th vertebrae), nonossified odontoid and calcaneum (unilateral) and partially ossified 5th sternebra. Although statistically significant fetal findings were present in the mid-dose group, there was a lack of a dose-response relationship in several observations. Further, because of the absence of significant litter findings, the lack of correlation to other indicators of inhibited fetal development, and comparisons to historical control data, the observed effects in the mid-dose group do not appear to be related to treatment.

The **Developmental Toxicity LOEL = 100 mg/kg/day and the Developmental Toxicity NOEL = 40 mg/kg/day** based decreased fetal body weight and increased incidence of delays in skeletal ossification.

The study is classified as Core - Minimum Data (Acceptable) and satisfies guideline requirements [§83-3(a)] for Teratology - Developmental Toxicity in the rat.

I. MATERIALS

A. Test Compound: PROXEL press paste Description: pale brown, damp powder Batch No.: ADH374793 BX973 Reference No: Y00180/020/001 Purity: 73.4% (w/w) [redacted]

B. Dosing Vehicle: 0.5% (w/v) hydroxy methyl propyl cellulose (HMPC) in 0.1% aqueous polysorbate 80.

C. Test Animals: Species: Rat Strain: Alpk:APfSD (Wistar-derived) Age: 12 weeks Weight (g): 217-292 (females) Source: Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK Housing: one/cage Feed: CT1 diet, Special Diets Services Ltd., *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature: 18 to 28°C; Humidity: 34-58%; Air changes: ≥ 12/hr; Light cycle: 12 hr light/12 hr dark

II. METHODS

A. Study Design: This study was designed to assess the developmental toxicity potential of Proxel press paste. Test compound was administered by oral gavage (10 ml/kg) at dosage levels of 0 (vehicle only), 10, 40 or 100 mg/kg. Animals were dosed once in the morning on gestation days (GD) 7 through 16, inclusive.

1. Mating: One female was placed in a cage overnight with a single male. Mating was confirmed the next morning by the presence of sperm (vaginal smear). The day of observation of sperm was designated as GD 1.

2. Group Arrangement: The animals were randomly assigned to the test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group	Dosage[a] (mg/kg/day)	Number Assigned
Control	0[b]	24
Low	10	24
Middle	40	24
High	100	24

[a] Doses corrected for percent purity of active ingredient.
[b] Control animals received vehicle only.

B. Dose Preparation and Analytical Chemistry: Bulk (2000 ml) dosing suspensions were prepared by mixing a weighed amount of Proxel press paste (corrected for percent purity of active ingredient) with vehicle; dosing solutions were

aliquoted into bottles and stored in the dark at 4°C until used. Samples of the low and high dosing solutions, taken at the start, middle and end of the aliquoting process, were analyzed to determine homogeneity. Aliquots of each dosing solution were analyzed before the start of and periodically during the study to verify the achieved concentration and stability.

C. Maternal Observations and Evaluations

1. Clinical observations: Animals were checked once daily for signs of toxicity, mortality and moribundity. Detailed clinical examinations were performed during the dosing period and at the time animals were weighed.

2. Body weights: Animals were weighed on GDs 1, 4, 7 through 16 (inclusive), 19 and 22.

3. Food consumption: Food consumption was measured over three day intervals. On GDs 1, 4, 7, 10, 13, 16, and 19, animals were presented with a weighed amount of food. At the end of each three day interval, the remaining food was weighed and the daily food consumption was determined.

4. Terminal observations: Animals were sacrificed with halothane on GD 22 and examined for gross abnormalities of the thoracic, abdominal and pelvic viscera. Animals which died or were sacrificed moribund during the study were also examined. After determining the gravid uterine weight, the number of live fetuses and intra-uterine deaths was determined. The number of *corpora lutea* in each ovary were also determined.

D. Fetal evaluations: Live fetuses were dissected from the uterus, weighed, killed by an intracardial injection (0.1 ml) of phenobarbital, and examined for externally abnormalities and for cleft palate. Visceral morphological abnormalities and sex were assessed at internal examination under magnification. After evisceration and fixation in methanol, the head of each fetus was sectioned at the fronto-parietal suture line and the brain examined. The carcasses were processed, stained with Alizarin Red S, and examined for skeletal abnormalities and degree of ossification. The study classified the abnormalities as major (rare and/or possibly lethal), minor (common deviations from normal) or variants (historically common variations). The individual bones of the *manus* and *pes* were also assessed and scored.

E. Statistical Analysis: Analysis of variance (ANOVA) was performed on parametric data; the percentage of pre- and post-implantation losses were double arcsine-transformed

before analysis. The litter was used as the basic unit of analysis as appropriate. Significant differences between individual treatment and control groups were evaluated using Student's t-test based on the error mean square of the analysis. Incidence data (proportional) were analyzed using Fisher's Exact Test. Non-pregnant or animals which died during the study were not included in the statistical analyses.

III. REGULATORY COMPLIANCE

A. Quality assurance: Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. Confidentiality claim: A statement of "no confidentiality claims" was provided.

C. Flagging Statement: The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

IV. RESULTS

A. Analytical Chemistry: Analysis of samples, taken at the start, middle and end of the aliquoting procedure, confirmed that the test compound was homogeneously distributed in the low- and high-dose suspensions; the analytical results were within 95.7 and 102.1% of the nominal concentrations. For samples of dose solutions taken before the start of the study, the achieved concentrations were within 92.0 and 97.5% of the target levels. Periodic analyses during the study, indicated that the dosing solutions remained stable (> 94.5% of nominal) for 34 days.

B. Maternal Observations and Evaluations

1. Clinical Observations: One high-dose animal died as a result of gavaging error on GD 17, all remaining animals survived until terminal sacrifice. Abnormal respiratory noise was observed in four mid-dose animals from GDs 11 through 22 and ten high-dose animals from GDs 8 through 22. No other treatment-related clinical observations were noted.

