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

008210

DEC 27 1990

OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM

**Subject:** Prodiamine Technical  
Identifying Number 55947-UR  
Tox Chem No. 727A  
HED Project No. 0-1689

**From:** John H.S. Chen, D.V.M. *John H.S. Chen 12/17/90*  
Review Section I  
Toxicology Branch II  
Health Effects Division (H7509C)

*Acute Det. 12  
Ac*

**To:** Joan I. Miller, PM 23  
Herbicide-Fungicide Branch  
Registration Division (H7505C)

**Thru:** Yiannakis M. Ioannou, Ph.D., Section Head *J.M. Ioannou 12/18/90*  
Review Section I  
Toxicology Branch II  
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief *Marcia van Gemert 12/19/90*  
Toxicology Branch II  
Health Effects Division (H7509C)

**Registrant:** Velsicol Chemical Corporation  
Chicago, IL 60611

**Action Requested:** Review and assessment of the acute toxicity studies with technical prodiamine and formulated prodiamine 75 WP, and the mouse lymphoma forward mutation assay with technical prodiamine.

Recommendation:

1. The following studies are adequate to support the Registrant's request:

A. Acute oral toxicity to rats of prodiamine technical (81-1). HRC 84704D/VCL47/AC. September 20, 1984. LD50 > 5 g/kg (both sexes); Toxicity Category IV; Core Minimum.

B. Acute dermal toxicity to rats of prodiamine technical (81-2). HRC 84705D/VCL48/AC. September 20, 1984. LD50 > 2 g/kg (both sexes); Toxicity Category III; Core Minimum.

C. Irritant effects on the rabbit eye of prodiamine technical (81-4). HRC 84623D/VCL50/SE. August 29, 1984. Minimally irritating to the rabbit eye; Toxicity Category III; Core Guideline.

D. Acute oral toxicity to rats of prodiamine 75 WP (81-1). HRC 84706D/VCL52/AC. September 20, 1984. LD50 > 5 g/kg (both sexes); Toxicity Category III; Core Minimum.

E. Acute dermal toxicity to rats of prodiamine 75 WP (81-2). HRC 84707D/VCL53/AC. September 20, 1984. LD50 > 2 g/kg (both sexes); Toxicity Category III; Core Minimum.

F. Dermal sensitization study of prodiamine 75 WP in rabbits (81-6). HRC 84865D/VCL57/SS. November 2, 1984. Not a sensitizer. Core Guideline.

G. Mouse lymphoma forward mutation assay with prodiamine technical (84-2). HLA 12200-0-431. June 27, 1990. Negative. Acceptable.

H. Irritant effects on the rabbit eye of prodiamine 75 WP (81-4). HRC 84624D/VCL55/SE. August 29, 1984. Minimally irritating to the eyes of rabbit. Toxicity Category III. Core Guideline.

2. The Registrant should be apprised of the deficiencies reported for the following studies:

A. Irritant effects on rabbit skin of prodiamine technical (81-5). August 15, 1984. HRC 84621D/VCL51/SE. Non-irritation to the rabbit skin. Toxicity Category IV. Supplementary.

B. Irritant effects on rabbit skin of prodiamine 75 WP (81-5). August 15, 1984. HRC 84622D/VCL56/SE. Non-irritation to the rabbit skin. Toxicity Category IV. Supplementary.

## Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. *John H.S. Chen 12/14/90*  
Section I, Toxicology Branch II (H7509C)

Secondary reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 12/18/90*  
Section I, Toxicology Branch II (H7509C)

**DATA EVALUATION REPORT**

**CHEMICAL:** Prodiamine

**Tox. Chem. No.:** 727A

**EPA File Symbol:** 55947-UR

**STUDY TYPE:** Mammalian cells in culture gene mutation assay  
in mouse lymphoma L5178Y cells

**ACCESSION NUMBER:**

MRID Number: 415581-01

**SYNONYMS/CAS No.:**

**SPONSOR:** Sandoz Crop Protection Corp., Des Plaines, IL 60018

**TESTING FACILITY:** Hazleton Laboratories America, Inc.  
Kensington, MD 20895

**TITLE OF REPORT:** Prodiamine technical in the L5178Y TK+/- mouse  
lymphoma forward mutation assay

**AUTHOR(S):** Robert R. Young

**STUDY NUMBER(S):** 12200-0-431

**REPORT ISSUED:** June 27, 1990

**CONCLUSION(S) - Executive Summary:**

Prodiamine technical was nonmutagenic in the in-vitro mouse lymphoma forward mutation assay with or without metabolic activation at the concentrations tested.

Concentrations tested: 0.5, 1, 4, 6, 8, 10, 13, 16, 20, & 50 ug/ml under the activated experiment; 1, 10, 40, 60, 80, 100, 130, 160, 200, & 500 ug/ml under the nonactivated experiment.

