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

008290

MAR 18 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Subject: Prodiamine Technical
Identifying No. 55947-UR
Tox Chem No. 727A
HED Project No. 0-1906

From: John H.S. Chen, D.V.M. John H S Chen 3/7/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

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

Thru: Yiannakis M. Ioannou, Ph.D., Section Head J.M. Ioannou 3/7/91
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 3/7/91
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Sandoz Crop Protection Corp., Des Plaines, IL

Action Requested: Review and evaluation of the subchronic oral toxicity study with prodiamine in rats and the metabolism study with prodiamine in rats

Recommendation:

(1) Subchronic Oral Toxicity with Prodiamine in Rats. Huntingdon Res. Ctr. Study No. VCL 41/85870. November 26, 1985. MRID No. 416084-02. Core Minimum (NOEL = 1200 ppm). The study satisfies guideline requirements (82-1) for a 13-week subchronic oral toxicity study in rodents; and (2) Metabolism Study with Prodiamine in Rats. Sandoz Crop Protection Corp. Study No. 480425-5. February 15, 1988. MRID No. 416084-01. Acceptable. The study provides adequate information to satisfy the guideline requirements (85-1) for a metabolism study in rats.

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EPA No.: 68D80056
DYNAMAC No.: 334-A
TASK No.: 3-34A
January 17, 1991

DATA EVALUATION RECORD

PRODIAMINE

Metabolism in Rats

STUDY IDENTIFICATION: Yu, C. C. Metabolism of prodiamine in rats. (Unpublished study No. 480425-5 performed by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 15, 1988.) MRID No. 416084-01.

APPROVED BY:

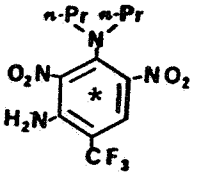
Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 1-16-91

1. CHEMICAL: N^3,N^3-Dipropyl-2,4-dinitro-6-trifluoromethyl-1,3-benzenediamine; prodiamine.

2. TEST MATERIAL: Unlabeled analytical grade prodiamine (>99% pure) and [^{14}C]prodiamine, labeled uniformly in the benzene ring, were used. The ^{14}C-labeled test material had a specific activity 25 mCi/mmol and a radiochemical purity of >98%. The structure and radiolabel position (*) of [^{14}C]prodiamine are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Yu, C. C. Metabolism of prodiamine in rats. (Unpublished study No. 480425-5 performed by Sandoz Crop Protection Corp., Des Plaines, IL; dated February 15, 1988.) MRID No. 416084-01.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Mary E. Cerny
Date: 1/16/91

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 1/16/91

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 1/16/91

John Chen, Ph.D.
EPA Reviewer
Review Section I
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Signature: John H. Chen
Date: 3/6/91

Yiannakis M. Ioannou, Ph.D., D.A.B.T.
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Date: 3/6/91

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7. CONCLUSIONS:

A. Prodiamine was absorbed and readily eliminated by rats given a single oral dose of 10 or 400 mg/kg of [^{14}C]prodiamine or a single oral dose of 10 mg/kg of the radioactive compound following 14 days of administration of 10 mg unlabeled prodiamine/kg/day. The total amount of radioactivity eliminated was similar for all animals. Elimination of ^{14}C was essentially complete within 4 days after dosing with the labeled compound, and the major route of elimination for all animals was the feces, which accounted for about 64 to 88% of the radioactive dose.

Absorption and rate of elimination were dose dependent. Animals given a single or repeated low dose (10 mg/kg) of [^{14}C]prodiamine excreted approximately 9 to 15.5% of the ^{14}C dose in the first 7 hours after compound administration; in contrast, high-dose rats eliminated <1%. Within 24 hours after dosing, all but the high-dose female rats had eliminated about 70% of the ^{14}C dose in the urine and feces (high-dose females excreted about 38% of the dose within 24 hours); within 96 hours, low- and repeated-dose rats had excreted approximately 27 to 32% of the ^{14}C dose in the urine and 64 to 68.5% in the feces, whereas high-dose animals (both males and females) had eliminated about 8 and 88% in the urine and feces, respectively. Tissues and carcasses contained small amounts of the ^{14}C dose--about 1.2% of the radioactivity administered to low- and repeated-dose animals and approximately 0.5% of that given to high-dose rats. Using both urinary excretion and tissue retention data, it was estimated that the amount of prodiamine absorbed by high-dose rats was approximately 26 to 37% of that absorbed by animals in the low- and repeated-dose groups.

Dose and duration of prodiamine administration did not affect the tissue ^{14}C distribution pattern; a slight sex-related difference in tissue ^{14}C concentrations was reported. Concentrations of radioactivity (as ppm ^{14}C equivalents) at 4 days after administration of [^{14}C]prodiamine were below 1 ppm for all tissues of low- and repeated-dose rats and were between 0.2 and 11 ppm for high-dose animals. Tissue residue concentrations, in descending order, were liver, fat, kidney, blood, lung, spleen, bone, gonads, heart, muscle, and brain. Residue levels

in females generally were higher than in males. Overall, tissue residue/retention data indicate that [^{14}C]prodiamine and its metabolites do not accumulate in the body to an appreciable extent.

Prodiamine was rapidly metabolized by N-dealkylation reactions to N,N-didespropyl. Metabolism of the test material also involved cyclization to form N-propyl benzimidazole A and N-propyl benzimidazole B. The N-propyl benzimidazoles were metabolized further via nitroreduction, N-dealkylation, and ring hydroxylation to form hydroxy benzimidazole. Other polar and conjugated metabolites were not identified.

B. This study provides adequate information, per EPA Guideline 85-1, on the metabolism of prodiamine in rats.

Items 8 through 11--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1) [^{14}C]Prodiamine was synthesized by Wizard Laboratories (Davis, CA) and had a specific activity of 25.0 mCi/mmol and a radiochemical purity >98%. Unlabeled analytical-grade prodiamine was greater than 99% pure and was supplied by the performing laboratory. Reference standards N-despropyl and N,N-didespropyl were analytical grade (99% pure) compounds; other reference metabolites were of technical grade (90 to 95% pure). Additional information, including batch or lot numbers and methods used to determine purity and specific activity, was not provided.

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

2) Male and female Sprague-Dawley rats (Charles River) were used. The animals were quarantined for at least 7 days before dosing and were between 7 and 9 weeks old at the start of the study. The average weight of the high-dose male rats was 255 g (CBI p. 52); other animal weights were not reported, but the protocol stated that weight variation among animals of the same sex should not exceed 10% of the mean weight (CBI p. 47).

