005656

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

JAN 12 1987

MEMORANDUM

SUBJECT: Registration of Endurance 65 WDG Herbicide CASWELL #727A

TO: Richard Mountfort (23)
Registration Division (TS-767C)

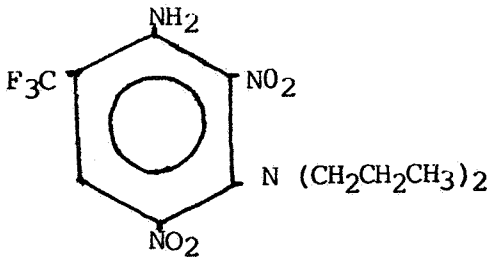
FROM: Winnie Teeters, Ph.D. Winnie Teeters 11-25-86
Pharmacologist, Section V
Tox./HED (TS-769C)

THRU: Laurence D. Chitlik, D.A.B.T. Laurence Chitlik for LDC 12/29/86
Head, Section V
Tox./HED (TS-769C)

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

CHEMICAL: Prodiamine; N^3,N^3-Di-n-propyl-2,4-dinitro-6-(trifluoromethyl)-m-phenylenediamine. (Formerly "Rydex" from U.S. Borax)



ACTION REQUESTED: Review submitted studies to support Vesicol Chemical Corporation's request for new chemical registration of Endurance 65 WDG Herbicide, and respond to the sponsor's discussion regarding oncogenicity studies to support non-crop uses.

RECOMMENDATIONS: Toxicology Branch does not find the requested registration supportable by the available data at this time. Prodiamine is structurally similar to Trifluralin, which has been classified (Trifluralin Registration Standard, August,1986) as a Category C (possible human) oncogen. The sponsor has not adequately addressed the possible oncogenicity of Prodiamine (for more detail refer to the discussion of this issue in this action). Furthermore, a required NOEL for developmental toxicity in rats has not been established,

and it has been recommended that an inconclusive mutagenicity test be repeated (see the relevant DERs in this action). Additionally, a requirement for an acute inhalation study with this specific product has not been met although these studies with the technical and 50 and 75% WPs are available.

Prodiamine is to be applied to common fruit, nut and vine crops, but it is not to be applied to orchards and vineyards that will produce a harvestable crop within one year after application; consequently, this use is considered by the Agency as a nonfood use (communication with R. Mountfort, the Product Manager for this chemical, 11-24-86). The registration requirements for Prodiamine, therefore, have been based upon this nonfood use.

Although there are several conditional requirements for this category of proposed use, the only ones applicable to Prodiamine are a specific request by the Agency for teratology studies, and a requirement for oncogenicity studies triggered by its chemical similarity to Trifluralin, a known oncogen. The status of these requirements were discussed above. With the exception of the above-mentioned data gaps (first paragraph under "Recommendations"), the sponsor has complied with the remaining toxicology data requirements for the applicable category of proposed uses.

005656

Summary of reviews for studies accompanying this action:

1. Acute oral toxicity to rats of Prodiamine 65 WDG, Huntingdon Res. Centre, Ltd, HRC Report #86386D/ VCL 106 AC, 5-27-86.

   The acute lethal oral oral dose of Prodiamine 65 WDG is greater than 5.0 g/kg. This corresponds to Toxicity Category IV. The study is classified as Core-Minimum.

2. Acute dermal toxicity to rats of Prodiamine 65 WDG, Huntingdon Res. Cent Ltd. HRC Report #86387D/ VCL 107/AC, 5-27-86.

   The acute lethal dermal dose to rats of Prodiamne 65 WDG is greater than 2.0 g/kg. This corresponds to Toxicity Category III. The study is classified as Core-Minimum.

3. Irritant effects on rabbit skin of Prodiamne 65 WDG, Huntingdon Res. Centre, Ltd., HRC #86244D/ VCL 109/SE, 3-26-86.

   Prodiamine 65 WDG applied to rabbit skin for four hours elicited transient, very slight erythem in 1/6 animals. This corresponds to Toxicity Category IV for dermal irritation. The study is classified as Core-Minimum.

4. Irritant Effects on the rabbit eye of Prodiamine 65 WDG, Huntingdon Res. Centre, Ltd., HRC #86241D/ VCL 108/SE, 4-10-86.

   Five of six rabbits had conjunctival redness and chemosis scores greater than 1, but the reactions had disappeared by four days after treatment. No corneal or iridal reactions were seen. These results correspond to Toxicity Category III for ocular irritation. The study is classified as Core-Minimum.

5. Delayed contact hypersensitivity in the guinea-pig with Prodiamine 65 WDG, Huntingdon Res. Centre, Ltd., HRC #86442D/ VCL 110/SS, 5-22-86.

   The test material was positive for induction of delayed contact hypersensitivity in guinea pigs. The study is classified as Core-Minimum.

6. L5178 TK +/- Mouse lymphoma mutagenesis assay, Microbiological Associates, Inc., # T2840.701, 5-20-85.

   Prodiamine Technical produced a weakly positive response, but only at toxic levels, in the absence of metabolic activation and a negative response in the presence of activation. The study is classified Inconclusive, and it is recommended that the assay be repeated.

7. Salmonella/mammalian microsome plate incorporation mutagenicity assay (Ames), Microbiological Associates, Inc., # T 4022.501, 6-27-85.

   Prodiamine was negative for mutation in each of the five tester strains, with or without metabolic activation, at dose levels up to 10,000 ug/plate (with moderate precipitation occurring at levels of 500 ug/plate and above). This study is classified as Acceptable.

8. Chromosome aberration assay in Chinese hamster ovary (CHO) cells, Microbiological Associates, Inc., #T 2840.337, 5-21-85.

   Prodiamine Technical did not induce a significant level of chromosone aberrations in the presence or absence of metabolic activation. The study is classified as Acceptable.

9. Salmonella/mammalian microsome plate incorporation mutagenicity assay (Ames Test), Microbiological Associates, Inc., # 2840.501, 4-8-85.

   Prodiamine Technical was negative for mutation in each of five tester strains, with or without metabolic activation, at dose levels up to 10,000 ug/plate (with moderate precipitation occurring at levels of 500 ug/plate and above). The study is classified as Acceptable.

10. Unscheduled DNA synthesis in rat primary hepatocytes, Microbiological Associates, Inc., # T 2840.380, 4-26-85.

    Prodiamine Technical did not cause a significant increase in the mean number of net nuclear grains, indicting that there was not an increase in unscheduled DNA synthesis. The study is classified as Acceptable.

11. A range-finding teratology study in rabbits with Prodiamine Technical, WIL Research Labs, Inc., # WIL 15152, 7-22-85,

    There were no maternal deaths; one 1000 mg/kg dam aborted. Maternal weight loss occurred during treatment at levels of 500 and 1000 mg/kg. Mean numbers of corpora lutea, implantation sites and viable fetuses were not affected at any level used. Levels of 100, 300 and 500 mg/kg were selected for the main study. The study is classified as Acceptable for a range-finding study.

12. A teratology study in rabbits with Prodiamine Technical, WIL Research Labs, Inc., # WIL 15153, 11-7-85.

    A NOEL for maternal toxicity was not established and the LEL is 300 mg/kg (LDT), based on an adverse effect on body weight gain. The fetal developmental toxicity NOEL is 500 mg/kg (HDT) and the LEL is greater than 500 mg/kg. The study is classified as Core-Minimum.

13. A range-finding teratology study in rats with Prodiamine Technical, WIL Research Labs., # WIL 15144, 3-28-85.

    Maternal body weight gain was decreased at 1000 mg/kg; no other notable signs of maternal toxicity were seen. There were no compound-related effects on fetal parameters. Dose levels of 100, 300 and 1000 mg/kg were selected for the main study. The study is classified as Acceptable as a range-finding study.

14. A teratology study in rats with Prodiamine Technical, WIL Research Labs., # WIL 15150, 11-11-85.

    The NOEL for maternal toxicity is 300 mg/kg and the LEL is 1000 mg/kg, based on depressed body weight gain.

A NOEL for developmental toxicity has not been established in this study based on the conclusion that there is a compound related increase in the incidence of ocular anomalies at the lowest dose tested, 100 mg/kg, which consequently, is considered the LEL for this effect. This study is classified as Core-Minimum, but it is necessary to establish a NOEL for developmental toxicity in another study. Since the sponsor has indicated that the ocular anomalies noted in the present study have been reported to have a genetic origin, any data provided to support this claim will be considered in a reevaluation of the present study.

Summary of data in Tox. Branch files (the PM has been notified that some of the available data for Prodiamine are not in these files).

The data for Prodiamine are presented in the attached "One-Liners" for this chemical.

Additionally, there are several studies for USB 3153 (Rydex), the same chemical structure as Prodiamine, all of which were conducted at IBT and have been classified as "Invalid" or "Core Supplementary Data"; consequently, none of these IBT-conducted studies can be used to meet current toxicology data requirements.