**Study:** Acceptable

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

1. Test Material: Name: Prodiamine technical  
Description (e.g. technical, nature, color, stability):  
orange crystals  
Batch #: C-85177 Purity: 94.3%  
Contaminants: if reported, list in CBI appendix  
Solvent used: DMSO  
Other comments:

2. Control Materials:

Negative: DMSO

Solvent/final concentration: DMSO

Positive: Non-activation (concentrations, solvent):

Ethylmethane sulfonate (EMS; 0.25 & 0.4 ul/ml)

Activation (concentrations, solvent):

3-methylcholanthrene (MCA; 2.5 & 4.0 ug/ml)

3. Activation: S9 derived from

Aroclor 1254  induced Male rat  liver  
 phenobarbital  non-induced  mouse  lung  
 none  hamster  other  
 other  other

If other, describe below

Describe S9 mix composition (if purchased, give details):

NADP (sodium salt) 3 mM  
Isocitrate 15 mM  
S9 homogenate 20 ul/ml

4. Test Cells: mammalian cells in culture

mouse lymphoma L5178Y cells  
 Chinese hamster ovary (CHO) cells  
 V79 cells (Chinese hamster lung fibroblasts)  
 other (list):

Properly maintained?  / N (circle one)

Periodically checked for Mycoplasma contamination?

/ N (circle one)

Periodically checked for karyotype stability?

/ N (circle one)

Periodically "cleansed" against high spontaneous background?

/ N (circle one)

MAMMALIAN CELLS IN CULTURE GENE MUTATION

5. Locus Examined:

thymidine kinase (TK)  
selection agent: \_\_\_\_\_ bromodeoxyuridine (BrdU)  
(give concentration) \_\_\_\_\_ 3 ug/ml fluorodeoxyuridine (FdU)  
trifluorothymidine (TFT)

\_\_\_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)  
Selection agent: \_\_\_\_\_ 8-azaguanine (8-AG)  
(give concentration) \_\_\_\_\_ 6-thioguanine (6-TG)

\_\_\_ Na<sup>+</sup>/K<sup>+</sup> ATPase  
Selection agent: \_\_\_\_\_ ouabain  
(give concentration)

\_\_\_ other (locus and/or selection agent; give details):

6. Test compound concentrations used:

Non-activated conditions: 1, 10, 40, 60, 80, 100, 130, 160, 200  
& 500 ug/ml

Activated conditions:

0.5, 1, 4, 6, 8, 10, 13, 16, 20, & 50 ug/ml

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound for:  
    4 hours (non-activated) 4 hours (activated)

b. Cells exposed to positive controls for:  
    4 hours (non-activated) 4 hours (activated)

c. Cells exposed to negative and/or solvent controls for:  
    4 hours (non-activated) 4 hours (activated)

d. After washing, cells cultured for 2 days  
(expression period) before cell selection

e. After expression, cells cultured for 10-14 days  
    in selection medium to determine numbers of mutants  
    and for 10-14 days without selection medium to  
    determine cloning efficiency

## MAMMALIAN CELLS IN CULTURE GENE MUTATION

2. Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; cell density during treatment; number of cells seeded for treatment and selection; subculture and feeding schedules, if necessary):

The procedure used was based on the method of Clive *et al* (Mutation Res. 31, 17-29, 1975; Mutation Res. 59, 61-108, 1979). The details of test procedure are attached. (pages 15-16).

3. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; reported results, e.g. cytotoxicity and solubility):

L5178Y cells were exposed to ten dose levels for 4 hours either in the presence or absence of S9 mix. Under the nonactivated assay, significant reductions in cell growth (10% of vehicle control cells) were observed at 0.125 mg/ml and higher. Under the activated assay, significant reductions in cell growth were observed at 0.0156 mg/ml and higher. This assay showed that the test material appears to be more toxic with metabolic activation than without activation. For this reason, a second activation cytotoxicity assay was performed using lower concentrations that ranged from 0.000195 mg/ml to 0.1 mg/ml. The test material was nontoxic at concentrations up to 0.000781 mg/ml followed by increasing toxicity that reached near-total, cell killing at 0.05 mg/ml. These results (Tables 1 and 2 attached) were used to select the dose levels of test material for the mutation assay.

## MAMMALIAN CELLS IN CULTURE GENE MUTATION

4. Mutagenicity assay (reported results, e.g. induction of mutant colonies - individual colony counts and/or summary given; mutant frequencies per  $10^6$  survivors; positive and background mutant frequencies; inclusion of concentration levels used; number of cultures per concentration; levels of cytotoxicity obtained; appropriateness of cloning efficiencies; include representative table, if appropriate):

In the nonactivated assay, no culture with acceptable levels of toxicity ( $> 10\%$  of vehicle control cells) had significantly elevated mutant frequencies (i.e., twice the averaged mutant frequency of vehicle control culture;  $2 \times 52.6 \times 10^{-6} = 105.2 \times 10^{-6}$ ). The five cultures with acceptable levels of toxicity (1, 10, 40, 60 & 80 ug/ml did not have a clear dose-related increase of mutant frequency with test material concentrations (Table 3 attached). The cultures treated with 100,, 160, and 500 ug/ml, while excluded from the evaluation due to excessive toxicity ( $< 10\%$  of relative cell growth), did have significant increases in mutant frequency. Because the reduced cloning efficiency at the time of selection contributed to the elevated mutant frequencies in cultures treated with 80 ug/ml and higher (cloning efficiency from 71.6% to 38.1%) and the absence of a positive correlation of mutant frequency with increasing test material concentration at dose levels with acceptable levels of toxicity, the test material was evaluated as negative for inducing forward mutations at the TK locus in L5178Y cells without metabolic activation (See Table 3).