3) Groups of five male and five female rats were randomly assigned to one of three oral dosing studies. Animals in the low-dose group received a single oral dose of 10 mg [^{14}C]prodiamine/kg [the No-Observed-Effect Level (NOEL)]. High-dose rats were given a single oral dose of 400 mg [^{14}C]prodiamine/kg (a dose level that produces some hematological alterations and liver weight gain). The repeated-dose group was administered oral doses of 10 mg unlabeled prodiamine/kg/day for 14 consecutive days followed by oral doses of 10 mg [^{14}C]prodiamine/kg within 24 hours after the last unlabeled dose was given. The vehicle for all prodiamine dosing solutions was Emulphor EL-620, a polyoxyethylated vegetable oil, and each rat in every study received a radioactive dose of approximately 25 μCi. All solutions were delivered by intubation. An intravenous dosing study was not conducted because of prodiamine's poor solubility (0.013 ppm) in water and physiological solutions.

Animals were placed in individual metabolism cages after administration of the radiolabeled test material. Animals were observed for signs of toxicity, and food consumption and water intake were measured daily. Urine and feces were collected separately at 7, 24, 48, 72, and 96 hours postdosing. Saturated mercuric chloride was added to each urine collection cup to prevent microbial degradation, and all excreta samples were kept frozen at -20°C until radioanalysis. Animals were killed 4 days after dosing, and the following were removed for ^{14}C determination: blood, bone, brain, fat, gonads, heart, kidney, liver, lung, muscle, and spleen. Residual carcasses were retained for counting.

4) Aliquots of urine (and other liquids) were assayed directly for ^{14}C content by liquid scintillation counting (LSC), whereas feces were combusted prior to radioassay. Pre-counting treatment of specific tissues (except for liver, as described below) was not included in the report, but a general description of radioassay methods stated that all solid samples were combusted before ^{14}C determinations were made. The efficiency of $[^{14}C]CO_2$ recovery was >97% and was monitored by combusting a known amount of [^{14}C]hexadecane. Counting efficiency was determined by an external pulse method and was generally between 65 and 95%.

5) Prior to metabolite analysis, aliquots of urine collected at 7 and 24 hours after dosing were freeze dried. The residue was extracted twice with acetone and centrifuged. The solid residue remaining after acetone extraction was extracted twice with methanol and centrifuged. The solid residue was dissolved in water and radioassayed. The acetone extracts were counted, and the methanol extracts were reduced to a small volume under nitrogen gas, counted, and analyzed for prodiamine metabolites.

Feces samples collected at 24 and 48 hours postdosing from three rats/sex/group were analyzed for prodiamine metabolites. Samples were mixed with 4 N HCl and water and were held at 81°C overnight for acid hydrolysis. Samples were then freeze dried, treated with methanol, and sonicated. Solid and liquid phases were separated by repeated centrifugations. The combined methanol extracts were evaporated, and additional methanol was used to dissolve the residue; acetone was added, and the sample was frozen overnight. The material was then centrifuged, and the supernatant was decanted and reduced in volume for metabolite analysis; the precipitate was dissolved in water and radioassayed. The solid remaining after the initial methanol extraction was blended with water, sonicated, and centrifuged; these steps were repeated, and both the solid and liquid phases were counted.

Liver samples were homogenized three times with acetone and centrifuged after each extraction. The acetone extracts (containing free metabolites) were combined and radioassayed. The remaining solid was treated with 1 N HCl, refluxed, and extracted three times with ethyl acetate; the ethyl acetate extracts (containing acid-released metabolites) were combined and counted. The aqueous and solid fractions were adjusted to pH 12 (using 50% NaOH), refluxed, and extracted three times with ethyl acetate. These extracts (containing base-released metabolites) were combined and radioassayed; the remaining aqueous phase and solids were centrifuged, and the ^{14}C content of each fraction was determined.

Prodiamine metabolites in urine and feces extracts were isolated and identified by thin-layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS). Because of the small amount of ^{14}C in liver extracts, no further analysis was performed.

Precoated silica gel TLC plates treated with a fluorescence indicator were used to separate prodiamine from its metabolites. Organic extracts of urine, feces, and liver samples were applied alone or with reference standards (Table I) to the plates and developed in one of two solvents (hexane:ethyl acetate, 70:30; or ethyl acetate:toluene:acetone:acetic acid:water, 85:3:2:5:5). Radioactive spots were detected by autoradiography or by a TLC linear analyzer; nonradioactive spots were detected directly under UV light.

Additionally, GC/MS was used to confirm the structures of prodiamine and N,N-didespropyl and to determine the structures of urinary metabolites U6 (hydroxy benzimidazole) and U8 (possibly a conjugate of prodiamine).

B. Protocol: The protocol included in this report is presented in the Appendix.

12. REPORTED RESULTS:

A. Neither food nor water intake was affected by consumption of any level of [^{14}C]prodiamine. Clinical signs of toxicity were not reported.

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____ The product confidential statement of formula.

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B. Between 95 and 100% of the ^{14}C administered was recovered from the urine, feces, and tissues at 96 hours after dosing (Table II). No sex-related differences in the recovery or elimination of radioactivity were reported, but the route and rate of elimination appeared to be dose related: elimination of ^{14}C initially was slower in high-dose rats, and high-dose animals excreted a larger percentage of the radioactive dose in the feces than other rats. Animals given a single or repeated low dose (10 mg/kg) of [^{14}C]prodiamine excreted approximately 9 to 15.5% of the ^{14}C dose in the first 7 hours after compound administration and between 54 and 64% during the 7- to 24-hour postdosing collection period (Table III). In contrast, high-dose rats eliminated <1% (males and females) and approximately 38% (females) and 70% (males) of the ^{14}C dose within 7 hours and between 7 and 24 hours postdosing, respectively. Elimination of radioactivity was nearly complete within 48 hours postdosing for all groups, and within 96 hours, low- and repeated-dose rats had excreted approximately 27 to 32% of the ^{14}C dose in the urine and 64 to 68.5% in the feces, whereas high-dose animals had eliminated about 8 and 88% in the urine and feces, respectively. Tissues of high-dose rats contained slightly less of the ^{14}C dose (0.4 to 0.55%) than tissues of low- and repeated-dose rats (1.15 to 1.4%).

C. Tissue ^{14}C residues were low for all animals, accounting for a total of 0.4 to 1.4% of the radiolabel administered at 96 hours postdosing (Table IV). The liver and kidney contained approximately 0.1 to 0.4% and 0.01 to 0.035% of the ^{14}C dose, respectively. Carcasses of all animals accounted for <1% of the radioactivity administered. All other tissues accounted for <0.005% of the radiolabeled dose. Overall, tissue ^{14}C levels--on a percent of dose basis--were about three times greater in low- and repeated-dose rats when compared with high-dose animals; no sex-related differences were noted.