Tox Chem No. 727A File Last Updated __ __ Current Date 8/15/86

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation LC_{50}- rat; Huntingdon Research Center; #VCL 49/84839; 6/18/84	Technical Prodiamine 68.20%	257489	LC_{50} > 0.256 g/m^3	III	Guideline 005267
Acute inhalation LC_{50}- rat; Huntingdon Research Center; #VCL 54/84865; 3/85	Prodiamine	257490	LC_{50} > 3.8 g/m_3	III	Guideline 005267
Acute oral LD_{50} - rat; Hazleton Lab.; #182-123; 11/22/78	Prodiamine 50%	253053	LD_{50} > 5000 mg/kg	IV	Guideline 005268
Acute dermal LD_{50} - rabbit; Hazleton Lab.; #182-124; 11/22/78	Prodiamine 50%	253053	LD_{50} > 10,000 mg/kg		Supplementary 005268
Acute inhalation LC_{50} - rat; Hazleton Lab.; #182-126; 11/22/78	Prodiamine 50%	253053	LC_{50} not given		Supplementary 005268
Primary dermal irr.-rabbit; Hazleton Lab.;#182-125;11/28/78	Prodiamine 50%	253053	24 hrs.: 6/6 erythema (sc. 1); 72 hrs.: clear. Irritation Score: 0.5	IV	Guideline 005268
Primary eye irr. - rabbit; Hazleton Lab.;#182-117; 1/8/79	Prodiamine 50%	253053	24 hrs.: 2/6 conj. redness (sc. 1); 48 hrs.: clear.	III	Guideline 005268
* MUTAGENICITY - AMES TEST; MICROBIOLOGICAL ASSOC.; * T2434.5C1; 4-13-84	RYDEX (PRODIAMINE)	253361	MUTAGENIC IN ABSENCE OF MA IN AT LEAST ONE his⁻ SALMONELLA STRAIN		ACCEPTABLE 005658

* ADDED BY REVIEWER

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V, Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T.
Section V, Tox. Branch (TS-769C)

12/2/86

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity

TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 263738

MRID NO.: — TOX. PROJ. NO.: 2250

TEST MATERIAL: Prodiamine 65 WDG Herbicide

SYNONYMS: Endurance 65 WDG Herbicide (Rydex)

STUDY NUMBER: HRC Report No. 86386D/ VCL 106 AC

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: Huntingdon Res. Centre, Ltd.

TITLE OF REPORT: Acute Oral Toxicity to Rats of Prodiamine 65 WDG

AUTHOR(S): S.R. Kynoch, B. Sc.

REPORT ISSUED: 5-27-86

CONCLUSIONS: The acute lethal oral dose to rats of Prodiamine 65 WDG is greater than 5.0 g/kg bodyweight. This corresponds to Toxicity Category IV.

Classification: core-minimum

A. MATERIALS:

1. Test compound: Prodiamine 65 WDG, (N^3,N^3 -dipropyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine),
Description: beige granular solid
Batch: IL 9001 (9/12/85)
Purity: not stated

2. Test animals: Species: rat
Strain: CFY (Remote Sprague-Dawley)
Age: approximately 4-6 weeks
Weight: 93-127g prior to dosing on Day 1
Source: Interfauna UK Ltd.

B. STUDY DESIGN:

A group of ten rats (five of each sex) was treated at 5.0 g/kg bodyweight by oral intubation of a 50% w/v concentration of Prodiamine 65 WDG in distilled water prepared on the day of dosing. The rats were fasted overnight prior to and approximately 4 hours after dosing. They were observed soon

after dosing and often that day, twice daily during the week and once/day on weekends for 14 days after dosing. Body weights were recorded on Days 1, 8 and 15. All rats were sacrificed on Day 15 and the abdominal and thoracic cavities were opened and the contents examined.

C. RESULTS:

There were no mortalities. Signs of reaction to treatment included pilo-erection, hunched posture, abnormal gait and the production of stained urine. Recovery, as judged by observations, was apparently complete by Day 3. All rats gained weight on Days 8 and 15. Terminal autopsy findings were stated to be normal (no data were presented).

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V, Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T.
Section V. Tox. Branch (TS-769C)

12/2/86

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 263738 MRID NO.: -- TOX. PROJ. NO.: 2250

TEST MATERIAL: Prodiamine 65 WDG Herbicide

SYNONYMS: Endurance 65 WDG Herbicide (Rydex)

STUDY NO.: HRC Report No. 86387D/VCL 107/AC

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: Huntingdon Res. Centre Ltd.

TITLE OF REPORT: Acute Dermal Toxicity to Rats of Prodiamine 65 WDG

AUTHOR(S): S.R. Kynoch, B. Sc.

REPORT ISSUED: 5-27-86

CONCLUSIONS: The acute lethal dermal dose to rats of Prodiamine 65 WDG is greater than 2.0 g/kg bodyweight. This corresponds to Toxicity Category III.

Classification: core-minimum

A. MATERIALS:

1. Test compound: Prodiamine 65 WDG, (N^3, N^3-dipropyl-2,4-dinitro-6-trifluoro-methyl-m-phenylenediamine)
   Description: beige granular powder
   Batch: 1L 9001 (9-12-85)
   Purity: not stated

2. Test animals: Species: rat
   Strain: CFY (Remote Sprague-Dawley)
   Age: Approximately 6-8 weeks
   Weight: 207-250g prior to dosing on Day 1
   Source: Interfauna UK Ltd.

B. STUDY DESIGN:

A group of ten rats (five of each sex) was treated at 2.0 g/kg bodyweight by spreading a 100% w/v paste of the test material in distilled water (2.0 ml/kg)

over an area of dorso-lumbar skin which had been clipped free of hair on the previous day. The treated area was promptly covered with gauze held in place with an impermeable dressing encircled around the trunk. After 24-hours of exposure, the treated skin was washed with water and dried. The rats were observed soon after dosing and often that day, twice each weekday and once/day on weekends for clinical signs. Treated areas of skin were examined daily for signs of dermal irritation and assessed according to a scoring system which graded erythema and eschar formation on a scale of zero to four and edema on a similar numerical scale. Body weights were recorded on study Days 1, 8 and 15. Rats were observed for 14 days after dosing, after which they were sacrificed and the abdominal and thoracic cavities were opened and the contents examined.

C. RESULTS:

There were no deaths nor any signs of systemic reaction to treatment. There also were no dermal reactions to treatment but the treated skin of all rats was stained yellow on Days 2, 3 and 4. All rats gained weight during the observation period and the report stated that terminal autopsy findings were normal.

Reviewed by: Winnie Teeters, Ph.D.
Section V, Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. 12/22/86
Section V. Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Irritation

TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 263738

MRID NO.: - TOX. PROJ. NO. 2250

TEST MATERIAL: Prodiamine 65 WDG Herbicide

SYNONYMS: Endurance 65 WDG Herbicide (Rydex)

STUDY NUMBER: HRC Report No.86244D/VCL 109/SE

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: Huntingdon Res. Centre Ltd.

TITLE OF REPORT: Irritant Effects On Rabbit Skin Of Prodiamine 65 WDG

AUTHOR(S): M.P. Liggett and B.I. Parcell

REPORT ISSUED: 3-26-86

CONCLUSIONS: Prodiamine 65 WDG applied to rabbit skin for four hours elicited transient, very slight erythema in 1/6 animals. This corresponds to Toxicity Category IV.

Classification: core-minimum

A. MATERIALS:

1. Test compound: Prodiamine 65 WDG (N^3, N^3-dipropyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine).
Description: beige granular solid
Batch: IL 9001 (9/12/85)
Purity: not stated

2. Test animals: Species: rabbit
Strain: New Zealand White
Age: approximately 10-13 weeks
Weight: 2.2-2.9 kg prior to treatment
Source: Rosemead Rabbits, Rosemead, Essex, England.

B. STUDY DESIGN:

A 0.5 g amount of test compound was applied under a gauze square moistened with 0.5 ml distilled water on an intact skin site of each of six rabbits. The site on the dorso-lumbar region had been clipped free of hair the day prior to treatment. Each site was occluded with "Elastplast" dressing for a period of

four hours after which the semi-occlusive dressing and the gauze pad were removed and the site was washed with distilled water. Site examination was made on Day 1 about 30 minutes after removal of the patch and on Days 2, 3 and 4. Scoring and grading for erythema and eschar formation and for edema was made using a scale from zero to four.

C. RESULTS:

Very slight erythema was noted for one rabbit on Day 2; this was the only reaction seen for the group throughout the observation period.

Individual data were present only through Day 4, since this period included two days of completely negative results.

Reviewed by: Winnie Teeters, Ph.D.
Section V, Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. 12/22/86
Section V, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Eye Irritation

TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 263738

MRID NO.: - TOX. PROJ. NO.: 2250

TEST MATERIAL: Prodiamine 65 WDG Herbicide

SYNONYMS: Endurance 65 WDG Herbicide (Rydex)

STUDY NUMBER: HRC Report No. 86241D/VCL 108/SE

SPONSOR: Vesical Chem. Corp.

TESTING FACILITY: Huntingdon Res. Centre Ltd.

TITLE OF REPORT: Irritant Effects on The Rabbit Eye Of Prodiamine 65 WDG.

AUTHOR(S): M.P.Ligett and B.I. Parcell

REPORT ISSUED: 4-10-86

CONCLUSIONS: Five of six rabbits had a score greater than 1 for conjunctival redness and chemosis; the reactions had disappeared in all of these subjects by four days after treatment. No corneal or iridal reactions were seen in any rabbit. These results correspond to a Toxicity Catagory of III for ocular irritation.

Classification: core-minimum

A. MATERIALS:

1. Test compound: Prodiamine 65 WDG (N^3, N^3-dipropyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine).
Description: beige granular solid
Batch: IL 9001 (9-12-85)
Purity: not stated

2. Test animals: Species: rabbit
Strain: New Zealand White
Age: approximately 10-15 weeks
Weight: 2.2-3.6 kg prior to treatment
Source: Rosemead Rabbits, Rosemead, Essex, England

B. STUDY DESIGN:
A 80 mg amount (0.1 ml volume) of test material which had been finely powdered was placed on the lower everted lid of one eye of each of six rabbits and the eyelids

were gently held together for one second. Examination of the eyes was made after 1 hour and 1, 2, 3, 4 and 7 days after instillation, aided by the use of a flashlight. Grading and scoring of the ocular reactions was made according to the system described on the following page, copied from the report.

C. RESULTS:

No corneal or iridal reactions were seen in any rabbit. Grades of 2 for conjunctival redness and chemosis were recorded for 5/6 rabbits at the one-hour reading; these effects had disappeared by the fourth day after instillation in all of these animals.

110201 PRODIAMINE RIN 1786-93

Page 15 is not included in this copy.

Pages _____ through _____ are not included.

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 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.

✓ 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.