In the activated assay, no statistically significant increases in mutant frequency above the solvent control were observed (Solvent control M.F. =  $48.4 \times 10^{-6}$ ; Prodiamine treated cultures, M.F. =  $56.4 \times 10^{-6}$  to  $78.1 \times 10^{-6}$ ). Therefore, prodiamine was evaluated as negative for inducing forward mutations at the TK locus in L5178Y cells in the presence of metabolic activation system. (Table 4 attached).

MAMMALIAN CELLS IN CULTURE GENE MUTATION

5. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

A. The positive control compounds (EMS and MCA) induced significant increases in the mutant frequency with respect to the corresponding solvent control by a mutation factor of at least 6.45 (i.e., Factors in all trials: EMS, 8.23-15.05; MCA, 6.45-11.17) indicating the assay was sensitive to known mutagens in the presence and absence of metabolic activation.

B. The background mutation frequencies for the solvent control under either the activated or the nonactivated system were found within the normal range of historical mouse lymphoma assay control mutant frequency data from the testing laboratory (Appendix A attached).

C. The highest concentrations accepted in this study (80 ug/ml under the nonactivated assay; 16 ug/ml under the activated assay) demonstrated the reduction of survival to 10.4% to 12.2% of that seen in solvent control for all the assays. The selection of highest concentrations of this material was considered to be adequate for this study.

D. The study, which has been conducted in accordance with the method of mouse lymphoma mutation assay described by Clive et al., appears adequate to generate valid results. The test compound, prodiamine technical, was nonmutagenic to the mouse lymphoma L5178Y cells under either the activated or the nonactivated assay system at the concentrations tested.

6. Was test performed under GLPs (is a quality assurance statement present)? (Y / N (circle one))

7. CBI appendix attached (Y / N (circle one))

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PRODIAMINE

RIN 1786-93

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Page \_\_\_ is not included in this copy.

Pages 10 through 16 are not included.

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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.
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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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CA. NEELI FILE

008210

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/14/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

### Data Evaluation Report

Study type: Acute oral-rats (81-1) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine technical

Study number: HRC 84704D/VCL 47/AC

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Acute Oral Toxicity to Rats of Prodiamine Technical.

Author(s): Sheena R. Kynoch  
Pamela A. Mullins

Report issued: September 20, 1984

#### Conclusions:

Under the conditions of this study, the acute oral LD<sub>50</sub> of Prodiamine technical was > 5.0 g/kg in male and female rats.

Toxicity Category IV

Core Classification: minimum

This study fulfills the guideline requirements (81-1) for an acute oral toxicity study in rats.

## I. MATERIALS

A. Test Material: Prodiamine technical; description: yellow powder; batch no. 4647-21; purity: 94.4%

B. Test Animals: Male and female HC/CFY (Remote Sprague-Dawley) rats; Source: Hacking and Churchill, Ltd., Huntingdon, Cambridgeshire. Age: 4-6 weeks. Weight: males: 96-103g; females, 101-113g.

## II. METHODS

Five Male and 5 female rats were employed in this study. Rats were given a standard laboratory rodent diet (Scientific Feeds LAD 1, Special Diet Services Ltd., Witham, Essex, England) and water *ad libitum*, with the exception of an overnight fast prior to dosing and approximately 4 hours after dosing. Rats were acclimated to the lab environment for a minimum of 5 days prior to start of the study, and were randomly allocated to cages within the treatment group. Rats were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

Prodiamine technical was administered by oral gavage as a 50% w/v dilution in 1% methylcellulose in a dose volume of 10 ml/kg at a dose of 5.0 g/kg. Animals were observed soon after dosing and at frequent intervals for the remainder of day 1 (day of dosing). For the remaining days, animals were observed at least twice daily. The nature, severity, approximate time of onset, and duration of each toxic sign was recorded. Body weights were recorded on days 1, 8, and 15 postexposure. Animals were killed on day 15 by cervical dislocation and were subjected to gross macroscopic examination. Any abnormal appearance of organs on gross examination was recorded.

## III. RESULTS

No mortality was observed in either male or female rats during the study. Shortly after dosing (time not provided), piloerection was observed in all treated rats. This was accompanied by hunched posture in two treated males and 1 treated female. According to the registrant (page 3 of report), recovery as judged by external appearance and behavior was complete by day 4. There was no apparent adverse effect of treatment on body weight gain, although control data were not provided. No abnormal gross pathologic findings were noted at autopsy on day 15.

## IV. CONCLUSIONS

Under the conditions of this study, the acute oral LD<sub>50</sub> of Prodiamine technical was > 5.0 g/kg in male and female rats.

Toxicity Category IV

#### V. CORE CLASSIFICATION

minimum

This study fulfills the guideline requirements (81-1) for an acute oral toxicity study in rats.

Reviewed by: Timothy F. McMahon, Ph.D. *J.F.M. 11/14/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *J.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

008210

### Data Evaluation Report

Study type: Acute dermal-rats (81-2) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine technical

Study number: HRC 84705D/VCL 48/AC

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Acute Dermal Toxicity to Rats of Prodiamine technical.