Concentrations of radioactivity (as ppm ^{14}C equivalents) also were low; levels were below 1 ppm for all tissues of low- and repeated-dose rats and were between 0.2 and 11 ppm for high-dose animals (Table V). Tissue ^{14}C residue concentrations, in descending order, were: liver, fat, kidney, blood, lung, spleen, bone, gonad, heart, muscle,

Table II. Total Percent Recovery of Radiocarbon Administered to Rats Dosed Orally with [^{14}C]Prodiamine

	Percent of ^{14}C dose administered at:					
	10 mg/kg (single)		400 mg/kg (single)		10 mg/kg (repeated)	
Fraction	Males	Females	Males	Females	Males	Females
Urine	31.77[a]	26.84	7.27	8.11	30.21	29.98
Feces	67.41	68.50	87.76	88.55	63.75	67.53
Tissue	1.15	1.37	0.403	0.55	1.17	1.37
Total	100.33	96.71	95.43	97.21	95.13	98.88

[a]Each value is the mean of five animals. Values are for 96 hours after dosing.

SOURCE: Adapted from CBI Tables IV and V, CBI pp. 21 and 22.

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and brain. Residual levels in high-dose rats were about 10 to 20 times higher than those of low- and repeated-dose animals; similarly, tissue ^{14}C concentrations in females generally were somewhat higher than in males. Tissue radioactivity levels were similar for low- and repeated-dose animals.

D. Prodiamine was rapidly metabolized by all animals, but the amount of test material metabolized was dose dependent: high-dose animals metabolized a much smaller proportion or percentage of the compound than animals given either single or repeated low oral doses of prodiamine.

Fecal radiocarbon accounted for approximately 64 to 88% of the ^{14}C administered. Acetone extraction of feces released about 20% of the radioactive dose (28 to 33% of the fecal ^{14}C) given to low- and repeated-dose groups and approximately 70% of that administered to high-dose rats (equivalent to 80% of the total fecal radiolabel). Unchanged prodiamine in the acetone-extracted feces accounted for about 50% of the ^{14}C dose given to high-dose rats but <1% of that given to low- and repeated-dose animals (Table VI). Two isomers of N-propyl benzimidazole (see Table I and Figure 1) were also isolated from the feces following acetone extraction. N-Propyl benzimidazole A (4-amino-2-ethyl-7-nitro-1-propyl-5-trifluoromethyl benzimidazole) represented approximately 0.1 and 0.3% of the 10- and 400-mg/kg doses, respectively. N-propyl benzimidazole B (6-amino-2-ethyl-7-nitro-1-propyl-5-trifluoromethyl benzimidazole) was associated with about 0.1% of the ^{14}C administered to low- and repeated-dose rats and about 1.2% of that given to high-dose animals. Between 11 and 20% of the ^{14}C dose remained at the TLC origin and consisted of polar metabolites; acid hydrolysis and further TLC analysis of this material indicated the presence of free (i.e., unconjugated) metabolites of prodiamine, N-propyl benzimidazole B, and other unidentified compounds. Approximately 25 to 30% and <10% of the fecal radioactivity (16 to 21 and 8% of the ^{14}C dose, respectively) precipitated after acetone treatment of excreta of rats given 10 or 400 mg [^{14}C]prodiamine/kg, respectively. Relatively large amounts of fecal radioactivity (33 to 40% of the total; 22% of the dose) were not extractable from low- and repeated-dose rats.

Several metabolites were isolated from the urine, but most were not identified. Urinary radiocarbon

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accounted for 7 to 32% of the ^{14}C dose; methanol extracts contained 22% of the dose administered to low- and repeated-dose rats and 6% of that given to high-dose animals. Prodiamine and N,N-didespropyl chromatographed together as metabolite U1 and accounted for 0.1 to 0.5% of the radioactive dose received by all animals (Table VII); the compounds were separated and identified by MS. Urinary metabolite U6 represented between 0.3 and 2.1% of the radioactivity administered; this compound was tentatively identified as hydroxy benzimidazole (4-hydroxy-6,7-diamino-2-ethyl-7-nitro-5-trifluoromethyl benzimidazole). Metabolite U8, proposed to be a conjugate of prodiamine, accounted for 6 to 11% of the low and repeated doses and about 2% of the high dose. Several other metabolites (U2, U3, U4, U7) each accounted for 0.1 to 1.4 of the ^{14}C administered; none of these compounds was identified. Material remaining at the origin of the TLC plate accounted for 2 to 12% of the radioactive dose; acid hydrolysis and subsequent TLC of this material demonstrated the same metabolite distribution pattern as that shown for metabolites U1 through U8. Acetone extracts of urine contained 0.3 to 5.2% of the administered radioactivity, and water-soluble material accounted for approximately 1 to 5.8%.

Liver radiocarbon represented 0.1 to 0.4% of the ^{14}C administered to any animal; most of this consisted of polar metabolites. Free metabolites accounted for 7 to 14% of the liver ^{14}C, and acid- and base-released metabolites represented about 5 to 9 and 3 to 6% of the hepatic radiolabel, respectively (Table VIII). Between 52 and 58% of the liver radioactivity was recovered from the aqueous phase following acid and base hydrolysis and extraction with ethyl acetate; approximately 10 to 18% was nonextractable. An insufficient amount of radiolabeled hepatic material precluded further analysis.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that [^{14}C]prodiamine was absorbed and readily eliminated by rats given a single oral dose of 10 or 400 mg/kg or repeated oral doses of 10 mg/kg. The total amount of radioactivity eliminated was similar for all animals; elimination of ^{14}C was essentially complete within

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4 days after dosing, and the major route of elimination for all animals was the feces, which accounted for about 64 to 88% of the radioactive dose.