2. Body Weight Gain: Maternal body weight gains during the pre-dosing, dosing and post-dosing periods are summarized in Table 2. Body weight gains of all groups prior to dosing were comparable. During the dosing period, body weight gain was reduced for the 40 and 100 mg/kg/day groups at each interval calculated; statistical significance was achieved only on at GDs 7-16 and 10-13 for the high-dose group. During the post-dosing period, body weight gains of the treated-animals were comparable to control values. Some recovery of

body weight reduction was evident for the high-dose group during the post-dosing period, but overall (GDs 1-22) body weight gains were still reduced 9% from control.

Table 2: Initial body weights (g) and weight gains (g) of maternal animals (Data taken from study Table 6)

Period	Gestation Day	Dosage (mg/kg/day)			
		0	10	40	100
Initial weight	1	251.5	252.1	255.8	254.9
Pre-dosing	1-7	35.8	32.9	33.9	34.9
Dosing	7-16	47.0	49.9	40.4	35.4**
	7-10	12.9	14.1	10.8	10.1
	10-13	16.9	16.7	15.8	9.4**
	13-16	17.2	19.1	13.8	15.9
Post-dosing	16-19	32.6	33.4	36.2	29.6
	19-22	24.0	23.7	17.3	27.6
	16-22	56.5	57.1	53.4	57.1
Overall gain	1-22	139.3	139.9	127.7	127.4

** $p \leq 0.01$, compared to control value

3. Food Consumption: Maternal food consumption during the pre-dosing, dosing and post-dosing periods is summarized in Table 3. These data were highly correlated to decreases in body weight gain for the high-dose group (Table 2). No significant differences were noted during the pre-dosing period. During the entire dosing period (GDs 7-16) and during GDs 10-13 and 13-16 of the dosing period, significantly lower food consumption was noted in high-dose animals. Decreased food consumption was also evident in high-dose animals during GDs 16-19 of the post-dosing period; food consumption returned to control values during GDs 19-22.

4. Gross Pathology: Gross pathological examination at terminal sacrifice revealed that three, high-dose animals had raised lesions on the surface of the non-glandular mucosa of the stomach. No other treatment-related effects were noted. Examination of the animal which died during the study confirmed that gavaging error was the cause of death. No abnormalities of the respiratory tract were reported for those mid- and high-dose females with clinical finding of respiratory noise.

Table 3: Maternal food consumption (g/day) (Data taken from study Table 7)

Period	Gestation Day	Dosage (mg/kg/day)			
		0	10	40	100
Pre-dosing	1-7	23.2	23.1	24.2	24.1
Dosing	7-16	28.4	28.5	27.5	26.1**
	7-10	26.6	26.4	26.3	26.1
	10-13	28.0	28.4	27.7	25.2**
	13-16	30.5	30.5	28.4	26.8**
Post-dosing	16-19	32.0	31.9	30.5	28.7**
	19-22	25.9	25.5	23.5	25.8
	16-22	29.0	28.7	27.0	27.3

** $p \leq 0.01$, compared to control value

5. Cesarean Section Data: The cesarean section data are presented in Table 4. A slight (3.6%), but statistically significant, decrease in the mean fetal body weight was observed in the high-dose group. Neither litter size nor viability were reduced by treatment.

C. Fetal Evaluations: Fetal external, visceral and skeletal observations are summarized in Tables 5 (major defects) and 6 (minor defects and variations). The fetal and litter incidences of external/visceral abnormalities in the treated groups were comparable to controls. Neither the incidence nor distribution of fetal malformations (major defects) were indicative of a response to treatment. The majority of observed skeletal abnormalities consisted of nonossified or partially ossified bones. The high-dose group showed statistically significant increases in the number of fetuses, but not litters, with a single nonossified cervical centrum (2nd through 4th vertebrae), nonossified odontoid and calcaneum (unilateral) and partially ossified 5th sternebra; the number of fetuses and litters with nonossified 5th cervical vertebral centrum was also statistically significantly higher. The mid-dose group showed significant increases in the fetal incidence of nonossified 3rd cervical vertebral centrum and odontoid, and in the number of fetuses with any skeletal variation; both the number and percentage of affected litters were comparable to controls.

Assessment of the individual bones of the *manus* and *pes* did not reveal any treatment-related differences. For the control through high-dose groups, respectively, the mean manus scores were 2.16, 2.09, 2.09 and 2.23; mean pes scores were 2.86, 2.77, 2.88, and 2.87.

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Table 4: Cesarean Section Data (Data summarized from Table 9 and Appendix 5 of the study)

Observations	Dosage (mg/kg/day)			
	0	10	40	100
Total Assigned	24	24	24	24
No. Died - Gravid	0	0	0	1
- Nongravid	0	0	0	0
No. Nongravid	0	1	0	0
No. (%) Gravid	24 (100)	23 (96)	24 (100)	24 (100)
With Nonviable Fetuses Only	0	0	0	0
With Viable Fetuses	24	23	24	23
Corpora Lutea/Litter, Mean (Total)	13.3 (320)	13.7 (314)	13.7 (329)	13.5 (311)
Implantation Sites/Litter, Mean (Total)	12.3 (295)	13.6 (297)	12.8 (306)	12.2 (286)
% Preimplantation Loss (No. females affected)	7.8 (10)	5.4 (8)	7.0 (10)	8.0 (8)
Resorptions/Litter, Mean (%) - Total	14 (4.7)	21 (7.1)	6 (2.0)	5 (1.7)
- Early	11 (3.7)	19 (6.4)	6 (2.0)	5 (1.7)
- Late	3 (1.0)	2 (0.7)	0 (0.0)	0 (0.0)
% Postimplantation Loss (No. females affected)	4.7 (10)	7.1 (12)	2.0 (5)	1.7 (5)
Fetuses/Litter, Mean (Total) - All	11.7 (281)	12.1 (276)	12.5 (300)	12.0 (281)
- Male	5.8 (139)	6.0 (137)	6.9 (166)	5.7 (130)
- Female	5.9 (142)	6.0 (139)	5.6 (134)	6.5 (151)
% Males	49.5	49.6	55.3	46.3
Mean Weights (g) - Litter	58.1	60.7	60.9	57.7
- Fetal Body Weight	4.97	5.04	4.90	4.79*
- Gravid Uterus	84.3	86.9	87.2	82.6