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V, Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. 12/3/86
Section V, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Delayed Dermal Sensitization TOX. CHEM. NO.: 727-A

ACCESSION NO.: 263738 MRID NO.: - TOX. PROJ. NO.: 2250

TEST MATERIAL: Prodiamine 65 WDG Herbicide

SYNONYMS: Endurance 65 WDG Herbicide (Rydex)

STUDY NO.: HRC Report No. 86442D/VCL 110/SS

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: Huntingdon Res. Centre Ltd.

TITLE OF REPORT: Delayed Contact Hypersensitivity In The Guinea-Pig With Prodiamine 65 WDG.

AUTHOR(S): S.R. Kynoch and P.A. Smith

REPORT ISSUED: 5-22-86

CONCLUSIONS: The test material was positive for the induction of delayed contact hypersensitivity in guinea pigs.

Classification: core-minimum

A. MATERIALS:

1. Test compound: Prodiamine 65 WDG (N^3, N^3 dipropyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine)
   Description: beige granular solid
   Batch: IL 9001 (9-12-85)
   Purity: not stated

2. Test animal: Species: guinea pig
   Strain: Hartley/Dunkin
   Age: not stated
   Weight: 400-493 at start of study
   Source: D. Hall, Newchurch, Staffordshire, England

B. STUDY DESIGN:

Twenty female guinea pigs were randomly allocated to a test or control group (10/group). They were weighed at the start and end of the test and observed daily for signs of ill health or toxic signs. In a preliminary test a concentrattion of 60 % w/w in distilled water for the test material was selected as the induction

and challenge applications.

Induction applications were made to the skin of the left shoulder which had been clipped free of hair. Patches (2 X 2 cm of surgical gauze, 3 layers thick) were saturated with approximately 0.5 ml of the prepared test material, placed on the skin and covered with impermeable tape which was secured by an adhesive bandage wound around the animal's torso and fixed with tape. Skin contact was maintained for approximately 6 hours. Dermal reactions were assessed for erythema and edema upon removal of the patches and 24 hours later by scoring the reactions on a scale of 1-4.

Nine induction applications were made, 3/week, over a period of 3 consecutive weeks. Control animals were treated similarily with water. A test material challenge patch prepared in a similar way was applied to the right flank of both test and control pigs for 6 hours, two weeks after the ninth induction application. Challenged sites were scored 24, 48 and 72 hours following patch removal. Hair was clipped again to aid the 48-hour reading. For a test animal to be considered positive the challenge reaction had to be more marked and/or more persistant than the maximum reaction noted in control pigs.

RESULTS:

Location of the test material induction applications 7, 8 and 9 had to be altered because of the dermal reactions present. None of the control inductions caused any reactions. Five of the 10 treated pigs showed a reaction to the challenge patch which was more severe and/or persistent that that noted for any control animal challenge reaction. Therefore, Prodiamine 65 WDG was judged to have elicited evidence of delayed contact sensitization in 5/10 animals tested.

POSITIVE CONTROL TEST:

Simultaneous to the above reviewed study, the same testing laboratory conducted a similar study using formalin as the positive control sensitizer and demonstrated that 6/10 animals gave evidence of delayed dermal contact sensitization to this substance.

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Irving Mauer, Ph.D.
Section VI, Tox. Branch (TS-769C)

11-07-86 12/3/86

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity

TOX. CHEM. NO.: 727 A

ACCESSION NUMBER: 260680

MRID NO.: - Tox.Proj.No. 2250

TEST MATERIAL: Prodiamine Technical, Lot No. C 84331, a yellow crystalline solid with purity of 96.3%.

SYNONYMS: (Rydex)

STUDY NUMBER(S): T2840.701

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates

TITLE OF REPORT: L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay

AUTHOR(S): A.M. Rogers-Back, Study Director

REPORT ISSUED: 5-20-85

PROCEDURES: The assay consists of a solubility or miscibility test to select a suitable solvent, followed by a toxicity test and then the mutagenesis test; the latter two tests are conducted with and without metabolic activation provided by Arochlor 1254- and 1242- stimulated microsomes from rat liver. Toxicity testing was conducted using seven Prodiamine Technical concentrations from .004 to 4000 ug/ml, two solvent controls, and checking for growth at 24 and 48 hours. A range of concentrations of Prodiamine Technical was used in the mutagenesis assay which also included two solvent controls and two concentrations each of ethyl methanesulfonate and 7, 12 dimethylbenz(a)anthracene as positive controls. A four-hour exposure period was followed by a two-day expression period. Cell populations were adjusted at 24 and 48 hours and appropriate cultures were cloned in restrictive medium. Following incubation, the plates were scored and mutation frequencies calculated. The report contained guidelines used in judging the significance of the activity of the test article and signed statements regarding quality assurance procedures.

RESULTS: The toxicity tests indicated complete toxicity at 40 ug/ml for the non-activated cultures and at 400 ug/ml for activated cultures. Consequently, Prodiamine Technical was tested at 0.54 to 40 ug/ml without activation and 5.4 to 400 ug/ml with activation, but the solvent control cultures had less than

50% cloning efficiency and another assay was conducted because plating efficiency must exceed 50% for the assay to be valid. In this second assay, concentrations of 4.0-300 ug/ml were used for activated cultures. Cloned non-activated cultures were treated with Prodiamine Technical concentrations of 1.3-17 ug/ml, producing suspension growth of 96-9%. Cloned activated cultures received concentrations of 4.0-23 ug/ml, producing growth of 94-14%. Higher concentrations were too toxic to clone. Cloning data are shown in appended Tables 2 and 4, taken from the report. Concentrations of 13 and 17 ug/ml in the non-activated cultures produced mutant frequencies of 1.5 and 3.6, respectively, compared to a mean control of 0.7, thus their mutant frequencies were 2.1 and 5.1 times, respectively, greater than the mean control. The Total Growth for these treatments were 18 and 1%, respectively. Mutant frequencies for the other non-activated cloned cultures were similar to the mean for the solvent controls. None of the cloned activated cultures had mutant frequencies that were significantly (at least two-fold) greater than that of the mean solvent controls.

The positive controls gave expected increases in mutant frequencies; the increase over control for ethyl methanesulfonate was over eight-fold at 0.5 ug/ml and those for dimethylbenzanthracene were 3-fold at 5 ug/ml and over 9-fold at 7.5 ug/ml.

DISCUSSION and CONCLUSIONS: In the protocol for this assay under Section 8.0 Evaluation of Test Results, criteria are given as guidelines in judging the significance of the activity of a test article. Paragraph 8.1 states that a test compound is to be considered POSITIVE "if there is a positive dose response and one or more of the doses in the 10% or greater Total Growth range exhibit a mutant frequency which is two-fold greater than the background level. All data including that from cultures with less than 10% Total Growth will be used to establish the dose response relationship". The requirements for an EQUIVOCAL result are "if there is no dose response but any one of more doses with 10% or greater Total Growth exhibit a two-fold increase in mutant frequency over background, or if there is a dose response but no culture exhibits a two-fold increase in mutant frequency over background".

The results from the 13 and 17 ug/ml non-activated cultures specifically meet the testing facility's protocol requirements for a POSITIVE result since there were both: 1) a positive dose response for these two levels and 2) one or more doses with greater than 10% Total Growth (the 13 ug/ml level) exhibited a mutant frequency which is two-fold greater than the background level. However, the lower dose level, which had acceptable growth, barely met the requirement for a doubling of mutant frequency (2.1 times compared to a 2.0 times requirement). Furthermore, the Total Growth in the 17 ug/ml culture, where the mutant frequency was 3.6-fold over control, was only 1%. For these reasons, it is concluded that the data from non-activated cultures should be considered as indicating a weekly positive response, and it is recommended that

that the assay be repeated.

The results of this assay indicate, therefore, that under the conditions of this test, Prodiamine Technical produced a weakly positive response, but only at toxic levels, in the absence of metabolic activation and a negative response in the presence of metabolic activation.

Classification: Inconclusive, and it is recommended that the assay be repeated.

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 21 through 22 are not included.

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 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.

__✓__ 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.

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Irving Mauer, Ph.D.
Section VI, Tox. Branch (TS-769C)

10-28-85

12/3/85

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity- Ames Test TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 260680 MRID NO.:- Tox.Proj.No. 2250

TEST MATERIAL: Prodiamine, lot no. C-85134, a gold-colored powder with purity of 92.9%.

SYNONYMS: (Rydex)

STUDY NUMBER(S): T4022.501

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test).

AUTHOR(S): T.E. Lawlor, Study Director

REPORT ISSUED: 6-27-85

PROCEDURES: The standard set of 5 Salmonella typhimurium his^- strains were exposed to test substance up to the maximum concentration (10 mg/plate) stated in the protocol in use by the testing facility, both in the absence and presence of metabolic activation (MA) provided by Arochlor 1254- stimulated microsomes from rat liver. Using TA 100 as the indicator strain, Prodiamine was checked for toxicity up to a concentration of 10 mg/plate, in the presence and absence of MA. A single experiment was conducted, employing triplicate plates per each of 5 dose levels of Prodiamine, a solvent control (DMSO), and effective concentrations of known mutagens, appropriate for each strain. It was stated that all criteria for a valid study as described in the facility's protocol were met. The report contained signed statements regarding quality assurance procedures.

RESULTS: The toxicity tests, including concentrations which caused

moderate precipitation (333 mg/plate and above without activation, and 667 mg/plate and above with activation) did not indicate any toxicity up to concentrations of 10 mg/plate. With Prodiamine there were no biologically meaningful increases in revertants (less than a doubling) at any dose for any of the strains, as seen in the table below, data taken from Report Tables 3, 4, 5, 6 and 7. The positive controls induced at least three-fold increases in revertants over the average value for the appropriate solvent control.

Averaged Revertants/Plate

Strain	Solvent control	Concentrations (ug/plate)				
		100	500	2500	5000	10000
TA 98 with S-9	20	31	22	23	25	20
without S-9	12	12	15	20	19	19
TA 100 with S-9	104	153	126	128	122	140
without S-9	84	88	92	82	98	90
TA 1535 with S-9	9	10	9	9	8	6
without S-9	17	21	20	10	8	10
TA 1537 with S-9	6	5	5	4	7	6
without S-9	4	6	3	4	6	5
TA 1538 with S-9	14	13	15	17	18	13
without S-9	9	8	9	12	11	15

CONCLUSIONS: Under the conditions of this study, Prodiamine was negative for mutation in each of the five tester strains, with or without metabolic activation, at dose levels up to 10,000 ug/plate (with moderate precipitation occurring at levels of 500 ug/plate and above).