Absorption and rate of elimination were dose dependent. Within 24 hours after dosing, all but the high-dose female rats had eliminated about 70% of the ^{14}C dose in the urine and feces (high-dose females excreted about 38% of the dose within 24 hours); within 96 hours, low- and repeated-dose rats had excreted approximately 27 to 32% of the ^{14}C dose in the urine and 64 to 68.5% in the feces, whereas high-dose animals (both males and females) had eliminated about 8 and 88% in the urine and feces, respectively. Thus, urinary levels of radioactivity in high-dose rats were about 25% of those of other animals, indicating that high-dose animals had absorbed only 25% of the prodiamine dose when compared with animals given the low dose. Tissues and carcasses contained about 1.2% of the radioactivity administered to low- and repeated-dose animals and about 0.5% of that given to high-dose rats. Using both urinary excretion and tissue retention data, the study author estimated that the proportion of administered prodiamine absorbed by high-dose rats was approximately 26 to 37% of that absorbed by animals in the low- and repeated-dose groups. The study author suggested that the lower percent excretion of total radioactivity in urine of high-dose rats may have been due to saturated urinary excretion but concluded, on the basis of tissue residue data and the presence of large amounts of unchanged parent compound in the feces of high-dose animals (50% of the ^{14}C dose versus <1% of the dose given to other animals), that reduced urinary ^{14}C levels resulted from reduced absorption of prodiamine by high-dose rats. The study author also indicated that unchanged parent compound in the feces represented unabsorbed prodiamine, rather than compound excreted via the bile, by citing results of a study in which <1% of a 1-mg/kg dose of [^{14}C]prodiamine given to bile duct-cannulated rats was eliminated unchanged in the bile (Nietschmann, D. A. 1985. Comparative metabolism of prodiamine, a dinitroaniline herbicide, in rats and goats. Master of Science thesis, Illinois Institute of Technology, Chicago, IL).

Sex, dose, and duration of prodiamine administration did not affect the tissue ^{14}C distribution pattern. Tissue residue concentrations, in descending order, were liver, fat, kidney, blood, lung,

spleen, bone, gonads, heart, muscle, and brain. Residue levels generally were higher in females than in males.

Prodiamine was rapidly metabolized by N-dealkylation reactions to N,N-didespropyl. Metabolism of the test material also involved cyclization to form N-propyl benzimidazole A and N-propyl benzimidazole B. The N-propyl benzimidazoles were metabolized further via nitroreduction, N-dealkylation, and ring hydroxylation to form hydroxy benzimidazole. These metabolites and prodiamine were also metabolized to polar and conjugated metabolites. The proposed metabolic pathways for prodiamine in the rat are shown in Figure 1.

B. A quality assurance statement, signed and dated March 16, 1988, and a statement of compliance with Good Laboratory Practices (GLPs), signed and dated March 16, 1988, and August 22, 1990, were included in the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

This study is acceptable per EPA Guidelines (85-1) and provides adequate information on the absorption, distribution, metabolism, and elimination of orally administered prodiamine in rats. A sufficient number of animals (five/sex/dose level) was used, and rats were given a single low, single high, and repeated low oral doses, as required by EPA. An intravenous study was not conducted because of prodiamine's poor solubility in aqueous solutions.

In general, the study author's conclusions are supported by the data presented. Absorption, metabolism, and rate of elimination of [^{14}C]prodiamine were dose related; among high-dose animals, the rate of elimination was also related to sex and was slowest in females. Elimination of radioactivity was essentially complete within 96 hours after dosing for all groups, and the major route of elimination was the feces, which accounted for about 64 to 88% of the ^{14}C administered. Elimination was delayed slightly in high-dose rats: animals given a single or repeated low dose (10 mg/kg) of [^{14}C]prodiamine excreted approximately 9 to 15.5% of the ^{14}C dose in the first 7 hours after compound administration, whereas high-dose rats eliminated <1%. In addition, high-dose rats eliminated approximately 53 to 87 times more

radioactivity during the 7- to 24-hour collection period than during the 0- to 7-hour period, whereas low- and repeated-dose animals excreted approximately four to six times more ^{14}C during the second versus first excreta collection period. Although the reason for this difference is not clear, these data suggest that uptake and excretion of very high doses of prodiamine are initially inhibited but then appear to be induced by some mechanism. Within 24 hours after dosing, all but the high-dose female rats had eliminated about 70% of the ^{14}C dose in the urine and feces (high-dose females excreted about 38% of the dose within 24 hours); within 96 hours, low- and repeated-dose rats had excreted approximately 27 to 32% of the ^{14}C dose in the urine and 64 to 68.5% in the feces, whereas high-dose animals (both males and females) had eliminated about 8 and 88% in the urine and feces, respectively.

Absorption of prodiamine appeared to be saturated in high-dose animals, based on (1) low urinary ^{14}C levels (as percent of dose), (2) recovery of most of the radioactive dose in the feces, and (3) the presence of large amounts (about 50% of the ^{14}C dose, compared with <1% of that given to other animals) of unchanged parent compound in the feces. An intravenous dosing study was not appropriate for this compound, but examination of biliary excretion would have supported these conclusions. Results of the previously conducted study in which <1% of 1-mg/kg dose of [^{14}C]prodiamine given to bile duct-cannulated rats was eliminated unchanged in the bile do not adequately support the author's conclusions because the patterns of absorption and metabolism of prodiamine in the rat appear to be highly dose related; thus, making conclusions about the absorption of a 10- or 400-mg/kg dose of the compound, based on the results of the metabolism of a 1-mg/kg dose, is inappropriate.

Tissue distribution of radioactivity and tissue residue levels were independent of dose level and dosing regimen, but tissue radioactivity levels in females were slightly higher than those in males. Overall, however, the data indicate that accumulation of prodiamine and its metabolites was minimal for all groups, and, as suggested by the study author, the recovery of a proportionately smaller amount of the radioactive dose from the tissues of high-dose rats, when compared with tissues of low- and repeated-dose animals, supported the conclusion that absorption was saturated in animals given the high dose.

Prodiamine was rapidly metabolized by N-dealkylation reactions to N,N-didespropyl. Metabolism of the test

benzimidazole A and N-propyl benzimidazole B. The N-propyl benzimidazoles were metabolized further via nitroreduction, N-dealkylation, and ring hydroxylation to form hydroxy benzimidazole. These metabolites and prodiamine were also metabolized to polar and conjugated metabolites. Metabolism of prodiamine was essentially complete for low- and repeated-dose rats; less than 1% of the dose was recovered unchanged from the urine and feces of these animals. In contrast, a large proportion of the high dose--approximately 50%--was not metabolized by rats. According to the study author, approximately 74% of the 1-mg/kg dose of [^{14}C]prodiamine administered to bile duct-cannulated animals (Nietschmann, 1985) was excreted via the bile, indicating that, for low-dose rats in particular, metabolism of prodiamine probably involves enterohepatic circulation. However, the data from the bile duct-cannulation study were not presented in the Sandoz report and can be used for speculation only. A deficiency in the Sandoz report is the inadequacy of the separation, chromatographic, and spectral analyses to extract, characterize, and/or identify a large proportion of the fecal radioactivity--approximately 50 and 20% of the 10- and 400-mg/kg doses, respectively. Most of the urinary radioactivity (about 70 to 80%; 6 to 26% of the ^{14}C dose) was identified or characterized.

Items 15 and 16--see footnote 1.