* $p \leq 0.05$ from control value

Table 5: Percentage and number of affected fetuses and litters with major malformations (Summarized from study Tables 10, 11, and 12)

Observation		Dosage (mg/kg/day)			
		0	10	40	100
No. Fetuses (Litters) Examined		281 (24)	276 (23)	300 (24)	281 (23)
External/Visceral Defects					
Total Major Defects	- No.	0 (0)	3 (3)	2 (2)	2 (2)
	- %	0.0 (0.0)	1.1 (13.0)	0.7 (8.3)	0.7 (8.7)
Abdomen: ascites	- No.	0 (0)	1 (1)	1 (1)	1 (1)
	- %	0.0 (0.0)	0.4 (4.4)	0.3 (4.2)	0.4 (4.2)
Liver: cysts attached	- No.	0 (0)	1 (1)	0 (0)	0 (0)
	- %	0.0 (0.0)	0.4 (4.4)	0.0 (0.0)	0.0 (0.0)
Torso: *situs inversus totalis*	- No.	0 (0)	1 (1)	0 (0)	0 (0)
	- %	0.0 (0.0)	0.4 (4.4)	0.0 (0.0)	0.0 (0.0)
Brain: lateral ventricles moderately dilated	- No.	0 (0)	0 (0)	1 (1)	0 (0)
	- %	0.0 (0.0)	0.0 (0.0)	0.3 (4.2)	0.0 (0.0)
Cleft lip: anophthalmia	- No.	0 (0)	0 (0)	0 (0)	1 (1)[a]
	- %	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (4.2)
Skeletal Defects					
Total Major Skeletal Defects	- No.	0 (0)	0 (0)	2 (2)	2 (2)
	- %	0.0 (0.0)	0.0 (0.0)	0.7 (8.3)	0.7 (8.7)
Fused mandibles	- No.	0 (0)	0 (0)	0 (0)	1 (1)[a]
	- %	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (4.2)
Major vertebral defect	- No.	0 (0)	0 (0)	1 (1)	1 (1)
	- %	0.0 (0.0)	0.0 (0.0)	0.3 (4.2)	0.4 (4.2)
Multiple minor defects of limbs and ribs	- No.	0 (0)	0 (0)	1 (1)	0 (0)
	- %	0.0 (0.0)	0.0 (0.0)	0.3 (4.2)	0.0 (0.0)

[a] A single fetus had both skeletal and visceral defects

Table 6: Percentage and number of affected fetuses and litters with minor malformations and variations (Summarized from study Tables 10 and 12)

Observation		Dosage (mg/kg/day)			
		0	10	40	100
No. Fetuses (Litters) Examined		281 (24)	276 (23)	300 (24)	281 (23)
External/Visceral Defects					
Total Affected	- No.	98[a] (24)	98 (23)	99 (23)	79 (23)
	- %	34.9 (100)	35.5 (100)	33.0 (95.8)	28.1 (100)
Minor Defects Only	- No.	38	26	28	21
	- %	13.5	9.4	9.3	7.5
Variations	- No.	65	78	85	61
	- %	23.1	28.3	28.3	21.7
Skeletal Defects					
Total Affected	- No.	268[a] (14)	267 (17)	295 (19)	273 (17)
	- %	95.4 (58.3)	96.7 (73.9)	98.3 (79.2)	97.2 (73.9)
Minor Defects Only	- No.	91	63	113	105
	- %	32.4	22.8	37.7	37.4
3rd Cervical centrum, nonossified	- No.	17 (11)	10 (7)	37** (13)	53** (15)
	- %	6.0 (45.8)	3.6 (30.4)	12.3† (54.2)	18.9† (65.2)
4th Cervical centrum, nonossified	- No.	11 (8)	7 (6)	21 (10)	26** (12)
	- %	3.9 (33.3)	2.5 (26.1)	7.0† (41.7)	9.3† (52.2)
5th Cervical centrum, nonossified	- No.	1 (1)	2 (2)	3 (3)	11** (6*)
	- %	0.4 (4.2)	0.7 (8.7)	1.0 (12.5)	3.9† (26.1)
Skeletal Variations	- No.	266 (24)	267 (23)	294* (24)	269 (23)
	- %	94.7 (100)	96.7 (100)	98.0 (100)	95.7 (96)
Odontoid, nonossified	- No.	68 (20)	66 (19)	103** (21)	92* (20)
	- %	24.2 (83.3)	23.9 (82.6)	34.3† (87.5)	32.7† (87.0)
2nd Cervical centrum, nonossified	- No.	97 (23)	73 (18)	124 (20)	118* (22)
	- %	34.5 (95.8)	26.4 (78.3)	41.3 (83.3)	42.0 (95.7)
5th Sternebra, partially ossified	- No.	63 (22)	58 (16)	79 (21)	95** (20)
	- %	22.4 (91.7)	21.0 (69.6)	26.3 (87.5)	33.8 (87.0)
Calcaneum, nonossified, unilateral	- No.	81 (17)	62 (17)	84 (21)	107* (19)
	- %	28.8 (70.8)	22.5 (73.9)	28.0 (87.5)	38.1 (82.6)

* $p \leq 0.05$, ** $p \leq 0.01$ compared to control values

[a] Data under the heading "No Abnormalities Detected" in Table 12 pages 60 (External/Visceral Defects) and 63 (Skeletal Defects) were incorrectly summarized; the total number and percentage of affected fetuses and litters are summarized in this table.

† Greater than historical control values (see Table 7)

V. DISCUSSION AND CONCLUSIONS

A. Maternal Toxicity: No treatment-related mortalities were observed during the study. Clinical signs, consisting of abnormal respiratory noises, were observed in four mid- and ten high-dose animals from GDs 11-22 and 8-22, respectively; no abnormalities of the respiratory tract were evident at necropsy in any of the affected animals. Body weight gain was significantly decreased at 100 mg/kg/day during GDs 7-16 and 10-13 of the dosing period, and to a lesser, but not significant, extent at 40 mg/kg/day. Food consumption was significantly decreased in high-dose animals during GDs 7-16, 10-13 and 13-16 of the dosing period and GDs 16-19 of the post-dosing period; the decrease in food consumption correlated well with the decrease in mean body weights.