Classification: Acceptable

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Irving Mauer, Ph.D. 11-02-86
Section VI, Tox. Branch (TS-769C)

12/3/86

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity

TOX. CHEM. NO.: 727 A

ACCESSION NUMBER: 260680

MRID NO.: - Tox.Proj.No. 2250

TEST MATERIAL: Prodiamine Technical, Lot No. C 84331, yellow crystals, with a purity of 96.3%.

SYNONYMS: (Rydex)

STUDY NUMBER(S): T2840.337

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

TITLE OF REPORT: Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells.

AUTHOR(S): D.L. Putnam, Study Director

REPORT ISSUED: 5-21-85

PROCEDURES: A cytotoxicity test in the presence and absence of metabolic activation by Arochlor-induced microsomes was performed to select dose levels of the test material for the chromosome aberration assay. Chinese hamster ovary (CHO) cells were seeded in duplicate and incubated for 18-24 hours after which they were treated with the test material in solvent (DMSO) or solvent alone for 16 hours for the non-activated system or for 2 hours for the activated system followed by 14 hours incubation after removing the treatment medium.

For the assay, the CHO cells were seeded in 2 sets of duplicate flasks for each treatment condition, incubated, then treated with test article with or without S-9 reaction mixture, control article in solvent or solvent alone. An untreated control consisting of cells in complete medium was also included. The cells were incubated as described above for the cytotoxicity test for the two conditions of treatment (with and without activation), harvested by trypsinization and used for estimation of toxicity. Medium was removed from a second set of duplicate flasks for each treatment condition and the cells were washed and refed with a medium containing 0.1 ug/ml of colcemid.

Two-three hours after colcemid addition, metaphase cells were harvested, fixed with Carnoy's solution and used for slide preparations which were stained with Giesma. Fifty metaphase cells were

25

scored in each duplicate treatment flask, for a total of 100 cells/ treatment.

Triethylenemelamine (1.0 ug/ml) was the positive control for non-activated cultures and cyclophosphamide (50 ug/ml) was the positive control for activated cultures.

Cytotoxic effects are expressed relative to the solvent control. The number and types of aberrations, the percentage of damaged cells and the frequency of structural aberrations were reported. Chromatid and chromosome gaps are presented but not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations/cell. Chi-square analysis, using a 2 X 2 contingency table, was used to determine significant differences between the number of cells with aberrations in the treatment and control groups. It was stated that the positive and negative controls fulfilled the requirements for a valid test, and the report contained signed statements regarding quality assurance procedures.

RESULTS: Six concentrations of the test material ranging from 1000 to 0.01 ug/ml were tested for toxicity. Concentrations of 1000 and 100 ug/ml gave relative cell survivals of 12 and < 1%, respectively, for non-activated cultures and 4 and 9%, respectively, for activated ones; consequently, concentrations of 60, 30, 15, 8 and 4 ug/ml were selected for the assay.

Survival, relative to the solvent control, ranged from 2-95% without activation and 20-103% with activation. The four highest doses with scorable metaphases were selected for evaluation of chromosome aberrations.

Prodiamine Technical in the absence of activation was toxic at levels of 60 and 30 ug/ml; no scorable metaphases were found for the higher level in either duplicate culture or in one duplicate for the lower level, so that all counted cells for the latter level came from one culture. The frequency of cells with structural aberrations was not significantly increased. The positive control induced 0.78 aberrations/cell and 50% of the cells had structural aberrations. These data can be seen in appended Table 5, copied from the report.

In the presence of activation, Prodiamine Technical was toxic to the monolayer at the level of 60 ug/ml and the slides from these cultures contained no scorable metaphases. For the other levels, the frequency of cells with structural aberrations was not significantly increased. The positive control induced 0.93 aberrations per cell, with 46% of cells scored containing structural damage. These data are presented in appended Table 6, copied from the report.

CONCLUSIONS: Prodiamine Technical, under the conditions of this test, did not induce a significant level of chromosome aberrations in the presence or absence of metabolic activation.

Classification: Acceptable.

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 27 through 28 are not included.

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 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.

__✓__ 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.

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Irving Mauer, Ph.D.
Section VI, Tox. Branch (TS-769C)

10-20-86
12/23/86

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity - Ames Test TOX. CHEM. NO.: 727 A

ACCESSION NUMBER: 260680 MRID NO.:- Tox.Proj.No. 2250

TEST MATERIAL: Prodiamine Technical, Lot No. C 84331, a yellow crystalline solid, with purity of 96.3%.

SYNONYMS: (Rydex)

STUDY NUMBER(S): T2840.501

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test).

AUTHOR(S): T.E. Lawlor, Study Director

REPORT ISSUED: 4-8-85

PROCEDURES: The standard set of 5 Salmonella typhimurium his$^-$ strains were exposed to test substance up to the maximum concentration (10 mg/plate) stated in the protocol in use by the testing facility, both in the absence and presence of metabolic activation (MA) provided by Arochlor 1254- stimulated microsomes from rat liver. Using TA 100 as the indicator strain, Prodiamine Technical was checked for toxicity up to a concentration of 10 mg/plate, in the presence and absence of MA. A single experiment was conducted, employing triplicate plates per each of 5 dose levels of Prodiamine Technical, a solvent control (DMSO), and effective concentrations of known mutagens, appropriate for each strain. It was stated that all criteria for a valid study as described in the facility's protocol were met. The report contained signed statements regarding quality assurance procedures.

RESULTS: The toxicity tests, including concentrations which caused

moderate precipitation (333 ug/plate and above without activation, and 667 ug/plate and above with activation) did not indicate any toxicity up to concentrations of 10 mg/plate. With Prodiamine Technical there were no biologically meaningful increases (less than a doubling) in revertants at any dose for any of the strains, as seen in the table below, data taken from Report Tables 3, 4, 5, 6 and 7. The positive controls induced at least three-fold increases in revertants over the average value for the appropriate solvent control.

Averaged Revertants/Plate

Strain	Solvent control	Concentrations (ug/plate)				
		100	500	2500	5000	10000
TA 98 with S-9	22	28	26	27	26	22
without S-9	18	19	13	21	23	27
TA 100 with S-9	104	143	125	114	132	137
without S-9	102	116	114	99	104	115
TA 1535 with S-9	10	9	9	8	9*	6
without S-9	17	19	16	15	14	11
TA 1537 with S-9	9	7	7	6	6	9
without S-9	9	5	8	4	9	8
TA 1538 with S-9	20	18	18	22	20	27
without S-9	12	11	16	11	14	14

*This value was 8 in the Report Table 5; correction by reviewer.

CONCLUSIONS: Under the conditions of this study, Prodiamine Technical was negative for mutation in each of the five tester strains, with or without metabolic activation, at dose levels up to 10,000 ug/plate (with moderate precipitation occurring at levels of 500 ug/plate and above).

Classification: Acceptable

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Irving Mauer, Ph.D. 10/20/86 12/3/86
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 260680 MRID NO.:- Tox.Proj.No.: 2250

TEST MATERIAL: Prodiamine Technical, Lot No. C-84331, a yellow crystalline solid with purity of 96.3%.

SYNONYMS: (Rydex)

STUDY NUMBER(S): T2840.380

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes.

AUTHOR(S): R.D. Curren, Study Director

REPORT ISSUED: 4-26-85

PROCEDURES: Primary hepatocytes were obtained from Sprague-Dawley rats. The assay begins with selection of a suitable solvent, followed by a preliminary toxicity test and then the assay itself, which is performed with a simultaneous toxicity test. Evaluation is based on the incorporation of tritiated-thymidine into the hepatocyte DNA, evidenced by the presence of silver grains over nuclei of cells which had been coated earlier with photographic emulsion. The cells are stained by hematoxylin-eosin and the nuclear and background grains counted by a colony counter. Toxicity data are presented as relative plating efficiencies, based on viable counts after exposure to the test material.

The solvent selected was DMSO. The positive control was dimethylbenzanthracene at concentrations of 3 and 10 ug/ml.

The cytotoxicity test utilized 10 doses ranging from 0.07-2000 ug/ml in 2 replicate cultures. Following 18-20 hours of exposure, the cells were washed, trypsinized, stained with trypan blue and counted in a hematocytometer. Both cultures were counted and relative survival was obtained by comparing the treated to control groups.

In the assay, 3 replicate seeded plates/level were treated with 7 dose levels ranging from 0.3-100 ug/ml of test material, positive controls, and the solvent, DMSO. In parallel with the

test plates, 3 cultures/level were treated with the same dilutions for a toxicity test; counts of viable cells were made after incubation to obtain relative survivals and relative toxicities. After incubation, the cells in the assay were washed, swelled and fixed. Coverslips were dried, covered with Kodak NTB emulsion and stored for 11 days, after which they were developed, fixed, and stained. Nuclear grains were counted in 25 cells in random areas on each of 3 coverslips/treatment. The net counts were determined by counting 3 nucleus-sized areas adjacent to each nucleus and subtracting the average cytoplasmic count from the nuclear count. For each treatment slide, the net nuclear counts were averaged and a standard deviation determined. Also reported are the Grand Mean (mean of the net nuclear counts from all 75 cells at each dose level) and standard deviation, and the percent of cells at each dose level which had $\geq$ 5 net nuclear counts.

The report contained signed statements regarding quality assurance procedures.

RESULTS: From the initial toxicity test it was found that Prodiamine Technical had a relative toxicity (RT) of 100.0% at 67 ug/ml and 13.9% at the next lower dose of 20 ug/ml. Consequently, 7 dose levels ranging form 100-0.3 ug/ml were selected for the assay.