APPENDIX

Protocol
(CBI pp. 46-51)

110201 PRODIAMINE RIN 1786-93

Page_____ is not included in this copy.

Pages 28 through 33 are not included.

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EPA No.: 68D80056
DYNAMAC No.: 334-B
TASK No.: 3-34B
February 28, 1991

DATA EVALUATION RECORD

PRODIAMINE

Subchronic Oral Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 2/27/91

34

EPA No.: 68D80056
DYNAMAC No.: 334-B
TASK No.: 3-34B
February 28, 1991

DATA EVALUATION RECORD

PRODIAMINE

Subchronic Oral Toxicity Study in Rats

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DATA EVALUATION RECORD

GUIDELINE § 82-1

STUDY TYPE: Subchronic oral toxicity study in rats.

MRID NUMBER: 416084-02.

TEST MATERIAL: Prodiamine.

SYNONYMS: N/A.

STUDY NUMBER: VCL 41/85870.

SPONSOR: Sandoz Corporation, East Hanover, NJ.

TESTING FACILITY: Huntingdon Research Center, Ltd., Huntingdon, Cambridge, England.

TITLE OF REPORT: Prodiamine Toxicity to Rats by Repeated Dietary Administration for 13 Weeks.

AUTHORS: Jones D.R., Powell L.A.J., Heywood R., Street A.E., and Gibson W.A.

REPORT ISSUED: November 26, 1985.

CONCLUSIONS:

Prodiamine was fed to male and female Sprague-Dawley rats at dose levels of 0, 400, 1200, or 4000 ppm (corresponding to 0, 26.7, 80.1, or 268.9 mg/kg/day for males and 0, 32, 97, or 324.9 mg/kg/day for females) for 13 weeks. No deaths occurred; high-dose animals exhibited yellow discoloration of fur and tails. Mean body weights and body weight gains of males and females fed 4000 ppm were reduced throughout the study; weight reduction was more pronounced in males. Food and water consumption were not individually measured. Minor reductions occurred in erythrocyte counts and hemoglobin concentrations of dosed animals; cholesterol levels were increased in these animals in a dose-related manner. This change was most pronounced in high-dose males and females. Urinary protein content was increased in high-dose males at 5 and 12 weeks. Absolute liver weights were increased in females fed 4000 ppm and relative liver weights were increased in males and females of this dose group. In addition, relative kidney weights were increased in males fed 4000 ppm. There were no compound-related macroscopic or microscopic pathological findings. Based on changes in body weights, organ weights, cholesterol, and urinary protein, the LOEL is 4000 ppm and the NOEL is 1200 ppm prodiamine.

Study Classification: Core Minimum. This study satisfies Guideline §82-1 requirements for a 13-week subchronic oral toxicity study in rodents.

A. MATERIALS:

1. Test Compound: Prodiamine; description: orange powder; batch No.: C-84268; purity: 91.3%.

2. Test Animals: Species: rat; strain: CD Sprague-Dawley; age: approximately 41 days at study initiation; weight: males--194 to 199 g; females--144 to 148 g at study initiation; source: Charles River Breeding Laboratories, Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Following a 6-day acclimation period, animals were assigned to the following test groups by computer randomization:

Test group	Dose in diet (ppm)	Main study (13 Weeks) Males	Females
1 Control	0	20	20
2 Low (LDT)	400	20	20
3 Mid (MDT)	1200	20	20
4 High (HDT)	4000	20	20

Prestudy animal health investigations were conducted on 10 male and 10 female rats. This consisted of routine hematology and macroscopic examinations; abnormal tissue was examined microscopically. Veterinary examinations were conducted on all test animals prior to animal assignment. A second acclimation period of 7 days was allowed between animal assignment and initiation of dosing. Animals were housed five/cage; temperature, humidity, and lighting conditions of the room were not provided.

2. Diet Preparation: Diets were prepared weekly; a concentrated premix of the diet was prepared by mixing the appropriate amount of test material directly with the rodent chow. A second premix for use in low- and mid-dose diets was prepared by diluting the original premix with untreated diet. The test diets were prepared by diluting the appropriate premix with the appropriate amount of untreated diet to give the required concentrations, and blending for a minimum of 7 minutes. Untreated diet was provided for the control animals. Prior to initiation of dosing, test diets were analyzed for homogeneity, stability, and concentration; concentration was also analyzed at week 13.

Diet Preparation Results: Diets containing 100 and 10,000 ppm prodiamine were homogeneous when tested and stable for 18 days at room temperature; however, these dietary levels were not used in the study, and data variability for stability of the 100 ppm dose level was attributed to lack of homogeneity. Dietary levels used in the study were not analyzed for homogeneity or stability. The concentrations of the test material in the diets were within 6% of nominal concentrations; the mean concentrations for two analyses at each of two intervals of analysis were 0, 407.8 ± 19.43, 1225 ± 50, and 3947.5 ± 74.11 ppm prodiamine for the 0, 400, 1200, and 4000 ppm diets, respectively.

3. Food and Water Consumption: Animals received food (Scientific Feeds Laboratory Animal Diet No. 2) and water ad libitum.

4. Statistics: Body weight, food consumption, water consumption, clinical biochemistry, and organ weights were tested for homogeneity of variance using Bartlett's test. Analysis of variance and the Student's t-test were performed for data with homogeneous variance; Williams test was incorporated for a dose-related response. For data with heterogeneous variance, the procedures utilized included data transformations, the Kruskal-Wallis analysis of ranks, and nonparametric equivalents of the t-test and William's test. Fisher's exact test and Mantel's test were used for those parameters in which the relative frequency of the mode was at least 75%. Organ weights were analyzed using analysis of covariance with bodyweight as the covariate.

5. Quality Assurance: A quality assurance statement was signed and dated November 19, 1985.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily (7 days/week) for signs of mortality. Animals were examined daily from study weeks 1 to 4 and weekly thereafter to study termination for signs of toxicity and behavioral changes. All rats were palpated for masses during the clinical examination.

   Results: No deaths occurred during the study. High-dose males and females exhibited yellow discoloration of the fur and tail beginning at weeks 5 and 14, respectively. Alopecia and red staining around the eyes was also found sporadically among these animals.