The Maternal Toxicity LOEL = 100 mg/kg/day and the Maternal Toxicity NOEL = 40 mg/kg/day, based on decreased body weight gain and decreased food consumption.

B. Developmental Toxicity: A slight, but statistically significant decrease in the mean fetal body weight was observed in the high-dose group, no other treatment-related differences in cesarean section data were noted. All pregnant dams delivered litters with viable fetuses. The mean numbers of corpora lutea and percent pre- and post-implantation loss were also comparable in all study groups.

Treatment-related increases in the incidence of skeletal, but not external or visceral, abnormalities were noted in high-dose group, where statistically significant increases in the litter and fetal incidence of nonossified 5th cervical vertebral centrum were observed. Also observed in the high-dose group were statistically significant increases in the number of fetuses, but not litters, with a single nonossified cervical centrum (2nd through 4th vertebrae), nonossified odontoid and calcaneum (unilateral) and partially ossified 5th sternebra. Comparison of the study results to historical control values (Table 7), indicates that the fetal incidence (litter incidences were not provided in the study) of nonossified cervical centra (3rd to 5th) and odontoid, but not partial ossification of the 5th sternebra or nonossified calcaneum, in the mid- and high-dose groups were greater than the historical control values. The significant increase in partially ossified 5th sternebra in the high-dose group was a result of an unusually low incidence in the concurrent control group when compared to the historical. Although statistically significant fetal findings were present in the mid-dose group, there was a lack of a dose-response relationship in several observations. Further, because of the lack of significant litter findings, the lack of fetal body weight decreases, and lack of decreases in manus/pes scores, the observed findings in the mid-dose group do not suggest

Table 7: Historical control values for skeletal abnormalities[a] (Values interpolated from data presented in Figures 3, 4, 5, 8, 10, and 11 of the study)

Observation [b]	Range (%)[c]
3rd Cervical centrum, nonossified	1.8 to 7.7
4th Cervical centrum, nonossified	1,5 to 4.0
5th Cervical centrum, nonossified	0.3 to 2.3
Odontoid, nonossified	15 to 31
5th Sternebra, partially ossified	29 to 41
Calcaneum, nonossified	33 to 84

[a] Historical data compiled from eight studies conducted within 3 years prior to the current study.
[b] Data not available for incidence of nonossified 2nd cervical centrum
[c] Percentage of fetuses affected

treatment-related effects. The terminal sacrifice at Day 22, rather than Day 21, may have masked some minimal delays in development at the mid-dose.

The **Developmental Toxicity LOEL = 100 mg/kg/day and the Developmental Toxicity NOEL = 40 mg/kg/day** based decreased fetal body weight and increased incidence of delays in skeletal ossification.

The study is classified as Core - Minimum Data (Acceptable) and satisfies guideline requirements [§83-3(a)] for Teratology - Developmental Toxicity in the rat.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 13 June 94 141114
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Byron T. Backus Byron T. Backus 6/17/94
Section II, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Gene Mutation - L5178Y Lymphoma Cells [§84-2(a)]

EPA ID NO's: MRID No.: 410221-03 (Original Submission)
428581-05 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST MATERIAL: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one

STUDY NUMBER: CTL/P/2115

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc, Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: PROXEL® Press Paste: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells

AUTHORS: M.F. Cross (Original submission)
M.E. Burt (Addendum)

REPORT ISSUED: 1 November 1988 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: Cytotoxicity and mutation frequency of Proxel press paste was evaluated in L5178Y (TK +/-) mouse lymphoma cells at 0.03 to 2.00 µg/ml without metabolic activation (-S9) and 1 to 64 µg/ml with activation (+S9). In three separate experiments, survival ranged from 9 to 53% at the highest dose -S9 and from 15 to 75% +S9. Within each experiment, cytotoxicity increased with increasing concentration of test compound, however, inter-experimental comparisons at the same dose levels produced varying results. All of the mutation frequencies without metabolic activation and essentially all with metabolic activation were less than two-times the negative control mutation frequency. There did not appear to be any strong inter-experimental correlation between the observed cytotoxicity and the mutation frequency. **No biologically significant increase in mutation frequency was noted at the thymidine kinase locus either with or without metabolic activation at any of the concentrations tested.**

This study is classified as Core - Acceptable Data and satisfies the guideline [§84-2a)] requirements for a gene mutation assay. 30

I. MATERIALS

A. Test Compound: PROXEL press paste Description: pale brown, damp powder Batch No.: ADH374793 BX973 Reference No: Y00180/020 Purity: 73.1% (w/w) [redacted]

B. Control Materials: Dimethylsulfoxide (DMSO) was used as the negative control material. Positive control materials were ethylmethanesulfonate (EMS) without metabolic activation and N-nitrosodimethylamine (DMN) with metabolic activation. EMS and DMN were dissolved in DMSO to yield final concentrations of 750 µg/ml and 0.9 µl/ml, respectively. The final concentration of DMSO in the media, after addition of either the test solutions or control solutions, was, in all cases, 1.0%.

C. Indicator Cells: L5178Y (-3.7.2c [TK+/-]) mouse lymphoma cells were used as the indicator cells and were stored in liquid nitrogen until use. Cultures were determined to be free of mycoplasma by electron microscopy.

D. Media: Maintenance culture medium (MCM10) was RPMI 1640 supplemented with L-glutamine, penicillin/streptomycin and 10% horse serum. As required by the protocol, the final concentration of horse serum was either 5% (MCM5) for the treatment stage or 20% (MCM20) for microwell plating.

E. Selecting Agent: Trifluorothymidine (TFT)

F. S9 Liver Fraction: The livers of Aroclor 1254-treated (500 mg/kg) Sprague-Dawley rats were used to prepare the S9 fraction. The S9 fractions were aliquoted out and rapidly frozen and stored at -70°C until use. A small sample of the S9 fraction was tested, in duplicate, for sterility by plating on nutrient agar plates and incubating for 48 hours. Before use an ampule of frozen S9 fraction was thawed at room temperature and stored at 4°C.

G. Cofactor Solution: A sterile, 100x solution of cofactor solution was prepared to yield final concentrations of 0.75 mM NADP (Na salt) and 12 mM glucose-6-phosphate.