In the parallel cytotoxicity test of the assay, the highest level of test compound caused an RT of 100.0% and the next lower level of 30 ug/ml had an RT of 87.9%, with an RT of 34.5% for the next lower level of 20 ug/ml. These data with those for other levels of the test compound and for the positive, solvent and untreated controls are shown in appended Table 2 copied from the report.

None of the levels of Prodiamine Technical caused a significant increase in the mean net nuclear counts while both levels of the positive control did. These data are presented in appended Table 3 taken from the report.

CONCLUSIONS: Under the conditions of this assay Prodiamine Technical did not cause a significant increase in the mean number of net nuclear grains, indicating that there was not an increase in unscheduled DNA synthesis.

Classification: Acceptable

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 33 through 34 are not included.

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 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.

✓ 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.

005656

Reviewed by: Winnie Teeters, Ph.D.
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. for LDC 12/23/86
Section V , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Teratology, Range-finding TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 263739 MRID NO.: - Tox. Proj. No. 2250

TEST MATERIAL: Prodiamine Technical

SYNONYMS: (Rydex)

STUDY NUMBER(S): WIL 15152

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: WIL Research Labs. Inc.

TITLE OF REPORT: A Range-Finding Teratology Study in Rabbits with Prodiamine Technical

AUTHOR(S): M.D. Nemec, B.S.

REPORT ISSUED: 7-22-85

CONCLUSIONS: No female died during the study; one high level rabbit aborted and was sacrificed on day 21 of gestation. Females in the 500 and 1000 mg/kg levels lost weight during most of the treatment period but gained more than controls posttreatment. Although postimplantation loss was increased in the high dose level, the value was within the range for historical controls. Mean numbers of corpora lutea, implantation sites and viable fetuses were not affected at any level.

The NOEL for maternal toxicity is 250 mg/kg and the LEL is 500 mg/kg, based on an adverse effect on body weight (i.e. an actual weight loss during most of the treatment period).

Doses of 100, 300 and 500 mg/kg were selected for the main study.

Classification: The study is classified as Supplementary Data since it is a range-finding study.

A. MATERIALS:

1. Test Compound: Prodiamine Technical, Description: light orange powder, Batch: #C-84331, Purity: 96.3%,

2. Test Animals: Species: Rabbit, Strain: New Zealand White, Age: 5 mo. Weight: 3.0-4.1 kg, Source: Hazleton-Dutchland, Inc.

B. STUDY DESIGN:

1. Animal Assignment:

Animals were assigned by a computer randomization procedure based on body weight stratification in a block design. The experimental design consisted of the following groups:

Groups	Dosage (mg/kg/day)	No. of Mated Females
1. Vehicle control	0	5
2. Low dose	50	5
3. Intermed.- 1	125	5
4. Intermed.- 2	250	5
5. Intermed.- 3	500	5
6. High dose	1000	5

2. Test Material Preparation and Dosing:

The vehicle, 0.5 % methylcellulose, was prepared by adding 5.0 g of the appropriate powder to 1000 ml of heated deionized water. It was prepared weekly and stored under refrigeration.

The appropriate amount of test material was weighed, made into a slurry with the vehicle, and diluted to various concentrations with additional vehicle. Fresh mixtures were prepared daily.

The test mixtures and the vehicle for the control group were administered by gavage once daily for 13 consecutive days, from gestation day 6 through 18, as a 4 ml/kg volume. Dosages were based on the most recent body weights.

3. Insemination Procedures:

All does were artificially inseminated with diluted semen which had been evaluted for motility (> 50%) and the final concentration was greater than 3 million sperm/ml. The does then received an IV injection of human chorionic gonadotropin (100 U.S.P. Units).

4. Animals received food (Purina Certified Rabbit Chow #5322) and water ad libitum.

5. No statistical analyses were performed.

6. Quality assurance was acomplished by inspections on Mar. 21, April 10 and June 12-13, 1985, A statement was signed by the QA Director that to the best of his knowledge there were no significant deviations from the Good Laboratory

Practice Regulations which affected the quality or integrity of the study.

C. METHODS and RESULTS:

1. Observations:

Methods: Clinical observations were recorded individually from day 0 through 29 of gestation.

Results: One doe in the high dose group aborted and was sacrificed on day 21 of gestation.

Hair loss, urogenital matting, soft stool and decreased urination were the recorded clinical observations; their distribution seemed unrelated to dosing except that hair loss was more frequent in the 500 and 1000 mg/kg levels.

2. Body Weight:

Methods: Females were weighed on gestation days 0, 6, 9, 12, 18, 24 and 29. Mean weights were calculated for these days and for 6-18, 6-29, 18-29 and 0-29 days of gestation.

Results: The 500 and 1000 mg/kg groups had lost weight by days 12 and 18 and were less than control on these days and on days 24 and 29 of gestation, on the basis of mean body weight. A comparison of body weight changes showed that these two groups gained less than the controls for the period of days 6-9 and lost weight in periods of days 9-12 and 12-18 of gestation whereas the other test groups and the controls gained during these periods. The weight changes (grams) for all groups for periods of days 6-18 and 18-29 are shown below.

Mg/kg	0	50	125	250	500	1000
Days 6-18	184	248	254	166	-21	-113
Days 18-29	200	113	198	290	301	293

On the basis of the actual loss of weight throughout the treatment period, it is concluded that the 500 and 1000mg/kg groups were adversely affected.

3. Necropsy and Uterine Examination:

Methods: All surviving females were sacrificed by an IV injection of T-61 Euthanasia Solution on gestation day 29. Contents of the thoracic and abdominal cavities were examined. The numbers of corpora lutea were recorded. The number and location of viable and non-viable fetuses, early and late resorptions and number of implantation sites were recorded. Uteri with no macroscopic evidence of implantation were placed in ammonium sulfide solution.

Results: The female which aborted had a pale, soft liver with accentuated lobular markings, red hepatization of all lung lobes and clear fluid in the thoracic cavity. Accentuation of liver lobular markings was also noted for 2, 2, 2, and 1 females in the control, 50, 125 and 1000 mg/kg groups, respectively.

One female each in the 50 and 500 mg/kg groups was non-gravid and negative for early implantation loss.

One female in the 1000 mg/kg group had 5 early resorptions and another had one late resorption; this caused the mean postimplantation loss per dam for this group (1.5) to be increased compared to the control (0.8). Yet, the value for this treated group is within the range of the WIL historical control data (0.3-1.9) for this species/strain.

There was no effect noted in the other treated groups for postimplantation loss and mean numbers of corpora lutea, implantation sites and viable fetuses nor in the high dose group for these parameters, except for the mentioned effect on postimplantation loss.

The NOEL for maternal toxicity is 250 mg/kg and the LEL is 500 mg/kg, based on an adverse effect on body weight (i.e. an actual weight loss during most of the treatment period).

Based on the results of this study, dose levels of 100, 300 and 500 mg/kg were selected for the main study.

005656

Reviewed by: Winnie Teeters
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. for LDC 12/24/85
Section V , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Rabbit teratology TOX. CHEM. NO.: 727 A

ACCESSION NUMBER: 263739 MRID NO.: - Tox. Proj. No. 2250

TEST MATERIAL: Prodiamine Technical

SYNONYMS: (Rydex)

STUDY NUMBER(S): WIL-15153

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: WIL Research Labs., Inc.

TITLE OF REPORT: A Teratology Study in Rabbits with Prodiamine Technical

AUTHOR(S): M.D. Nemec, B.S.

REPORT ISSUED: 11-7-85

CONCLUSIONS: A NOEL for maternal toxicity was not established and the LEL is 100 mg/kg (LDT), based on an adverse effect on body weight gain. The fetal developmental toxicity NOEL is 500 mg/kg (HDT) and the LEL is greater than 500 mg/kg.

Classification: core-minimum

A. MATERIALS:

1. Test compound: Prodiamine Technical, Description: Light orange powder, Batch: #C-84331, Purity: 96.3%
C-84268 91.3%
Z-0009 91.3%

2. Test animals: Species: Rabbit, Strain: New Zealand White, Age: approximately 5 1/2 mo., Weight: 3.066-4.707g, Source: Hazleton-Dutchland, Inc.

B. STUDY DESIGN:

1. Animal assignment:

Animals were assigned, by a computer randomization procedure based on body weight stratification, to the following groups:

Test Group	Dose (mg/kg)	Vol.	No. of mated females
1 Control	0	4ml/kg	18
2 Low	100	"	18
3 Mid	300	"	18
4 High	500	"	18

2. Test and Control Material Preparation and Dosing:

The vehicle was 0.5% aqueous methylcellulose prepared fresh at least weekly and stored under refrigeration.

A slurry was made from the vehicle and appropriate weighed quantities of Prodiamine, then diluted to final concentration with additional vehicle. Dosing preparations were prepared fresh daily.

Dosing was accomplished by gastric intubation once daily for 13 consecutive days (6 through 18 of gestation) using a volume of 4 ml/kg based on the most recent body weights.

3. Insemination Procedures:

Semen was collected from resident males of the same strain and source as the females and evaluated for concentration and motility. Semen with greater than 50% motility and 3 million motile sperm/ml was used. An aliquot of 0.25-0.50 ml was deposited into the anterior vagina of each female after which she received an IV injection of 100 units of human chorionic gonadotropin.

C. METHODS AND RESULTS:

1. Observations and Survival:

Methods: Individual clinical observations were recorded from days 0-29 of gestation; they included examinations for appearance, behavior, signs of toxicity, moribundity and mortality.

Results: Although it was stated that individual observations were recorded, only data summarized by groups were reported.

No rabbits died; four aborted and were sacrificed prior to scheduled time, they were: one from the control group on gestation day 28; two from the low level, both on day 29; and one from the high level on day 26. The incidence was not dose-related and is considered to represent spontaneous occurrences.

Decreased defecation and urination were the most prevalently noted signs; the incidence/number of affected animals were markedly greater for the high level (100/13 for defecation and 92/13 for urination) compared to the controls (19/8 and 27/8, respectively) and slightly greater for the mid level (34/11 and 35/9, respectively).