2. Body Weight: Rats were weighed at study initiation and weekly thereafter.

   Results: Representative data on mean body weights and body weight gains are presented in Tables 1 and 2. Mean body weights and body weight gains of high-dose males and females were slightly depressed (4 to 10%) throughout the dosing period when compared to concurrent controls. The depression of body weight was significant in high-dose males at study week 7 ($p < 0.01$) and 13 ($p < 0.05$). The body weight gains, as calculated by the reviewers, were significantly depressed in high-dose males from weeks 0 to 7 (14% depression, $p < 0.01$); body weight gains were only slightly depressed from weeks 7 to 13. The overall body

TABLE 1. Representative Results of Mean Body Weights for Rats Fed Prodiamine for 13 Weeks

Dose Group (ppm)	Mean Body Weights (g ± S.D.) at Week: 0	7	13
		Males	
0	197.5 ± 7.72	481.2 ± 39.40	562.1 ± 54.90
400	196.9 ± 10.09	469.1 ± 43.84	550.4 ± 53.03
1200	198.5 ± 10.09	475.7 ± 40.31	561.3 ± 64.89
4000	194.5 ± 10.14	437.2 ± 50.04**[a]	512.3 ± 67.99*[b]
		Females	
0	147.7 ± 11.23	247.2 ± 28.83	275.6 ± 34.47
400	144.4 ± 8.90	250.7 ± 20.27	275.2 ± 23.63
1200	145.9 ± 7.45	249.5 ± 19.89	280.5 ± 21.46
4000	144.3 ± 11.37	233.7 ± 22.54	257.7 ± 27.61

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

[a]The mean body weight of high-dose males at week 7 was not found to be significant by the study authors; these data were recalculated by the reviewers and found to be significant at $p < 0.01$ using analysis of variance and Dunnett's test.

[b]The mean body weight of high-dose males at study week 13 was not found to be significant by the study authors; these data were reevaluated by the reviewers and found to be significant at $p < 0.05$ using analysis of variance and Dunnett's test.

TABLE 2. Representative Results of Mean Body Weight Gains for Rats Fed Prodiamine for 13 Weeks

Dose Group (ppm)	Mean Body Weights (g ± S.D.) Between Weeks: 0-7	7-13	0-13
		Males	
0	283.7 ± 35.42	81.0 ± 21.73	364.7 ± 50.10
400	272.3 ± 38.57 (96)[a]	81.3 ± 21.18 (100)	353.5 ± 47.76 (97)
1200	277.2 ± 33.49 (98)	85.6 ± 32.51 (106)	362.8 ± 59.03 (99)
4000	242.7 ± 42.62** (86)	75.1 ± 25.35 (93)	317.8 ± 61.74* (87)
		Females	
0	99.5 ± 18.71	28.4 ± 15.27	127.9 ± 25.70
400	106.4 ± 15.27 (107)	24.5 ± 8.35 (86)	130.9 ± 19.28 (102)
1200	103.6 ± 14.84 (104)	31.0 ± 9.47 (109)	134.6 ± 16.84 (105)
4000	89.4 ± 16.89 (90)	24.0 ± 10.47 (85)	113.4 ± 21.48[b] (89)

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

[a]Numbers in parentheses equal percent of control body weight gain.

[b]The body weight gain of high-dose females from weeks 0-13 was found to be significant at $p < 0.05$ by the study authors; these data were reevaluated by the reviewers and were not found to be significant using analyisis of variance and Dunnett's test.

weight gains of these animals were depressed by 13% from weeks 0 to 13 when compared to concurrent controls ($p < 0.05$). The body weight gains of high-dose females were depressed by 10 (weeks 0 to 7) to 15% (weeks 7 to 13); the reviewers found the body weight depression of these animals to be nonsignificant.

3. Food Consumption and Compound Intake: Food consumption was determined for each cage of five rats, and the diet consumption was calculated for each rat on a weekly basis (g/rat/week). Efficiency and compound intake were calculated from the consumption and body weight gain data. Water consumption was measured during weeks 1, 4, 8, and 11.

   Results: Total food consumption and compound intake of prodiamine are presented in Table 3. Total food consumption of high-dose males was slightly but significantly ($p < 0.05$, 94% of control consumption) depressed when compared to concurrent controls. These data were based on food consumption/cage of five rats. The food consumption of other dosed groups was similar to that of concurrent controls. Food efficiency of high-dose males and females was slightly inferior when compared to that of concurrent controls. Mean compound intakes were 26.7, 80.1, and 268.9 mg/kg/day for males, and 32.0, 97.0, and 324.9 mg/kg/day for females fed 400, 1200, or 4000 ppm, respectively. Water consumption, based on five rats/cage, was increased in high-dose males (11%) and decreased in high-dose females (7%) when compared to controls during study week 11. No individually measured data were reported for food or water consumption.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to study initiation and during week 13. Prior to examination, the pupils of the eyes were dilated using a tropicamide ophthalmic solution.

   Results: No ocular lesions described were considered to be a result of dosing.

5. Hematology and Clinical Chemistry: Blood was collected from the orbital sinus prior to study initiation and at weeks 6 and 13 for hematology and clinical analysis from 10 rats/sex/dose. The CHECKED (X) parameters were examined:

TABLE 3. Achieved Compound Intake of Prodiamine and Total Food Consumption of Rats Dosed Orally for 13 Weeks[a]

Dose Group (ppm)	Mean Compound Intake (mg/kg/day) at Week: 1	7	13	Total food consumption (g/rat) Weeks 1 to 13
			Males	
0	--	--	--	2662 ± 36.5
400	45.2	24.7	18.8	2615 ± 77.2
1200	138.6	73.1	55.1	2646 ± 123.8
4000	446.2	252.0	189.5	2496 ± 82.7*
			Females	
0	--	--	--	1769 ± 84.5
400	45.1	30.3	25.0	1782 ± 73.9
1200	137.8	92.7	74.0	1814 ± 91.5
4000	435.2	315.0	255.8	1728 ± 77.8

[a]Based on food consumption and compound intake of five rats/cage; no individual animal data were provided by the study authors.

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count
X	Hemoglobin (HGB)†		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†		Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†		
X	Platelet count†	X	Mean corpuscular volume (MCV)
	Reticulocyte count (RETIC)	X	Coagulation:thrombotest (TT)
X	Red cell morphology		

Results: Table 4 summarizes mean hematological data of rats fed prodiamine for 13 weeks. Hemoglobin concentrations and erythrocyte counts of mid- and high-dose females were slightly but significantly ($p < 0.05$) depressed (hemoglobin depressed 3 and 4% and erythrocyte counts depressed 7 and 5%, respectively) when compared to concurrent controls at study week 6; associated slight but significant ($p < 0.05$) depressions also occurred in the MCV and MCHC of these animals. However, no changes occurred in hematocrit levels at 6 or 13 weeks in dosed males or females. Hemoglobin concentration of high-dose females was slightly but significantly ($p < 0.05$) depressed (3%) at study week 13. Hemoglobin concentrations of all dosed males were significantly depressed ($p < 0.05$, 4% depression at low dose; $p < 0.01$, 5 and 4% depression at mid- and high-dose, respectively) at study week 13; however, at study week 6, the hemoglobin concentration of high-dose males was significantly ($p < 0.01$) increased when compared to concurrent controls. Erythrocyte counts of these animals were slightly but nonsignificantly depressed (2 to 5%) at this time. All changes in hemoglobin concentration and erythrocyte counts were within the range of strain-matched historical controls.[1] In addition, since these changes were variable and only slight when compared to concurrent controls, the reviewers consider these findings to be of questionable biological importance.