II. STUDY DESIGN

A. Dose Selection: A preliminary cytotoxicity assay was carried out to determine the doses of test compound to be used in the main study. The range of doses for the main study would include a low-dose, which would produce slight or no decrease in cell survival, and a high-dose, which would markedly decrease (≥ 90%) cell survival.

B. Main Study: The main study consisted of evaluating the cytotoxicity and mutagenicity of the test compound over a broad range of concentrations. Negative (solvent) and

positive (EMS and DMN) controls were run concurrently with the main study. The test compound were evaluated with and without metabolic (S9) activation.

1. Cytotoxicity (survival) assay: For determination of cytotoxicity, aliquots (200 μl) of test solutions in DMSO were added to 20 ml of a suspension culture containing 5 to 6 x 10^5 cells/ml. The test solutions, and controls were assayed in duplicate. The cultures were placed in a CO_2 incubator at 37°C and rotated constantly during the four-hour incubation. At the completion of the incubation, the cells were washed twice (centrifugation at 250 x g for 5 min with resuspension of the pellet in 20 ml of fresh media) to remove the test compound. For the final resuspension, 50 ml of MCM10 was used. An aliquot of each cell suspension was taken and serially diluted with MCM20 to a cell density of 8 cells/ml. Aliquots (200 μl) of each cell suspension was pipetted into two 96-well microplates and incubated for 10 to 12 days, at which time the percent survival was determined using the following equation:

$$\%Survival = \frac{-\ln\frac{\text{No. of Treatment Negative Wells}}{\text{Total No. of Treatment Wells}}}{-\ln\frac{\text{No. of Solvent Control Negative Wells}}{\text{Total No. of Solvent Control Wells}}}$$

where ln = natural log.

To evaluate the effect of metabolic activation, the above procedure was repeated with the addition of 200 μl of cofactor mixture and an aliquot (volume not specified) of the S9 liver fraction to each of the initial 20 ml of suspension cultures (5 - 6 x 10^5 cells/ml).

2. Mutagenicity assay: To evaluate the mutagenicity potential of the test compound, the remaining cell culture suspensions [after removal of an aliquot for the determination of cytotoxicity, above] were incubated at 37°C for 72 hours to allow any mutated cells to express themselves. During the expression time, cell counts were determined daily and dilutions performed, as necessary, to maintain a cell density of 2 x 10^5 cells/ml in a volume of 50 ml. At the end of the expression time, an aliquot of each of the cell suspensions was taken and serially diluted with MCM20 to a density of 8 cells/ml. The remaining cell suspension was divided in two and serially diluted with MCM20 to a cell density of 1 x 10^4 cells/ml in a volume of 100 ml. The 1 x 10^4 cells/ml dilution was used to assess mutagenicity in the presence of 4 μg/ml TFT; the 8 cell/ml dilution was used to assess viability (no added TFT). Each of the TFT-treated cultures were aliquoted (200 μl/well) into two 96-well microplates.

To assess viability, aliquots (200µl/well) of each of the 8 cells/ml suspensions were pipetted into two 96-well microplates (1.6 cells/well). All microplates were placed in a CO_2 incubator, at 37°C, for 11 to 14 days, at which time the number of negative wells were determined.
The mutation frequency (MF) was calculated as follows:

$$MF = \frac{-\ln\frac{\text{No. of Negative Wells (+TFT)}}{\text{Total No. of Wells (+TFT)}}}{-\ln\frac{\text{No. of Negative Wells (-TFT)}}{\text{Total No. of Wells (-TFT)}}} \times \text{Dilution Factor}$$

where ln is the natural log.

C. Response Criteria

1. Positive treatment response: A response is considered positive when both of the following criteria are met: (1) A statistically significant, dose-related increase in the mutation frequency at dose levels which do not produce excessive toxicity (> 10% survival) and (2) a significant increase in the absolute numbers of mutants compared to negative control. The response must be confirmed by repeat experiments.

2. Negative treatment response: A response is considered negative when either of the following criteria are met: (1) No statistically significant dose-related increase is present OR (2) a statistically significant dose-related increase in the mutation frequency at dose levels which show excessive toxicity (≤ 10% survival) without an increase in the absolute numbers of mutants over negative controls. The response must be confirmed by repeat experiments.

3. Positive control response: Both positive controls must produce statistically significant increases in mutation frequency with and without metabolic activation. Lack of a significant response in the presence of a treatment-related effect does not necessarily invalidate the experiment; results, however, must be confirmed by a repeat experiment.

4. Negative control response: Negative controls should be within the historical values for spontaneous mutation frequency of 0.8 to 6.0 x 10^{-6} mutants/survivor.

5. Cloning efficiencies: Post-expression cloning efficiencies for the negative control viability plates should be ≥ 50%.

D. Statistical Evaluations: Data were analyzed using logit regression with the number of empty wells taken as the dependent variable. Changes in the mutation frequency with

dose were analyzed for trend.

III. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

IV. RESULTS AND CONCLUSIONS: The study results are summarized in the Attachment 1 to this report. Cytotoxicity and mutation frequency were evaluated in three independent experiments covering concentration ranges of 0.03 to 1.00 μg/ml, 0.06 to 2.00 μg/ml, and 0.6 to 1.1 μg/ml without metabolic activation and 4 to 32 μg/ml, 1 to 32 μg/ml, and 15 to 64 μg/ml with metabolic activation. Low concentrations produced survival rates comparable to that of the negative controls. High-doses of 1.1 μg/ml (without activation) and 64 μg/ml (with activation) resulted in 9% and 15% survival, respectively. In general, within each experiment, cytotoxicity increased with increasing concentration of test compound. Inter-experimental comparisons at the same dose levels, however, produced varying results. The mean cloning efficiencies for the viability negative controls ranged from 84 to 119%, which were within the acceptable range (Table 1).