Hair loss appeared to be evenly distributed among the groups.

The incidences of "red material" found on the cage papers of 2, 1, 1, and 1 rabbits did not correspond to the number of abortions, which were 1, 2, 0 and 1 for the control, low, mid and high dose groups, respectively.

2. Body Weights:

Methods: Maternal body weights were recorded individually on gestation days 0, 6, 12, 18, 24 and 29 and mean weights were calculated for these days and mean changes for the corresponding intervals and for 6-18, 18-29 and 0-29 days of gestation.

Results: Body weight gain was markedly decreased in the high dose group, moderately affected in the mid group and clearly decreased in the low group. Weight changes for the groups are shown in the following table (Table 4 of the report).

Throughout treatment, gains for the low, mid and high groups were considerably less than for controls and the mid and high groups lost weight between gestation days 12-18 and 6-18, when their weight changes were statistically significantly different from the control group ($p<0.05$ and $p<0.01$, respectively, for the two groups at both intervals). This adverse effect persisted for the high group, although not statistically significant, for the interval 18-24 days. Both the mid and high groups gained considerably more than the control and low groups for the period 24-29 days of gestation. Body weight gains for the entire study (days 0-29 of gestation) for the treated groups were less than for the control, but were significantly less ($p<0.05$) only for the high group.

3. Food consumption:

Methods: Individual food consumption was recorded daily from days 0-29 of gestation. Food intake was calculated as g/animal/day and as g/kg/day for corresponding body weight gain intervals.

110201 PRODIAMINE RIN 1786-93

Page 42 is not included in this copy.

Pages _____ through _____ are not included.

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 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.

✓ 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.

Results: Food consumption data generally corresponded to weight gain data except that significantly different values were only obtained for the high dose group. For this group, food consumption was less, compared to controls, for periods 12-18, 18-24 and 6-18 days of gestation ($p<0.01$ for g/animal/day for each interval). During treatment days 6-18 and the posttreatment days 18-29, food consumption was lower for the low and mid dose groups also, compared to the controls, but the differences were not significantly different.

4. Gestation Day 29 Cesarean Section Data:

Methods: All surviving females were sacrificed on gestation 29 by an intravenous injection of T-61 Euthanasia Solution. The contents of the thoracic and abdominal cavities were examined. The number of corpora lutea of each ovary and the number and location of viable and nonviable fetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of implantation were opened and placed in 10% ammonium sulfide solution. Dams which aborted were sacrificed that day and examined as for those at scheduled sacrifice. Each fetus was weighed individually, sexed and tagged for identification.

Results: There were no dead fetuses for dams sacrificed on day 29 of gestation. The mean number of implantation sites, corpora lutea and viable fetuses were comparable between treated groups and the control. There was some slight variation in the sex ratios among the groups but the differences were not notable. The mean number of early and late resorptions per litter for the control group was only 0.1 for each type and treated groups had more early resorptions (0.7, 1.2 and 0.4) and equal or greater late resorptions (0.2, 0.1 and 0.7, respectively, for the low, mid and high dose groups), resulting in higher postimplantation loss for treated groups which was somewhat trend-like for dose response relationship (0.3, 0.9, 1.3 and 1.1 for controls, low, mid and high dose groups, respectively). Mean fetal weights and the mean numbers of live fetuses/litter were comparable among the groups. None of the data for these parameters was significantly different between control and treated groups. These fetal data are shown in the following table, No. 7 copied from the report.

5. Necropsy Examination:

Methods: The methods for this examination have already been stated in Number 4, above, for cesarean section data.

Results: Four dams aborted: a control on day 28; two low dose dams, both on day 29; and a high dose dam on day 26 of gestation. There were no notable findings for the control and high dose dams which aborted. One aborted dam of the low dose group had a pale liver and biliary stasis; the other aborted dam of this group had a herniated umbilical cord with cyst-like formation and accentuation of hepatic lobular markings.

The most prevalent findings at scheduled necropsy were ascities,

110201 PRODIAMINE RIN 1786-93

Page 44 is not included in this copy.

Pages _____ through _____ are not included.

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 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.

✓ 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.

---

pulmonary congestion, soft mesenteric lymph nodes (some with fluid) and accentuation of hepatic lobular markings. The incidence ~~did not~~ did not appear to have any relationship to treatment.

The incidence of nongravid does was 3, 0, 3 and 8 for the control, low, mid and high dose groups, respectively; all were negative for sulfide staining. From the 18 does in the high dose group, only 9 litters were available for examination at gestation day 29.

6. Fetal Morphological Data:

Methods: Each fetus was subjected to an external examination, to include but not be limited to examination of the eyes, palate and external orifices. The crown-rump length of late resorptions was measured and the tissues were discarded. Each fetus was examined by a modification of the Staples fresh dissection technique. The brain was examined following a mid-coronal slice. Eviscerated fetuses were skinned, fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S. External, visceral and skeletal findings were recorded as developmental variations or malformations.

Results: The following table, Table 8 of the report, presents the number of fetuses and litters with malformations. The only findings with an incidence of greater than a single fetus/group were skeletal vertebral anomalies in two fetuses each of the control and mid level groups and 2 fetuses with cardiovascular anomalies and 4 with cataracts, all in the low dose group. The 4 fetuses with cataracts came from one litter but the other malformations for this group occurred in one fetus/litter. There was no dose-response relationship for any of these findings nor were any of the dosed groups statistically significantly different compared with the controls.

The most prevalent visceral variations were those of major blood vessels and absent or small gallbladder; for the blood vessels the incidences (litters) were 18(8), 13(7), 17(10) and 1(1) and for the gallbladder they were 1(1), 3(2), 5(5) and 2(2) for the control, low, mid and high dose groups, respectively. The percent incidence for visceral and skeletal variations are shown in the next table (Table 11 of the report). There were no external variations. The most prevalent skeletal variations were those for 13th ribs (rudimentary and full), 27 presacral vertebrae, bent hyoid arches, and 7th cervical ribs. For all but one of these findings there either was an inverse litter percentage dose-response relationship or the control group had the highest incidence; but for malaligned sternebrae the percentages were 7.1, 12.5, 13.3 and 22.2 for the control, low, mid and high dose groups, respectively. The only significant ($p < 0.05$) differences from controls for the litter variation incidences were a decrease in the high dose for major blood vessel variations (control-8, low dose-1) and an increase in the incidence of unossified 5th or 6th sternebrae for the low dose (control-1, low dose-6). For each of the skeletal variations mentioned above, the historical control had a higher range (litter percentage) than the highest values

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 46 through 47 are not included.

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 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.

__✓__ 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.

---

for any group in the present study, even including that for the low dose. With consideration for the lack of a dose-response relationship and comparison to historical control data, none of the variation data indicate a compound-related effect.

7. Discussion and Conclusions:

At the doses used (100, 300 and 500 mg/kg/day), the compound was not lethal to any of the rabbits. The four abortions (1 control, 2 low dose and 1 high dose) were not dose-related and were considered spontaneous. During treatment days 6-18 of gestation the mid and high dose does showed significant dose-related body weight losses. During this period body weight gain was reduced in the low level also but not to a significant extent. After cessation of treatment there was a delayed rebound effect on body weight for the mid and high dose groups for days 24-29 since their gains were more than those of the control and low dose groups for this period. Although food consumption was lower during and after treatment for all treated groups, the differences were significant only for the high dose group. The adverse effects on body weight and food consumption for this group are evidence that a maximum tolerated dose was used. Decreased defecation and urination noted for the mid and high dose groups probably can be associated with the adverse effects noted on body weight and food consumption.

There were no indications that the necropsy findings were related to treatment since the incidence of the most prevalent findings of ascities, pulmonary congestion, soft mesenteric lymph nodes and accentuated hepatic lobular markings showed no relationship to dose. However, the total incidence for ascities (16/72, 22%) and pulmonary congestion/hydrothorax (13/72, 16%) raises some doubt regarding the health status of the selected rabbits.

All the fetuses were alive at scheduled dam sacrifice and there were no compound-related effects on the mean numbers of implantation sites, corpora lutea, viable fetuses or mean fetal weights. Although postimplantation loss was slightly higher for all treated groups than for the concurrent control, the differences were not significant, the effect was not strictly dose-related (0.3, 0.9, 1.3 and 1.1 for control, low, mid and high dose groups, respectively), the control appeared low when compared to historical control data (1.0 with a range of 0.3-1.9) and the values for the treated groups were within the range for the historical control (see Appendix A which has been copied and attached to this review). Unfortunately, the historical control data were only summarized rather than being provided both for individual studies and in summary form.

There did not appear to be an effect of the compound on fetal development as evaluated by external, visceral or skeletal examinations. Although some of the treated groups had a higher incidence than the control for some malformations and variations, for these data there was either: no dose-response relationship; no significant difference from control or, for the variation data, the range for

the historical control showed a higher litter percentage incidence than found for treated groups in the present study. The last situation applies to the only litter incidence which had any semblance of a dose-response effect, that for malaligned sternebrae for which the incidence was 7.1, 12.5, 13.3 and 22.2 % for the control, low, mid and high dose groups, respectively. Yet the historical control had a range up to 55% for this finding.

The number of litters available from the high dose level was only nine. This number is fewer than required (12) and thereby reduces the sensitivity of the test. This low number is acceptable for this study, however, since there is no indication of a fetal effect.

The decreased maternal body weight gain during treatment seen for the low level, although not significantly different from control, usually would be taken as evidence for a compound effect because of the marked dose-related effect on body weight noted for the mid and high dose levels; however, because small body weight variations are quite common, particularly for this species, one has less confidence accepting a non-significant decrease as a true compound effect. Yet, the gains shown by this group for several periods are considerably less than for the controls: only 51% of control gain for days 6-12, 22% for days 12-18, 38% for days 6-18 and 58% for the entire study, days 0-29. These are substantial differences to attribute just to normal weight variability. Consequently, it is concluded that a NOEL for maternal toxicity has not been established and the LEL is 300 mg/kg (LDT), based on an adverse effect on body weight gain.