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[1]Hazleton Laboratories, 1984. Representative Historical Control Data. Hematology Reference Ranges for Sprague-Dawley Rats.

†Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies.

TABLE 4. Representative Hematology Results (± S.D.) for Rats Fed Prodiamine for 13 Weeks

Parameter/ Week	Exposure Level (ppm)							
	Males				Females			
	0	400	1200	4000	0	400	1200	4000
Hemoglobin (g/dL)								
6	14.8 ± 0.89	14.9 ± 0.32	15.0 ± 0.68	15.8 ± 0.84**	15.1 ± 0.62	15.2 ± 0.67	14.6 ± 0.47*	14.5 ± 0.43*
13	16.3 ± 0.62	15.7 ± 0.36*	15.5 ± 0.44**	15.6 ± 0.78**	15.9 ± 0.47	15.8 ± 0.66	15.6 ± 0.37	15.4 ± 0.30*
Erythrocyte Count (10^6/mm^3)								
6	7.6 ± 0.59	7.4 ± 0.21	7.6 ± 0.44	8.1 ± 0.63	7.5 ± 0.36	7.3 ± 0.38	7.0 ± 0.28*	7.1 ± 0.45*
13	8.2 ± 0.35	8.0 ± 0.29	8.1 ± 0.32	7.8 ± 0.66	7.4 ± 0.32	7.5 ± 0.41	7.5 ± 0.40	7.5 ± 0.15
Hematocrit (%)								
6	50 ± 2.8	51 ± 1.1	50 ± 2.0	50 ± 1.6	49 ± 1.6	50 ± 1.3	49 ± 1.1	49 ± 1.4
13	53 ± 2.0	52 ± 1.6	51 ± 1.5	51 ± 3.1	50 ± 1.9	50 ± 2.2	50 ± 1.3	49 ± 1.3

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

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b. Clinical Chemistry:

	Electrolytes
X	Calcium†
X	Chloride†
	Magnesium
X	Phosphorus†
X	Potassium†
X	Sodium†

	Enzymes
X	Alkaline phosphatase (ALP)
	Cholinesterase
	Creatine phosphokinase
X	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (SGPT)†
X	Serum aspartate aminotransferase (SGOT)†
	Gamma glutamyltransferase (GGT)

	Other
X	Albumin†
	Albumin/globulin ratio
X	Blood creatinine†
X	Blood urea nitrogen†
X	Cholesterol†
X	Globulins
X	Glucose†
X	Total bilirubin†
	Direct bilirubin
X	Total protein†
	Triglycerides

Results: Table 5 summarizes mean clinical chemistry data of rats fed prodiamine. Mean cholesterol and total protein levels of high-dose males and females were significantly increased ($p < 0.01$) in a dose-related manner when compared to concurrent controls at study week 6; cholesterol levels were increased by 51 and 38% for males and females, respectively, and total protein by 6% for both sexes. Albumin levels were slightly but significantly ($p < 0.01$) increased (5%) in high-dose females, and globulin levels were slightly but significantly ($p < 0.01$) increased (10%) in high-dose males at this time. At study week 13, cholesterol levels of high-dose males ($p < 0.05$, 25% increase) and all dosed females ($p < 0.01$, 22, 32, and 35% increase at the low, mid, and high dose levels, respectively) were significantly increased, total protein levels of all dosed males ($p < 0.05$, 4, 6, and 4% increase at the low, mid, and high dose levels, respectively) and high-dose females ($p < 0.05$, 6% increase), and albumin levels of all dosed males ($p < 0.01$, 8% increase) were significantly increased in a dose-related manner. Changes in total protein, albumin, and globulin appear to be within the range of strain-matched historical controls.[2] Increases

[2] Hazleton Laboratories, 1984.

†Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies.

TABLE 5. Representative Clinical Chemistry Results (± S.D.) for Rats Fed Prodiamine for 13 Weeks

Parameter/ Week	Exposure Level (ppm)							
	Males				Females			
	0	400	1200	4000	0	400	1200	4000
Cholesterol (mg/dL)								
6	53 ± 8.2	55 ± 6.5	58 ± 3.4	80 ± 15.1**	64 ± 8.7	63 ± 10.4	67 ± 7.8	88 ± 13.1**
13	56 ± 8.1	60 ± 16.3	64 ± 8.1	70 ± 12.2*	63 ± 8.0	77 ± 8.8**	83 ± 11.5**	85 ± 12.3**
Total Protein (g/dL)								
6	7.0 ± 0.22	7.1 ± 0.21	7.2 ± 0.23	7.4 ± 0.21**	7.0 ± 0.36	6.9 ± 0.31	7.1 ± 0.23	7.4 ± 0.27**
13	6.9 ± 0.28	7.2 ± 0.30*	7.3 ± 0.25*	7.2 ± 0.27*	7.2 ± 0.34	7.3 ± 0.40	7.2 ± 0.25	7.6 ± 0.45*
Albumin (g/dL)								
6	3.9 ± 0.08	4.0 ± 0.16	4.0 ± 0.13	4.0 ± 0.15	4.1 ± 0.26	3.8 ± 0.09	4.1 ± 0.16	4.3 ± 0.18**
13	3.6 ± 0.14	3.9 ± 0.07**	3.9 ± 0.22**	3.9 ± 0.12**	4.1 ± 0.22	4.3 ± 0.32	4.3 ± 0.21	4.4 ± 0.31
Globulin (g/dL)								
6	3.1 ± 0.22	3.1 ± 0.10	3.2 ± 0.18	3.4 ± 0.13**	3.0 ± 0.18	3.1 ± 0.26	3.0 ± 0.16	3.1 ± 0.17
13	3.3 ± 0.23	3.3 ± 0.29	3.4 ± 0.18	3.2 ± 0.18	3.0 ± 0.40	3.0 ± 0.24	3.0 ± 0.15	3.2 ± 0.21

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

in cholesterol appear to be related to dosing, but are of questionable toxicological significance in the Sprague-Dawley rat. Since these changes in clinical chemistry parameters were not accompanied by any correlating histologic findings, the reviewers consider these changes to be of questionable toxicological significance.