Table 1: Cloning Efficiencies (C.E.) for Negative Control Viability Plates[a]

Experiment	Total Negative Wells[b]	C.E.[c] (%)
1 -S9	44	92
+S9	29	118
2 -S9	67.5	65
+S9	28.5	119
3 -S9	50	84
+S9	30	116

a Data extracted from Appendices I through N of the study.

b Total negative wells/192 wells (2 x 96-well plates), values are mean of the two controls

c

$$C.E. = \frac{-\ln \frac{\text{No. of Negative Wells}}{\text{Total Wells Plated}}}{\text{No. of Cells/Well}} \times 100$$

Significant increases in the mutation frequencies were noted in each experiment both with and without metabolic activation; the increases were low (generally below 6 x 10^{-4}), however, and not considered biologically significant. All of the mutation frequencies without metabolic activation and essentially all with metabolic activation were less than two-times the negative control frequency. Only one dose level (16 μg/ml, Experiment 2 with metabolic activation) had a mutation frequency of 2.5 greater than the negative control. There did not appear to be any strong inter-experimental correlation between the observed cytotoxicity and the mutation frequency.

The positive control responses were adequate to validate the sensitivity of the assay to detect genetic mutation.

Classification: Acceptable

This study satisfies the guideline [§84-2a)] requirements for an *in vitro* forward gene mutation assay at the thymidine kinase locus in L5178Y mouse lymphoma cells.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 1 June 94
Section IV, Tox. Branch II (7509C)

Secondary Reviewer: Byron T. Backus, Ph.D. Byron T. Backus 6/1/94
Section II, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* micronucleus test - mice [§84-2(b)]

EPA ID NO's: MRID No.: 410221-04 (Original Submission)
428581-06 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST MATERIAL: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one

STUDY NUMBER: CTL/P/2113

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc, Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: PROXEL® Press Paste: An Evaluation in the Mouse Micronucleus Test

AUTHORS: T. Sheldon, C.R. Richardson, V. Randall and S.L. Beck (Original Submission)
M.E. Burt (Addendum)

REPORT ISSUED: 5 September 1988 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: The test compound, at 245 or 392 mg/kg (males) and 331 or 529 mg/kg (females), was administered by oral gavage to male and female mice (5/dose/sex). After treatment, bone marrow was harvested 24 hrs for all study groups and at 48 and 72 hrs for the high-dose animals.

Based on the observed mortality, the test compound was evaluated at an adequate top dose. No significant differences were noted in the percentage of PCE/NCE, indicating a lack of cytotoxicity to the bone marrow. **No significant increases in the frequency of micronucleated polychromatic erythrocytes were noted in the bone marrow at any of the times tested.**

This study is classified as Core - Acceptable Data and satisfies the guideline [§84-2b)] requirements for an *in vivo* mouse micronucleus study.

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I. MATERIALS

A. Test Compound: PROXEL press paste Description: pale brown, damp powder Batch No.: ADH374793 BX973 Reference No: Y00180/020 Purity: 73.4% (w/w) [redacted]

B. Control Materials

1. Vehicle Control: Vehicle (0.5% hydroxypropyl methyl cellulose in 0.1% Polysorbate 80) was administered in a dose volume of 10 ml/kg.

2. Positive Control: Cyclophosphamide was dissolved in vehicle to yield a dose of 65 mg/kg when administered in a dose volume of 10 ml/kg.

C. Test Animals: Species: Mouse Strain: C57BL/6JfCD-1/Alpk Age: 10-14 weeks Weight (g): 20.6 - 27.2 (males), 17.0 - 20.9 (females) Source: Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK Housing: 5/cage Feed: Porton Combined Diet, Special Diets Services Ltd., Stepfield, Witham, Essex, UK *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature: 19 to 22°C; Humidity: 32 to 53%; Air changes: 15/hr; Light/dark cycle: 12 hr/12 hr

II. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

III. METHODS

A. Preliminary Study: A preliminary study was carried out to determine the median lethal dose (MLD) in male and female mice. In a range-finding study, female mice (2/dose) were orally gavaged with 200, 1000, or 2000 mg/kg. Based on the results of this study, dose levels of 200, 600 and 900 were selected for determination of the MLD using 5 animals/dose. MLD, based on cumulative mortality over four days, was calculated using probit analysis. Doses were not corrected for percent purity of active ingredient of the test compound.

B. Study Design

1. For the main study, animals (5/dose/sex/group) were assigned to treatment, positive control and vehicle control groups (Table 1). Treated animals received test compound at doses of either 50% or 80% of the MLD. Because of the expected mortality at the 80% MLD, an additional five "spare" animals/sex were included in the high-dose group. All dosing solutions were administered in a volume of 10 ml/kg.

Table 1: Study Design

Group	Harvest Time (hrs)	Dose	
		Male	Female
Vehicle control[a]	24	10 ml/kg	10 ml/kg
Positive control[b]	24	65 mg/kg	65 mg/kg
Treatment[c]	24	245 mg/kg	331 mg/kg
	24, 48, 72	392 mg/kg	529 mg/kg

[a] 0.5% Hydroxypropyl methyl cellulose in 0.1% Polysorbate 80
[b] Cyclophosphamide
[c] Doses not corrected for percent purity of active ingredient.

2. Cell preparation: Bone marrow cells were isolated from femurs using a 6% (w/v) albumin solution in normal saline. The marrow suspensions were smeared onto microscope slides, allowed to air dry and stained with polychrome methylene blue and eosin. For each animal, approximately 1000 polychromatic erythrocytes (PCE) were scored and the frequency of micronucleated polychromatic erythrocytes (MPE) determined. The percent PCE in approximately 1000 erythrocytes was also determined.

C. Statistical Analysis: The incidence of MPE and percent PCE/NCE were evaluated statistically using analysis of variance (ANOVA). Using the general linear model procedure (SAS), the least square mean for each study group was determined; pari-wise data comparisons were carried out using one-sided Students t-test.

IV. RESULTS

A. Determination of the Median Lethal Dose and Dose Selection: Based on the results of the preliminary study, the MLDs were determined to be 490 mg/kg for males and 661 mg/kg for females. For the main study, the low and high doses, equivalent to 50 and 80% of the MLD, used were 245 and 392 mg/kg for males, respectively, and 331 and 529 mg/kg for females, respectively.