The NOEL for fetal developmental toxicity is the high dose, 500 mg/kg, since there was no convincing evidence for any fetal effect, and the LEL for this parameter is greater than 500 mg/kg, the highest dose tested.

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 50 through 55 are not included.

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 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.

____ 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.

005656

Reviewed by: Winnie Teeters
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. Concur for LDC 12/23/86
Section V , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Range-finding teratology TOX. CHEM. NO.: 727A

ACCESSION NUMBER: 263739 MRID NO.: - Tox. Proj. No. 2250

TEST MATERIAL: Prodiamine Technical

SYNONYMS: (Rydex)

STUDY NUMBER(S): WIL-15144

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: Wil Research Labs, Inc.

TITLE OF REPORT: A Range-finding Teratology Study in Rats with Prodiamine Technical

AUTHOR(S): M.D. Nemec, B.S.

REPORT ISSUED: 3-28-85

CONCLUSIONS: At the highest dose level of 1000 mg/kg/day, Prodiamine Technical decreased maternal body weight gain, particularly during the first 3 days of treatment (gestation days 6-9). No other notable signs of maternal toxicity were noted. There were no compound-related effects on fetal intrauterine survival, mean postimplantation loss and the mean numbers of corpora lutea, implantation sites and viable fetuses. Dose levels of 100, 300 and 1000 mg/kg were selected for the main study.

The NOEL for maternal toxicity is 500 mg/kg and the LEL is 1000 mg/kg, based on a slight adverse effect on body weight gain.

Classification: The study is classified as Supplementary Data since it is a range-finding study.

005656

A. Materials:

1. Test compound: Prodiamine Technical; Description: Yellowish- reddish orange powder, Batch: #84268, Purity: 91.3%

2. Test animals: Species: Rats, Strain: CRL COBS CD, Age: 13 weeks, Weight: 225-278 g, Source: Charles River Breeding Labs, Inc.

B. Study Design:

1. Animal assignment:

Animals were assigned consecutively in a block design to groups containing 5 rats each, as follows:

Test Group	Dose (mg/kg/day)	No. of Mated Females
1. Control	0	5
2. Low dose	50	5
3. Intermed-1	125	5
4. Intermed-2	250	5
5. Intermed-3	500	5
6. High dose	1000	5

2. Test Material Preparation and Dosing:

The appropriate amount of material was weighed and a slurry was prepared using 100% Mazola Corn Oil. Test mixtures were prepared daily. They were not adjusted for purity.

Dosing was orally by gavage, once daily for 10 consecutive days from gestation day 6 through 15. Control rats received corn oil on a comparable regimen. Doses were based on the most recent body weights.

3. Animals received food (Purina Certified Rodent Chow # 5002) and water ad libitum.

4. No statistical analyses were performed.

5. Quality Assurance was accomplished by inspections on Feb. 6, 7 and 13. A statement to the effect that to the best of his knowledge there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study was signed by the QA Director.

C. Methods and Results:

1. Observations:

Methods: Animals were inspected daily from days 0 through 20 of gestation and additionally 1 hour after each dose for signs of toxicity and clinical effect and for mortality and moribundity.

Results: All animals survived to scheduled sacrifice. No clinical signs of toxicity were observed other than sporatic hair loss, which occurred in all groups but was most prevalent in the 50 and 100 mg/kg groups, and one occurrence each of yellow urogenital staining and red vaginal discharge in the

500 mg/kg group.

2. Body Weights:

Methods: Females were weighed on gestation days 0, 6, 9, 12, 16 and 20.

Results: Mean body weights on these days do not reveal any obvious compound-related effect. However, examination of mean weight changes reveals that for treatment days 6-16, all test groups consistently gained less (not dose-related) than the control; this was also true for days 6-20 and 0-20, with the lowest gains for these two periods being for the high dose. All test groups also gained less for gestation days 6-9 (13, 11, 9, 7, 9 and 3 grams, respectively, for control, 50, 125, 250, 500 and 1000 mg/kg levels). See attached Table 4, copied from the report.

3. Gestation Day 20 Cesarean Section Data and Maternal Necropsy Examinations.

Methods: All animals were sacrificed by carbon dioxide asphixiation on gestation 20 and the abdominal and thoracic cavities were opened and the contents examined. The number of corpora lutea on each ovary, the number and location of viable and nonviable fetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no evidence of macroscopic implantation were excised, opened and placed in ammonium sulfide solution.

Results: There was no compound effect noted on fetal intrauterine survival, mean postimplantation loss and the mean numbers of corpora lutea, implantation sites and viable fetuses. Also, there were no apparent compound-related effects noted at necropsy of the dams.

110201 PRODIAMINE RIN 1786-93

Page 59 is not included in this copy.

Pages _____ through _____ are not included.

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 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.

__✓__ 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.

005656

Reviewed by: Winnie Teeters
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. Cleared for CBE 12/24/86
Section V , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Teratology in Rats TOX. CHEM. NO.: 72A

ACCESSION NUMBER: 263739 MRID NO.: - Tox. Proj No. 2250

TEST MATERIAL: Prodiamine Technical

SYNONYMS: (Rydex)

STUDY NUMBER(S): WIL-15150

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: WIL Research Labs., Inc.

TITLE OF REPORT: A Teratology Study in Rats with Prodiamine Technical

AUTHOR(S): M.D. Nemac, B.S.

REPORT ISSUED: 11-11-85

CONCLUSIONS: The NOEL for maternal toxicity is 300 mg/kg and the LEL is 1000 mg/kg, based on depressed body weight gain.

A NOEL for developmental toxicity has not been determined in this study based on the conclusion that there is a compound related increase in the incidence of ocular anomalies at the lowest dose tested, 100 mg/kg, which, consequently, is considered the LEL for this effect.

Classification: Core-minimum, but it is necessary to establish a NOEL for developmental toxicity in another study. Since the sponsor has indicated that the ocular anomalies noted in the present study have been reported to have a genetic origin, any data provided to support this claim will be considered in a reevaluation of the present study.

A. MATERIALS:

1. Test compound: Prodiamine Technical, Description: Yellowish- orange powder, Batch # 84268, Purity 91.3%

2. Test animals: Species: Rat, Strain: CRL:CD (SB) BR, Age: 12 weeks, Weight: 220-272 g, Source: Charles River Breeding Labs, Inc.

B. STUDY DESIGN:

1. Animal assignment:

Animals were assigned consecutively in a block design to the following groups:

Group	Dose (mg/kg/day)	No. of mated females
Control	0	25
Low	100	25
Mid	300	25
High	1000	25

2. Test material preparation and dosing:

The appropriate amount of material was weighed and a slurry was prepared using 100% Mazola Corn Oil. Test mixtures were prepared daily. They were not adjusted for purity.

Dosing was orally by gavage, once daily for 10 consecutive days from gestation day 6 through 15. Control rats received corn oil on a comparable regimen. Doses were based on the most recent body weights.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistical Methods:
Two-tailed tests for a minimum significance level of 5% comparing the treatment group to the control group were conducted; means with standard deviations are presented. Fetal sex ratios were analyzed using the Chi-square test with Yates' correction factor. Malformation and variation data were analyzed by Fisher's Exact Test. The numbers of early and late resorptions, dead fetuses and post-implantation losses were analyzed by the Mann-Whitney U-Test. Mean numbers of corpora lutea, total implantations and viable fetuses, mean fetal weight and maternal body weight, and maternal body weight gain were analyzed by a one-way analysis of variance and Dunnett's test.

5. Quality assurance was accomplished by several inspections. A statement, to the effect that to the best of his knowledge there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study, was signed by the QA Director.

C. METHODS and RESULTS:

1. Observations:

Methods: Animals were inspected daily from days 0 through 20 of gestation and

additionally 1 hour after each dose.

Results: Survival was 100% in all study groups. Alopecia, the primary clinical observation, was noted in each group, including the control, but with higher frequency in the mid and high dose groups. Yellow urogenital staining was observed sporatically in 5 rats in the high dose group during gestation days 7-20 and orange-colored urine was seen after dosing in each treated group.

2. Body weight:

Methods: Females were weighed on gestation days 0, 6, 9, 12, 16 and 20.

Results: When mean body weights during gestation days 0-20 were compared, there were no statistically significant differences between the control and each test group (see following Table 3, copied from the report), but treated groups weighed less than the controls for days 9, 12, 16 and 20 and the differences were greater (up to 4% decrease) for the high level. When body weight changes, a more sensitive weight parameter for this test, were compared (see following Table 4, copied from the report), the mid and high dose groups gained less than the controls for days 6-9 ($p<0.05$ for each) and days 6-16 ($p<0.05$ and 0.01 for these treated groups, respectively). Although weight changes for all treated groups for days 6-20 and 0-20 were also lower than controls, they were not statistically different.

3. Gestation Day 20 Cesarean Section Data and Maternal Necropsy Examination:

Methods: All females were sacrificed by carbon dioxide asphyxiation on gestation day 20. The abdominal and thoracic cavity contents were examined. The uterus and ovaries were exposed and the number of corpora lutea on each ovary, the number and location of fetuses (viable and nonviable), early and late resorptions and total number of implantation sites were recorded. Uteri with no evidence of macroscopic implantation were stained with ammonium sulfide.

Results: There were no statistically significant differences between the control and test groups for fetal sex ratios, mean numbers of viable fetuses and implantation sites, and mean fetal weights, but the mean number of corpora lutea was lower in each treated group (statistically significant [$p< 0.05$] only for the low dose [100 mg/kg/day group]). The mean post-implantation loss for the low dose group was also lower ($p<0.05$) than for the control (0.9, 0.4, 0.7 and 0.7 for the control, low, mid and high dose groups, respectively). These data are shown in the following Table 5, copied from the report.

Five dams each in the control and mid dose groups and four each in the low and high dose groups were non-gravid; all were ammonium sulfide negative.