Author(s): Sheena R. Kynoch  
Pamela A. Mullins

Report issued: September 20, 1984

#### Conclusions:

Under the conditions of this study, the acute dermal LD<sub>50</sub> of Prodiamine technical was > 2.0 g/kg in male and female rats.

Toxicity Category III

Core Classification: minimum

This study fulfills the guideline requirements (81-2) for an acute dermal toxicity study in rats.

## I. MATERIALS

A. Test Material: Prodiamine technical; description: yellow powder; batch no. 4647-21; purity: 94.4%

B. Test Animals: Male and female HC/CFY (Remote Sprague-Dawley) rats; Source: Hacking and Churchill, Ltd., Huntingdon, Cambridgeshire. Age: 6-8 weeks. Weight: males: 230-238g; females, 200-233g.

## II. METHODS

Five Male and 5 female rats were employed in this study. Rats were given a standard laboratory rodent diet (Scientific Feeds LAD 1, Special Diet Services Ltd., Witham, Essex, England) and water *ad libitum*. Rats were acclimated to the lab environment for a minimum of 5 days prior to start of the study, and were randomly allocated to cages within the treatment group. Rats were housed in temperature and humidity controlled rooms with a 12-hour light/dark cycle.

One day prior to treatment, hair was removed from the dorso-lumbar region of each rat with electric clippers, exposing an area equivalent to 10% of total body surface. Prodiamine technical was prepared as a 66.7% w/v paste in 1% methylcellulose and administered by spreading evenly over the prepared skin at a dose of 2.0 mg/kg. Dose volume did not exceed 3.0 ml/kg. The treated area was promptly covered with gauze which was held in place with an impermeable dressing wrapped around the trunk of each rat.

After 24 hours of exposure, the dressings were carefully removed and the treated skin area decontaminated by washing in warm water and blotting dry with absorbent paper.

Animals were observed soon after dosing and at frequent intervals for the remainder of day 1 (day of dosing). For the remaining days, animals were observed at least twice daily. The nature, severity, approximate time of onset, and duration of each toxic sign was recorded. Body weights were recorded on days 1, 8, and 15 postexposure. Treated areas of skin were observed daily for signs of dermal irritation.

Animals were killed on day 15 by cervical dislocation and were subjected to gross macroscopic examination. Any abnormal appearance of organs on gross examination was recorded.

## III. RESULTS

No mortality was observed in either male or female rats during the study. No signs of toxicity or skin reactions were observed in any animal. There was no apparent adverse effect of treatment on body weight gain, although control data were not provided. No abnormal gross pathologic findings were noted at autopsy on day 15.

#### **IV. CONCLUSIONS**

Under the conditions of this study, the acute dermal LD<sub>50</sub> of Prodiamine technical was > 2.0 g/kg in male and female rats.

Toxicity Category III

#### **V. CORE CLASSIFICATION**

minimum

This study fulfills the guideline requirements (81-2) for an acute dermal toxicity study in rats.

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/15/80*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/80*  
Section I, Toxicology Branch II (HFAS) (H7509C)

008210

### Data Evaluation Report

Study type: Primary eye irritation-rabbits (81-4) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine technical

Study number: HRC 84623D/VCL 50/SE

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Irritant Effects on the Rabbit Eye of Prodiamine technical.

Author(s): Michael P. Liggett  
Brenda I. Parcell

Report issued: August 29, 1984

#### Conclusions:

Prodiamine technical was minimally irritating to the eyes of white rabbits under the conditions of this study.

Toxicity Category III

Core Classification: guideline

This study fulfills the guideline requirements (81-4) for a primary eye irritation study in rabbits.

## I. MATERIALS

A. Test Material: Prodiamine technical; description: yellow powder; batch no. 4647-21; purity: 94.4%.

B. Test Animals: New Zealand White Rabbits; Source: Pemberley Rabbits, Cottenham, Cambridgeshire; Age: 9-12 weeks. Weight: 2.1-2.6kg

## II. METHODS

Three male and 3 female rabbits were employed in this study. Rabbits were given a standard laboratory rabbit diet (SDS standard rabbit diet) and tap water *ad libitum*. Rabbits were individually housed in metal cages, and acclimated to the lab environment prior to study initiation. Rabbits were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

Prior to instillation of test material, the eyes of each rabbit were examined for pre-existing corneal damage or conjunctival inflammation. A 60 mg amount of prodiamine technical (the amount in 0.1ml) was placed into the lower everted lid of one eye of each rabbit. The eyelids were then held together for one second before releasing. The untreated eye of each rabbit served as a control.

Examination of the eyes was made at 1 hour and at 1, 2, 3, 4, and 7 days after instillation, using a hand held torch (page 64 of report). Grading and scoring of ocular lesions was performed using the Draize scale (page 65 of report).

## III. RESULTS

No corneal opacity or iritis was observed in any rabbit at any time of ocular examination following test article administration. Slight conjunctival redness, chemosis, and discharge was observed in all rabbits at 1 and 24 hours after test article administration. Slight conjunctival redness was observed in 6 of 6 rabbits at 48 hours, and slight conjunctival chemosis in 4 of 6 rabbits at 48 hours. No ocular toxicity was present at 72 hours, with the exception of slight conjunctival redness in one rabbit.