B. Mortality: Lethality, observed in the high-dose animals (including the "spares"), occured in 2/20 males and 6/20 females.

C. Micronucleus Assay: The results of the micronucleus test are summarized in Table 2. For treated animals, the only statistically significant finding was an elevated number of micronuclei in the 392 mg/kg males at the 48 hr time point, relative to the 48-hr control. The value of 2.6, however, was lower than the control value (3.0) at 24 hr, suggesting that the effect was not treatment-related.

None of the results, when combined by sex, was statistically significantly different from the respective control values. The positive control group responded appropriately, with statistically significant increases in the number of micronuclei at 24 hrs. The percent of PCE for treatment and positive control animals were comparable to control values.

Table 2: Micronucleus Test Results (Summarized from study Tables 1, 2, 3, and 4)

Group	Dose (mg/kg)	Sex[a]	MPE/1000 PCE			% PCE		
			24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Vehicle control	0	M	3.0	1.0	1.8	34.4	42.2	49.5
		F	1.2	1.6	0.8	43.4	52.0	52.6
		M+F	2.1	1.3	1.3	38.9	47.1	51.0
Positive control	65	M	16.6**			50.4		
		F	13.4**			47.0		
		M+F	15.0**			48.7		
Treatment	245	M	3.2			42.9		
	331	F	1.0			54.6		
		M+F	2.1			48.8		
	392	M	3.4	2.6*	1.2	39.1	47.4	50.3
	529	F	0.6	1.6	1.8	39.4	43.4	51.2[b]
		M+F	2.0	2.1	1.4	39.2	45.4	50.7[c]

[a] M+F = Data combined by sex
[b] n = 4
[c] n = 9
* $p \leq 0.05$, ** $p \leq 0.01$

IV. CONCLUSIONS: The test compound, at 245 or 392 mg/kg (males) and 331 or 529 mg/kg (females), was administered by oral gavage to male and female mice (5/dose/sex). After treatment, bone marrow was harvested 24 hrs for all study groups and at 48 and 72 hrs for the high-dose animals. Based on the observed mortality, the test compound was tested at an adequate top dose.

Compared to the vehicle control, no significant increases in the frequencies of micronucleated cells were noted. Significant increases in the incidence of micronucleated cells was noted in the positive controls, validating the sensitivity of the assay procedure. No significant differences were noted in the percentage of PCEs, indicating a lack of cytotoxicity to the bone marrow.

Classification: Acceptable

This study satisfies the guideline [§84-2b)] requirements for an *in vivo* mouse micronucleus study.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 1 June 94
Section IV, Tox. Branch II (7509C)

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Secondary Reviewer: Byron T. Backus, Ph.D. Byron T. Backus 6/1/94
Section II, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity: UDS assay in primary rat hepatocytes (§84-4)

EPA ID NO's: MRID No.: 410221-05 (Original Submission)
428581-07 (Addendum)
Pesticide Chemical Code: 098901
Toxicology Chemical Code: 079A
DP Barcode: D194530
Submission No.: S446850

TEST MATERIAL: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one

STUDY NUMBER: CTL/P/2208

SPONSOR: ZENECA, Inc., Specialties
(ICI Americas, Inc, Specialty Chemicals)
Wilmington, DE 19897

TESTING LAB: ZENECA Central Toxicology Laboratory
(ICI Central Toxicology Laboratory)
Alderley Park, Macclesfield
Cheshire, UK

REPORT TITLE: PROXEL® Press Paste: Assessment for the Induction of Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures

AUTHORS: R.W. Trueman (Original Submission)
M.E. Burt (Addendum)

REPORT ISSUED: 31 October 1988 (Original submission)
28 June 1993 (Addendum)

EXECUTIVE SUMMARY: The test compound was evaluated over a concentration range of 10^{-8} to 10^{-2} M. At 10^{-4} M and higher cytotoxicity was observed. UDS was therefore assessed over a range of 10^{-8} to 10^{-5} M; at 10^{-5} M, some cytotoxicity was observed. The percent of cells in repair and the mean nuclear, cytoplasmic and net grain counts for control and treated cells were comparable to each other. The positive control cells showed a higher percent of cells in repair and marked increases in the mean nuclear, cytoplasmic and net grain counts, compared to the solvent control. **The test compound did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.**

Classification: Acceptable

This study satisfies the guideline (§84-4) requirements for an unscheduled DNA synthesis assay in primary rat hepatocytes.

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I. MATERIALS

A. Test Compound: PROXEL press paste Description: pale brown, damp powder Batch No.: ADH374793 BX973 Reference No: Y00180/020 Purity: 73.1% (w/w) [redacted]

B. Control Materials

Negative control: Dimethylsulfoxide (DMSO)
Positive control: 6-p-Dimethylaminophenylazo-benthiazole (6BT) in DMSO

C. Test Animals: Species: Rat Strain: Male, Alderly Park (Alpk:APfSD) Age: Weight (g): 270 - 278 Source: Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK Housing: 3/cage Feed: Porton Combined Diet, Special Diets Services Ltd., Stepfield, Witham, Essex, UK *ad libitum* Water: Tap water, *ad libitum* Environment: Temperature, humidity, air changes, light/dark cycle: not given

II. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

III. METHODS

A. Hepatocytes Isolation and Culturing: The portal veins of untreated, anesthetized animals were cannulated and the livers sequentially perfused *in situ* with the following buffers (composition of buffers are given in Table 1):

450 ml of Buffer 1 until washed free of blood
200 ml of Buffer 2 at a rate of 40 ml/min
200 ml of Collagenase Solution (recirculated through the liver). Perfusion rate was decreased to 20 ml/min after 1 min.

When the perfusion was completed (loss of the reticular pattern), the livers were excised, placed in a glass beaker, and minced into uniform pieces to form a crude homogenate.