Cystic kidneys were noted in 1, 4 and 3 dams of the control, mid and high dose groups, respectively. One dam each in the low and high dose groups showed hydronephrosis. One control dam had a fused placenta at two sites and a high dose dam showed inflammation of the uterus and vagina.

4. Fetal Morphological Data:

Methods: Each fetus was weighed, sexed, tagged and examined externally.

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 63 through 65 are not included.

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 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.

__✓__ 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.

Late resorptions were discarded after measurement of crown-rump length. Approximately one-half of the fetuses from each dam were placed in Bouin's fixative for soft tissue examination via Wilson's sectioning technique. The remaining fetuses were eviscerated, fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for skeletal examination.

Results: Several fetuses had external malformations. Omphalocele was noted in all groups except the low dose; the incidences (litter) were 1 (1), 0 (0), 4 (2) and 1 (1) for the control, low, mid and high doses, respectively. Cleft palate was noted in one fetus of the high dose group. Microphthalmia and/or anophthalmia was noted in two fetuses of the same litter in the low dose group and in four fetuses (2/litter) of the high dose group; no control fetus showed this anomaly.

The number of fetuses and litters with malformations and the corresponding percentages are shown in the following two tables (#6 and #7) copied from the report. None of the incidencies for treated groups were statistically significantly different from the controls.

Only one fetus (high dose) had a visceral malformation, a malpositioned testicle; one of his littermates was the pup in this group having an omphalocele.

Two skeletal malformations: vertebral anomaly (with or without associated rib anomaly) and sternoschisis, were noted; they both occurred in the same high dose pup (which also had microphthalmia). Vertebral anomalies were also noted in one pup each in the control and low dose groups.

The only variations noted were those for the urinary tract and skeleton, with the latter being much more prevalent. The only visceral variation was the finding of "renal papilla(e) not developed and/or distended ureter(s)"; treated groups had an incidence comparable to or less than the control (see following Table 9, taken from the report, for all variation percentages). Several skeletal variations were seen; the most frequent were: 14th rudimentary ribs, unossified 5th or 6th sternebrae and malaligned sternebrae. For each of these, a treated group had a higher incidence than the control; sternebral variations were found most often in the low dose and the rudimentary ribs most often in the mid dose. Consequently, there was not an apparent dose-response relationship discernable among these skeletal variation data.

5. Discussion and Conclusions:

There was a definite adverse effect on body weight gain for the mid and high dose groups during the period of 6-9 days of gestation, and the low dose group showed the same effect but to a much lesser degree (9, 7, 3, and 3 g mean gain for the control, low, mid and high dose groups, respectively). Gains for these two groups were also significantly lower for days 6-16. But this early period (days 6-9) was the only period for which there was an obvious difference between the low and mid dose groups; in fact, for all other periods the gains for the mid dose group were the same (days 6-16) or greater than for the low dose group. The changes in body weight for the mid group amounted to only a 3% decrease. Although the depressed body weight for the high dose group was low also, only a 4% decrease, this group's difference in gain reached the 0.01 level of significance and a depressed gain was consistently seen after treatment began. Consequently, it is concluded that the high dose group was the only one showing a biologically significant adverse effect on weight gain.

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 67 through 69 are not included.

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 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.

✓ 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.

The fetal data for treated groups which were significantly different from the controls were lower mean values for postimplantation loss and corpora lutea only for the low dose group; consequently, there was no biological significance attached to these data. In fact, the control group for the present study, although within the range for the historical control data, had somewhat higher values for each of these parameters. Historical data are presented in Appendix A, copied from the report and attached to this review.

In the present study some treated groups had a higher incidence of external malformations, i.e., omphalocele in the mid group and ocular anomalies in the low and high dose groups, than the control incidence (see Table 6). The range of percent incidence of the historical control for omphalocele was up to 0.4 for fetuses and up to 5.0 for litters (the overall incidence is actually .06% for fetuses and .88% for litters); these values are lower than found for the mid dose group of the present study: 0.4, 0.0, 1.5 and 0.4 for fetuses and 5.0, 0.0, 10.0 and 4.8 for litters for the control, low, mid and high dose groups, respectively. However, there is no dose-response relationship in the data of the present study since only the mid level has an increased incidence compared to the concurrent and historical controls. Furthermore, in the mid dose, three of the four affected pups were from the same litter. This situation is of less concern than would have prevailed had the pups each been in a separate litter. Likewise, the range of percent incidence of the historical control for microphthalmia and/or anophthalmia was lower (up to 0.3% for fetuses and up to 4.3% for litters, but overall incidence is actually .009% for fetuses and .13% for litters) than found in the present study (0.0, 0.7, 0.0 and 1.5% for fetuses and 0.0, 4.8, 0.0 and 9.5% for litters for the control, low, mid and high dose groups, respectively). But, again, there is not a clear dose-response relationship for the findings in the present study since the mid group had no incidence for these ocular anomalies. For this finding, the six affected pups were evenly distributed among three litters: one low dose litter and two high dose litters. Furthermore, the incidence in the high dose group in this study is five-fold greater for fetal incidence and two-fold greater for litter incidence than for the historical control range (but by using the overall historical control incidence as the basis for comparison, the fold differences would be 166 for fetuses and 73 for litters). Consequently, it is concluded that a compound related effect on developmental toxicity has been demonstrated by the data of the present study and the effect was seen at the lowest level. The sponsor noted that these ocular malformations have been reported to occur in this strain as a congenital anomaly which is inherited as an autosomal recessive trait (Kinney et al., Teratology 26:203, 1982); but the incidence must be very low since in the provided historical data it was only 0.13%.

Some treated groups showed a higher percentage incidence for some skeletal variations but there was not a clear dose-response relationship. Furthermore, the prevalent variations are rather common ones and each had a higher historical control incidence than found in the treated groups of the present study so no biological significance was attached to these findings.

The NOEL for maternal toxicity is 300 mg/kg and the LEL is 1000 mg/kg, based on depressed body weight gain.

A NOEL for developmental toxicity has not been determined in this study based on the conclusion that there is a compound related increase in the incidence of ocular anomalies at the lowest dose tested, 100 mg/kg, which, consequently is considered the LEL for this effect.

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 71 through 78 are not included.

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 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.

____ 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.

005656

SUBJECT: Technical Prodiamine, Discussion of Oncogenicity Studies to Support Non-Crop Uses

TOX. CHEM. NO.: 727-A

ACCESSION: 263732

TOX. PROJ. NO.: 2250

DATE: July, 1986

SPONSOR: Sandoz Crop Protection Corp.

This presentation begins with material copied from Footnote 21 of EPA Guidelines for oncogenicity studies stating the criteria for determining when these studies are required. This was followed by the statement that EPA's letter of Jan. 17, 1986 had referenced this footnote with regard to prodiamine.

There follows then a summation of the results from mutagenicity assays of prodiamine, which includes the five studies reviewed in this action and an earlier study which was referenced in this action. The earlier study was an Ames test which was positive in tester strain TA-1538 (memo of Mauer to Mountfort, July 28, 1984). It is explained in this presentation that the prodiamine in this referenced assay was that produced by U.S. Borax and that it is uncertain what process was used for the tested material since Borax was evaluating different processes at that time. The current mutagenicity studies involve Sandoz Crop Protection material. There are five of these studies with the following results:

1. Ames test with Prodiamine Technical (96.3% purity), Study # T2840.501-Negative

2. Ames Test with Prodiamine (92.9% purity), Study # T4022.501-Negative

3. Mouse Lymphoma Assay with Prodiamine Technical (96.3% purity), Study # T2840.701- Weakly positive at toxic concentrations without activation, and negative with activation.

4. Chromosome aberration in Chinese hamster ovary cells with Prodiamine Technical (96.3% purity), Study # T2840.337-Negative.

5. Unscheduled DNA synthesis in rat primary hepatocytes with Prodiamine Technical (96.3% purity), Study # T2840.380-Negative.

In summary, therefore, there have been three bacterial mutation tests (Ames), one of which used Borax Prodiamine and was positive in tester strain TA 1538, and two tests, each using a Sandoz Prodiamine (96.3 or 92.9% purity) which were negative on all 5 common tester strains, including strain TA 1538. Additionally, Sandoz Prodiamine was negative in two other mutagenicity assays (chromosome aberration and unscheduled DNA synthesis), and weakly positive (at toxic concentrations) in a third assay, the mouse lymphoma test.

The final section of this discussion presentation is about the "Oncogenicity History".

First, there is reference to EPA's evaluation of the oncogenicity of trifluralin as contained in the "Trifluralin, Position Document 4", in which it is concluded that trifluralin is not a mouse oncogen but there is evidence to suggest it is a rat oncogen. (Trifluralin and Prodiamine have similar chemical structures.)

Next, the oncogenicity studies with prodiamine are discussed. These include two IBT studies which have been reviewed by EPA. The rat study, IBT No. 621-06644, has been classified as Core-Supplementary Data (memo of Teeters to Mountfort, March 28, 1983). The mouse study, IBT No. 651-07145, was evaluated as Invalid (Burin, 3-2-83).

The sponsor states that "there were no compound related effects in any of the parameters investigated at the highest level tested, 600 ppm", in the rat study and that "no evidence of carcinogenicity was demonstrated at dietary levels up to 10,000 ppm prodiamine" in the mouse study, and concludes that "there is no current evidence available which suggests prodiamine is carcinogenic in either rodent species or mutagenic in any test systems utilized".

This reviewer essentially agrees with the sponsor regarding mutagenicity evaluation of the Sandoz Prodiamine (with the caveat that the weakly positive test should be repeated). But it is appalling that the sponsor would make any conclusions whatsoever regarding oncogenicity potential in view of the generally recognized suspect nature of the source of the experimental data, and, particularly, with having the benefit of the EPA classification of these two studies. With these classifications, neither study can meet the regulatory requirements for an oncogenicity study. Consequently, these two studies should not be used as supporting data by the sponsor for his statement that "there is no current evidence available to suggest that prodiamine is carcinogenic in either rodent species".