## OCULAR RESPONSES TO PRODIAMINE TECHNICAL

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<u>Response</u>	<u>1hr</u>	<u>24hr</u>	<u>48hr</u>	<u>72hr</u>
Corneal Opacity	0/6	0/6	0/6	0/6
Iritis	0/6	0/6	0/6	0/6
Conjunctiva				
Redness	6/6	6/6	6/6	1/6
Chemosis	6/6	6/6	4/6	0/6
Discharge	6/6	6/6	0/6	0/6

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Data from Table 1, page 67 of registrant report.

#### IV. CONCLUSIONS

Under the conditions of this study, prodiamine technical was minimally irritating to the eyes of white rabbits.

Toxicity Category III

#### V. CORE CLASSIFICATION

guideline

This study fulfills the guideline requirements (81-4) for a primary eye irritation study in rabbits.

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/4/80*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/80*  
Section I, Toxicology Branch II (HFAS) (H7509C)

008210

### Data Evaluation Report

Study type: Acute oral-rats (81-1) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine 75WP

Study number: HRC 84706D/VCL 52/AC

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Acute Oral Toxicity to Rats of Prodiamine 75WP.

Author(s): Sheena R. Kynoch  
Pamela A. Mullins

Report issued: September 20, 1984

#### Conclusions:

Under the conditions of this study, the acute oral LD<sub>50</sub> of Prodiamine 75WP was > 5.0 g/kg in male and female rats.

Toxicity Category IV

Core Classification: minimum

This study fulfills the guideline requirements (81-1) for an acute oral toxicity study in rats.

## I. MATERIALS

A. Test Material: Prodiamine 75WP; description: not given; batch no. 4647-23; purity: 75.1%

B. Test Animals: Male and female HC/CFY (Remote Sprague-Dawley) rats; Source: Hacking and Churchill, Ltd., Huntingdon, Cambridgeshire. Age: 4-6 weeks. Weight: males: 96-118g; females, 100-113g.

## II. METHODS

Five Male and 5 female rats were employed in this study. Rats were given a standard laboratory rodent diet (Scientific Feeds LAD 1, Special Diet Services Ltd., Witham, Essex, England) and water *ad libitum*, with the exception of an overnight fast prior to dosing and approximately 4 hours after dosing. Rats were acclimated to the lab environment for a minimum of 5 days prior to start of the study, and were randomly allocated to cages within the treatment group. Rats were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

Prodiamine 75WP was administered by oral gavage as a 50% w/v dilution in 1% methylcellulose in a dose volume of 10 ml/kg at a dose of 5.0 g/kg. Animals were observed soon after dosing and at frequent intervals for the remainder of day 1 (day of dosing). For the remaining days, animals were observed at least twice daily. The nature, severity, approximate time of onset, and duration of each toxic sign was recorded. Body weights were recorded on days 1, 8, and 15 postexposure. Animals were killed on day 15 by cervical dislocation and were subjected to gross macroscopic examination. Any abnormal appearance of organs on gross examination was recorded.

## III. RESULTS

No mortality was observed in either male or female rats during the study. Following dosing (time not provided), piloerection was observed in all treated rats. According to the registrant (page 3 of report), recovery as judged by external appearance and behavior was complete by day 4. There was no apparent adverse effect of treatment on body weight gain, although control data were not provided. No abnormal gross pathologic findings were noted at autopsy on day 15.

#### IV. CONCLUSIONS

Under the conditions of this study, the acute oral LD<sub>50</sub> of Prodiamine 75WP was > 5.0 g/kg in male and female rats.

Toxicity Category IV

#### V. CORE CLASSIFICATION

minimum

This study fulfills the guideline requirements (81-1) for an acute oral toxicity study in rats.

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/14/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

### Data Evaluation Report

Study type: Acute dermal-rats (81-2) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine 75WP

Study number: HRC 84707D/VCL 53/AC

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Acute Dermal Toxicity to Rats of Prodiamine 75WP.

Author(s): Sheena R. Kynoch  
Pamela A. Mullins

Report issued: September 20, 1984

#### Conclusions:

Under the conditions of this study, the acute dermal LD<sub>50</sub> of Prodiamine 75WP was > 2.0 g/kg in male and female rats.

Toxicity Category III

Core Classification: minimum

This study fulfills the guideline requirements (81-2) for an acute dermal toxicity study in rats.

## I. MATERIALS

A. Test Material: Prodiamine 75WP; description: yellow powder; batch no. 4647-23; purity: 75.1%.

B. Test Animals: Male and female HC/CFY (Remote Sprague-Dawley) rats; Source: Hacking and Churchill, Ltd., Huntingdon, Cambridgeshire. Age: 6-8 weeks. Weight: males: 228-244g; females, 201-221g.