The crude tissue homogenate was diluted with approximately 75 ml of WEC and filtered through nylon netting (150 µm), which was then rinsed with WEC to give a final volume of 120 ml. The cell suspension was centrifuged (2 min, 40 x g) at 4°C; the packed cells were washed and recentrifuged two times using WEC. The final pellet was resuspended in 25 to 30 ml of WEC. Cell viability was determined using the trypan blue exclusion method. The concentration of live

cells in the suspension was adjusted to 1.5×10^5 cells/ml and 3 ml seeded into each of three, 3 cm petri dishes containing a 25 mm round plastic cover slip. Cells were allowed to attach to the cover slip during a 1½ to 2¾ hour incubation at 37°C under an atmosphere of 5% CO_2/air. The culture medium was removed and the attached cells rinsed with 2 ml of WEI.

Table 1: Composition of Buffers and Media

Buffer/Media	Composition
Buffer 1	150 mM NaCl, 3.71 mM $NaHCO_3$, 4.84 mM Na_2HPO_4, 4.97 mM KCl, 1.24 mM KH_2PO_4, 0.62 mM $MgSO_4 \cdot 7H_2O$, 0.62 mM $MgSO_4 \cdot 6H_2O$, 10 mg/l phenol red (Na salt) Adjusted to pH 7.4 Gassed with 5% CO_2 in oxygen for five minutes
Buffer 2	142 mM NaCl, 24 mM $NaHCO_3$, 1.24 mM KH_2PO_4, 4.37 mM KCl, 0.62 mM $MgSO_4 \cdot 7H_2O$, 0.62 mM $MgSO_4 \cdot 6H_2O$, 10 mg/l phenol red (Na salt) Adjusted to pH 7.4 Gassed with 5% CO_2 in oxygen for 10 minutes
Ca^{++} Solution	769 mM $CaCl_2 \cdot 2H_2O$
Collagenase Solution	50 mg Collagenase 1 ml Ca^{++} solution 10 ml Buffer 2
Williams Medium 'E' Incomplete (WEI)	500 ml Williams medium 'E' 10 ml 200 mM L-glutamine 10 ml Penicillin and streptomycin (10,000 U/ml)
Williams Medium 'E' Complete (WEC)	500 ml WEI 50 ml Fetal calf serum
^{3}H-Thymidine solution (WEI^{3}H)	100 µl (3.7 MBq, 100 µCi) methyl-^{3}H-thymidine 10 ml WEI
Williams Medium 'E' + Thymidine (WEIT)	500 ml WEI 30.5 mg Thymidine (0.25 mM final)
Test Compound Media (one for each dose level)	50 µl of 60 mM, 6 mM, 600 µM, 60 µM, 6 µM, 0.6 µM or 0.06 µM test compound in DMSO (Final: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, or 10^{-9} M, not corrected for % purity of a.i.) 10 ml WEI^{3}H
Positive Control Media	50 µl of 6 mM, 600 µM, 60 µM, or 6 µM (Final: 10^{-4}, 10^{-5}, 10^{-6}, or 10^{-7} M) 10 ml WEI^{3}H
Solvent Control Medium	50 µl DMSO 10 ml WEI^{3}H
Media Control	10 ml WEI^{3}H only

B. UDS Assay: Following attachment of the cells, the medium was aspirated off and replaced with 3 ml of test compound media, positive control media, solvent control media, or media control; each dose level and control was assayed in triplicate. Following a 17 to 20 hr incubation at 37 °C under a 5% CO_2/air atmosphere, each dish was washed(3x) with 2 ml of WEIT. A final 3 ml aliquot was added to each dish and incubated for an additional 24 hrs. After the final incubation, the media was aspirated off and the cells washed with either WEI or normal saline. Cells were fixed by adding a 2 ml aliquot of glacial acetic acid:absolute alcohol (1:3, v/v) to each dish and after 10 min the solution was removed. The fixation process was repeated three times followed by four rinses with distilled water. The cover slips were air dried and mounted on microscope slides. Following autoradiography, cells were stained. At least 25 (normally 50) morphologically unaltered cells were examined on each slide using an automated image analyzer. For each cell the nuclear and cytoplasmic grain counts were determined and the net (nuclear - cytoplasmic) grain count calculated; the percent of cells in repair was also determined.

The entire experiment was repeated at least once to confirm that the results were reproducible.

C. Response Criteria

1. Positive response: A value of 5 or greater for the mean grain counts and greater than 20% of the cells under repair indicates a positive genotoxic effect.

2. Negative response: A value of 0 for the mean grain counts and less than 20% of the cells under repair indicates a negative genotoxic effect.

D. Statistical Analysis: Means and standard errors were determined.

IV. RESULTS and CONCLUSIONS: Cytotoxicity was observed at 10^{-4} M test compound and higher. Some cytotoxicity was observed at 10^{-5} M, however, there were sufficient number of cells with normal morphology to permit evaluation. UDS was therefore assessed over a range of test concentrations of 10^{-8} to 10^{-5} M (Table 2). The mean nuclear, cytoplasmic and net grain counts for control and treated cells were comparable to each other. No differences were noted in the percent of control and treated cells in repair.

The positive control cells showed a marked increase in the mean nuclear, cytoplasmic and net grain counts, compared to the solvent control. The percent of cells in repair was also markedly higher in the positive control cells.

Table 2: UDS Study Results[a]

Group	Concentration (M)	% Cells in Repair	Grain Counts (mean)		
			Nuclear	Cytoplasmic	Net
Treatment	10^{-5} 10^{-6} 10^{-7} 10^{-8}	2, 3 3, 4 2, 5 1, 4	18.7, 16.2 15.1, 17.2 17.8, 17.0 17.2, 14.7	24.0, 22.2 18.9, 22.2 23.0, 22.6 22.8, 18.7	-5.27, -6.00 -3.78, -5.06 -5.20, -5.54 -5.59, -4.04
Positive Control	10^{-7}	89, 91	67.5, 50.6	27.3, 26.2	40.19, 24.36
Solvent Control		2, 5	17.7, 18.3	21.9, 25.1	-4.20, -6.85
Medium Control		1, 4	15.5, 18.2	21.1, 23.5	-5.58, -5.28

[a] The results of the initial and repeat study are presented (Experiment 1, Experiment 2). Data taken from study Tables 1 and 2.

Results of the repeat experiment were comparable to those of the first.

The test compound did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Classification: Acceptable

This study satisfies the guideline [§84-4] requirements for an unscheduled DNA synthesis assay in primary rat hepatocytes.