## II. METHODS

Five Male and 5 female rats were employed in this study. Rats were given a standard laboratory rodent diet (Scientific Feeds LAD 1, Special Diet Services Ltd., Witham, Essex, England) and water *ad libitum*. Rats were acclimated to the lab environment for a minimum of 5 days prior to start of the study, and were randomly allocated to cages within the treatment group. Rats were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

One day prior to treatment, hair was removed from the dorso-lumbar region of each rat with electric clippers, exposing an area equivalent to 10% of total body surface. Prodiamine 75WP was prepared as a 66.7% w/v paste in 1% methylcellulose and administered by spreading evenly over the prepared skin at a dose of 2.0 mg/kg. Dose volume did not exceed 3.0 ml/kg. The treated area was promptly covered with gauze which was held in place with an impermeable dressing wrapped around the trunk of each rat.

After 24 hours of exposure, the dressings were carefully removed and the treated skin area decontaminated by washing in warm water and blotting dry with absorbent paper.

Animals were observed soon after dosing and at frequent intervals for the remainder of day 1 (day of dosing). For the remaining days, animals were observed at least twice daily. The nature, severity, approximate time of onset, and duration of each toxic sign was recorded. Body weights were recorded on days 1, 8, and 15 postexposure. Treated areas of skin were observed daily for signs of dermal irritation.

Animals were killed on day 15 by cervical dislocation and were subjected to gross macroscopic examination. Any abnormal appearance of organs on gross examination was recorded.

## III. RESULTS

No mortality was observed in either male or female rats during the study. No signs of toxicity or skin reactions were observed in any animal. There was no apparent adverse effect of treatment on body weight gain, although control data were not provided. No abnormal gross pathologic findings were noted at autopsy on day 15.

#### IV. CONCLUSIONS

Under the conditions of this study, the acute dermal LD<sub>50</sub> of Prodiamine 75WP was > 2.0 g/kg in male and female rats.

Toxicity Category III

#### V. CORE CLASSIFICATION

minimum

This study fulfills the guideline requirements (81-2) for an acute dermal toxicity study in rats.

008210

Reviewed by: Timothy F. McMahon, Ph.D. *11/14/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *J.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

**Data Evaluation Report**

Study type: Dermal sensitization-guinea pig (81-6)

Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine 75WP

Study number: HRC 84865D/VCL 57/SS

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Irritant Effects on Rabbit Skin of Prodiamine 75WP

Author(s): Jennifer A. Seaber

Report issued: November 2, 1984

**Conclusions:**

Dermal sensitization was not observed with Prodiamine 75 WP in female guinea pigs under the conditions of this study.

**Core Classification: guideline**

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

## I. MATERIALS

A. Test Material: Prodiamine 75WP; description: yellow powder; batch no. 4647-23; purity: 75.1%.

B. Test Animals: Female albino guinea pigs (Dunkin/Hartley strain); Source: D. Hall, Newchurch, Staffordshire; Age: not stated; weight: test animals- 484-548g; controls- 463-548g.

## II. METHODS

### General:

Guinea pigs were acclimated to the laboratory environment prior to the start of the study. Animals had free access to a Vitamin-C enriched guinea pig diet (SDS Services Limited) and tap water. Animals were housed in suspended cages with wire mesh floors in a temperature and humidity controlled animal room. A 12 hour light/dark cycle was used. Prior to the main study, a preliminary study was performed with a range of dilutions of Prodiamine 75WP in order to identify 1) a minimally irritating concentration of prodiamine 75WP suitable for the induction phase, and 2) a non-irritating concentration of Prodiamine 75WP for the challenge phase. Based upon these preliminary studies, a concentration of 50% Prodiamine 75WP in acetone was selected for both the induction and challenge phases of the study.

### Induction:

Prior to each induction application, the skin on the left shoulder region of each guinea pig was clipped free of hair using electric clippers. A 2 x 2 cm square patch of surgical gauze was saturated with approximately 0.5ml Prodiamine solution (50% w/w in acetone). The patch was placed on the skin and covered by a length of impermeable plastic adhesive tape ("Blenderm"), which was in turn secured by an elastic adhesive bandage ("Elastoplast") wrapped around the torso of the animal and fixed with "Sleek" impervious plastic adhesive tape. Dermal reactions were assessed for erythema and edema upon bandage removal (0 hour reading) and 24 hours after bandage removal according to the Draize scale.

A total of nine induction applications were performed three times a week for three weeks. Duration of each induction application was 6 hours.

Control animals were treated in a similar fashion as test animals during induction, with the exception that test compound was omitted from the application.

### Challenge:

Two weeks following the last induction application, guinea pigs were challenged with Prodiamine 50% w/w in acetone. Hair was clipped from a 5 x 5 cm area of the right flank of each animal, and test material was applied as described above for induction. Duration of challenge exposure was 6 hours. Challenge reactions were evaluated at 24, 48 and 72

hours after patch removal.

A test animal was considered to have evidence of dermal sensitization if the dermal reaction at challenge was definitely more marked and/or persistent than the maximum reaction observed in control animals.

### III. RESULTS

In control guinea pigs, localized dermal reactions were observed in three animals during the first three induction applications from 0 hour readings. Localized dermal reaction was observed in one control guinea pig after the sixth induction application and in one guinea pig after the ninth induction application. From 24 hour observations, only localized dermal reactions were observed in three guinea pigs after the second induction application.

In test article treated guinea pigs, slight erythema was observed from the 0 hour reading in two animals after the sixth induction application. This slight reaction persisted in one of the guinea pigs (#2071) through the ninth (last) induction application. Localized dermal reaction was also observed from the 0 hour reading in two other test article treated guinea pigs (#2075 and 2076) after the eighth and ninth induction application, while slight edema was observed in one test article treated guinea pig after the ninth induction application.

From the 24 hour reading, the slight erythema in animal #2071 was still present from the sixth through the ninth induction application. Animals #2075 and 2076 still displayed the localized dermal reaction, while one additional animal (#2074) showed localized dermal reaction after the eighth and ninth induction application and one animal (#2079) showed localized dermal reaction after the ninth induction application.

Results of challenge application in test article treated guinea pigs showed no positive reaction in any animal at any time point of observation after challenge.

### IV. CONCLUSIONS

Dermal sensitization was not observed with Prodiamine 75 WP in female guinea pigs under the conditions of this study.

### V. CORE CLASSIFICATION

guideline

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

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/14/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

008210

### Data Evaluation Report

Study type: Primary eye irritation-rabbits (81-4) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine 75WP

Study number: HRC 84624D/VCL 55/SE

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Irritant Effects on the Rabbit Eye of Prodiamine 75WP.

Author(s): Michael P. Liggett  
Brenda I. Parcell

Report issued: August 29, 1984

#### Conclusions:

Prodiamine 75WP was minimally irritating to the eyes of white rabbits under the conditions of this study.

Toxicity Category III

Core Classification: guideline

This study fulfills the guideline requirements (81-4) for a primary eye irritation study in rabbits.

## I. MATERIALS

A. Test Material: Prodiamine 75WP; description: yellow powder; batch no. 4647-23; purity: 75.1%.

B. Test Animals: New Zealand White Rabbits; Source: Pemberley Rabbits, Cottenham, Cambridgeshire; Age: 9-11 weeks. Weight: 2.1-2.5kg

## II. METHODS

Three male and 3 female rabbits were employed in this study. Rabbits were given a standard laboratory rabbit diet (SDS standard rabbit diet) and tap water *ad libitum*. Rabbits were individually housed in metal cages, and acclimated to the lab environment prior to study initiation. Rabbits were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

Prior to instillation of test material, the eyes of each rabbit were examined for pre-existing corneal damage or conjunctival inflammation. A 45 mg amount of Prodiamine 75WP (the amount in 0.1ml) was placed into the lower everted lid of one eye of each rabbit. The eyelids were then held together for one second before releasing. The untreated eye of each rabbit served as a control.

Examination of the eyes was made at 1 hour and at 1, 2, 3, 4, and 7 days after instillation, using a hand held torch (page 74 of report). Grading and scoring of ocular lesions was performed using the Draize scale (page 75 of report).

## III. RESULTS

No corneal opacity or iritis was observed in any rabbit at any time of ocular examination following test article administration. Slight conjunctival redness, chemosis, and discharge was observed in all rabbits at 1 and 24 hours after test article administration. Slight conjunctival redness was observed in 3 of 6 rabbits at 48 hours. No ocular toxicity was present at 72 hours.

## OCULAR RESPONSES TO PRODIAMINE 75WP

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<u>Response</u>	<u>1hr</u>	<u>24hr</u>	<u>48hr</u>	<u>72hr</u>
Corneal Opacity	0/6	0/6	0/6	0/6
Iritis	0/6	0/6	0/6	0/6
Conjunctiva				
Redness	6/6	6/6	3/6	1/6
Chemosis	6/6	6/6	0/6	0/6
Discharge	6/6	6/6	0/6	0/6

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Data from Table 1, page 77 of registrant report.

#### IV. CONCLUSIONS

Prodiamine 75WP was minimally irritating to the eyes of white rabbits under the conditions of this study.

Toxicity Category III

#### V. CORE CLASSIFICATION

guideline

This study fulfills the guideline requirements (81-4) for a primary eye irritation study in rabbits.

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

008210

### Data Evaluation Report

Study type: Primary dermal irritation-rabbits (81-5) Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine technical

Study number: HRC 84621D/VCL 51/SE

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Irritant Effects on Rabbit Skin of Prodiamine technical

Author(s): Michael P. Liggett  
Brenda I. Parcell

Report issued: August 15, 1984

#### Conclusions:

Prodiamine technical was non-irritating to the skin of white rabbits under the conditions of this study.

Toxicity Category IV

Core Classification: supplementary

This study does not fulfill the guideline requirements (81-5) for a primary dermal irritation study in rabbits.

An explanation is requested for the conclusion that no dermal irritation was observed from dermal application of prodiamine technical if assessment of irritation was incomplete due to staining of the test site by the test material. No staining was reported from either the acute

dermal toxicity study or the delayed contact hypersensitivity study.

## I. MATERIALS

A. Test Material: Prodiamine technical; description: yellow powder; batch no. 4647-21; purity: 94.4%.

B. Test Animals: New Zealand White Rabbits; Source: Pemberley Rabbits, Cottenham, Cambridgeshire; Age: 9-12 weeks. Weight: 2.1-2.7kg

## II. METHODS

One male and 5 female rabbits were employed in this study. Rabbits were given a standard laboratory rabbit diet (SDS standard rabbit diet) and tap water *ad libitum*. Rabbits were individually housed in metal cages, and acclimated to the lab environment prior to study initiation. Rabbits were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

Twenty-four hours prior to application of test substance, hair was removed from the dorso-lumbar region of each rabbit with electric clippers to expose an area approximately 10 cm<sup>2</sup>. Prodiamine technical (0.5g) was applied undiluted to the skin site under a 2.5 cm<sup>2</sup> gauze pad moistened with 0.5ml distilled water. Each treatment site (one per rabbit) was then occluded with "Elastoplast" elastic adhesive dressing for four hours. Animals were unrestrained in their cages during this period.

After four hours, the "Elastoplast" dressing and gauze pad were removed. The treatment site was washed with distilled water to remove any residual test substance. Dermal reactions were scored using the Draize scale on day 1 (approximately 30 minutes after dressing removal), and on days 2, 3, and 4.

## III. RESULTS

Assessment of edema was not possible due to staining of the skin by test material. No evidence of erythema was observed at any time point following application of test material.

## IV. CONCLUSIONS

Prodiamine technical was non-irritating to the skin of white rabbits under the conditions of this study.

Toxicity Category IV

## V. CORE CLASSIFICATION

supplementary

This study does not fulfill the guideline requirements (81-5) for a primary dermal irritation study in rabbits.

An explanation is requested for the conclusion that no dermal irritation was observed from dermal application of prodiamine technical if assessment of irritation was incomplete due to staining of the test site by the test material. No staining was reported from either the acute dermal toxicity study or the delayed contact hypersensitivity study.

Reviewed by: Timothy F. McMahon, Ph.D. *T.F.M. 11/14/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)  
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. *Y.M.I. 11/15/90*  
Section I, Toxicology Branch II (HFAS) (H7509C)

008210

### Data Evaluation Report

Study type: Primary dermal irritation-rabbits (81-5)

Tox. Chem. No.: 727A

EPA accession number: 256459

Test material: Prodiamine 75WP

Study number: HRC 84622D/VCL 56/SE

Testing Facility: Huntingdon Research Centre plc  
Huntingdon, Cambridgeshire, England

Sponsor: Velsicol Chemical Corporation  
Chicago, Illinois 60611

Title of report: Irritant Effects on Rabbit Skin of Prodiamine 75WP

Author(s): Michael P. Liggett  
Brenda I. Parcell

Report issued: August 15, 1984

#### Conclusions:

Prodiamine 75WP was non-irritating to the skin of white rabbits under the conditions of this study.

Toxicity Category IV

Core Classification: supplementary

This study does not fulfill the guideline requirements (81-5) for a primary dermal irritation study in rabbits.

An explanation is requested for the conclusion that no dermal irritation was observed from dermal application of prodiamine technical if assessment of irritation was incomplete due to staining of the test site by the test material. No staining was reported from either the acute

dermal toxicity study or the delayed contact hypersensitivity study.

## I. MATERIALS

A. Test Material: Prodiamine 75WP; description: yellow powder; batch no. 4647-23; purity: 75.1%.

B. Test Animals: New Zealand White Rabbits; Source: Pemberley Rabbits, Cottenham, Cambridgeshire; Age: 9-12 weeks. Weight: 1.9-2.6kg

## II. METHODS

Six male rabbits were employed in this study. Rabbits were given a standard laboratory rabbit diet (SDS standard rabbit diet) and tap water *ad libitum*. Rabbits were individually housed in metal cages, and acclimated to the lab environment prior to study initiation. Rabbits were housed in temperature and humidity controlled rooms with a 12 hour light/dark cycle.

Twenty-four hours prior to application of test substance, hair was removed from the dorso-lumbar region of each rabbit with electric clippers to expose an area approximately 10 cm<sup>2</sup>. Prodiamine 75WP (0.5g) was applied undiluted to the skin site under a 2.5 cm<sup>2</sup> gauze pad moistened with 0.5ml distilled water. Each treatment site (one per rabbit) was then occluded with "Elastoplast" elastic adhesive dressing for four hours. Animals were unrestrained in their cages during this period.

After four hours, the "Elastoplast" dressing and gauze pad were removed. The treatment site was washed with distilled water to remove any residual test substance. Dermal reactions were scored using the Draize scale on day 1 (approximately 30 minutes after dressing removal), and on days 2, 3, and 4.

## III. RESULTS

Assessment of edema was not possible due to staining of the skin by test material. No evidence of erythema was observed at any time point following application of test material.

## IV. CONCLUSIONS

Prodiamine 75 WP was non-irritating to the skin of white rabbits under the conditions of this study.

Toxicity Category IV

## V. CORE CLASSIFICATION

supplementary

This study does not fulfill the guideline requirements (81-5) for a primary dermal irritation study in rabbits.

An explanation is requested for the conclusion that no dermal irritation was observed from dermal application of prodiamine technical if assessment of irritation was incomplete due to staining of the test site by the test material. No staining was reported from either the acute dermal toxicity study or the delayed contact hypersensitivity study.