6. Urinalysis: Urine was collected from 10 fasted rats/sex/dose at weeks 5 and 12. The CHECKED (X) parameters were examined:

	Appearance†		Bilirubin†
X	Volume†	X	Blood (qualitative)†
X	Specific gravity†		Nitrate
X	pH	X	Urobilinogen (qualitative)
X	Sediment (microscopic)†	X	Bile pigments (qualitative)
X	Protein†	X	Total Reducing Substances (qualitative)
X	Glucose (qualitative)†		
X	Ketones (qualitative)†		

Results: Representative urinalysis data are presented in Table 6. The urinary protein content of high-dose males was significantly ($p < 0.01$) increased by 23 and 93% at study weeks 5 and 12, respectively, when compared to concurrent controls. The urinary protein content of low- and mid-dose males was slightly increased by 38 and 31%, respectively, at study week 12.

7. Sacrifice and Pathology: All animals that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

†Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies.

TABLE 6. Urinary Protein Content of Rats Fed Prodiamine for 13 Weeks

Dietary Level (ppm)	Mean Protein Content (mg/dL ± S.D.) at Week: 5		12	
	Males	Females	Males	Females
0	80 ± 6.7	0	45 ± 21.2	0
400	87 ± 9.5	0	62 ± 27.4	1 ± 3.2
1200	78 ± 12.3	0	59 ± 28.8	0
4000	98 ± 4.2**	0	87 ± 20.6**	0

**Significantly different from control values at $p < 0.01$.

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

Frozen sections of liver were stained with Oil Red O (ORO) and examined for lipid content. Sections of kidney were stained with ORO or Periodic Acid-Schiff reagent (PAS). Histological examination of all tissues was conducted on control and high-dose animals. The liver, kidney, heart, and gross lesions of low- and mid-dose animals were examined.

Results:

a. Organ Weights: Table 7 presents data for liver, kidney, and spleen weights. Liver weights were increased 14% in high-dose females (significant at $p < 0.05$) and liver-to-body weights were increased

†Recommended by Subdivision F (November 1984) Guidelines for subchronic oral toxicity studies.

TABLE 7. Absolute and Relative Liver, Kidney, and Spleen Weights (Mean ± S.D.) in Rats Fed Prodiamine for 13 Weeks

Dose Group (ppm)	Males		Females	
	Organ Weight (g)	Organ/Body Weight (%)	Organ Weight (g)	Organ/Body Weight (%)
		Liver		
0	22.9 ± 3.53	4.1 ± 0.47	10.6 ± 1.52	3.9 ± 0.40
400	23.8 ± 2.79	4.4 ± 0.56	11.1 ± 1.53	4.1 ± 0.44
1200	23.6 ± 3.03	4.3 ± 0.45	11.6 ± 1.51	4.2 ± 0.54
4000	23.7 ± 4.04[a]	4.7 ± 0.57**[b]	12.1 ± 1.64*[a]	4.7 ± 0.45**[b]
		Kidney		
0	4.16 ± 0.50	0.75 ± 0.07	2.41 ± 0.26	0.89 ± 0.08
400	4.32 ± 0.47	0.80 ± 0.10	2.42 ± 0.21	0.89 ± 0.09
1200	4.34 ± 0.41	0.80 ± 0.10	2.32 ± 0.20	0.84 ± 0.07
4000	4.28 ± 0.48[a]	0.85 ± 0.07**[b]	2.33 ± 0.37	0.91 ± 0.11
		Spleen		
0	0.72 ± 0.12	0.13 ± 0.02	0.45 ± 0.07	0.16 ± 0.02
400	0.79 ± 0.13	0.15 ± 0.02	0.45 ± 0.07	0.17 ± 0.02
1200	0.80 ± 0.13	0.15 ± 0.03	0.42 ± 0.12	0.15 ± 0.05
4000	0.71 ± 0.10	0.14 ± 0.02	0.48 ± 0.09[c]	0.19 ± 0.03

[a]Organ weights reported by the study authors to be significant at $p < 0.01$ using analysis of covariance; organ weights recalculated by the reviewers and found to be nonsignificant or significant at $p < 0.05$ using analysis of variance and Dunnett's test.

[b]Organ to body weight ratios were calculated by the reviewers and found to be significant at $p < 0.01$ using analysis of variance and Dunnett's test.

[c]Spleen weights reported by the study authors to be significant at $p < 0.05$; spleen weights were recalculated by the reviewers using analysis of variance and Dunnett's test and were found to be nonsignificant.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

15 and 21% in high-dose males and females, respectively (significant at $p < 0.01$), when compared to concurrent controls. Kidney-to-body weight ratios were increased 13% in high-dose males; this increase was significant at $p < 0.01$. Histopathologic examinations of the organs did not reveal any alterations that would correlate with these organ weight changes.

b. Gross Pathology: There were no macroscopic pathological changes that were considered to be compound related by the study authors.

c. Microscopic Pathology: There were no microscopic pathological changes that were considered to be compound related by the study authors or that corresponded to the increased liver, kidney, or spleen weights of dosed animals. All changes found were considered to be of no toxicological significance.

D. STUDY AUTHORS' CONCLUSIONS:

The 13-week dietary administration of prodiamine to male and female Sprague-Dawley rats at dose levels of 0, 400, 1200, or 4000 ppm resulted in reduced body weight gain and minor changes in the hemoglobin, cholesterol, and protein of high-dose males and females. In addition, the urinary protein content of high-dose males was increased. Minor changes in hemoglobin, cholesterol, and protein levels were seen in low- and mid-dose animals. Liver and kidney weights were increased in high-dose males, and liver and spleen weights were increased in high-dose females. No compound-related findings were observed histologically. Based on the results of this study, dosage levels for an oncogenicity study in rats were set at 50, 200, 800, and 3200 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate; however, food and water consumption were based on determinations on cages of five rats. These group determinations were divided by five to assess these parameters on an individual basis; food and water consumption were not individually measured. The reviewers do not consider this method of reporting of food and water consumption to be valid. Individual determinations of these data should have been conducted. Group determinations reported slight changes in these parameters in high-dose animals.

Dietary levels used in the study were not analyzed for homogeneity or stability. In addition, the data for stability of a 100-ppm dose level diet were variable. The study authors attributed this variation in stability to the lack of homogeneity of the original preparations. However, homogeneity analyses of this test diet were within 4% of nominal.

The body weight gains and organ-to-body weight ratios were calculated by the reviewers. The statistical calculations of many body and organ weights reported by the study authors were considered by the reviewers to be incorrect. These corrections are noted in Tables 1, 2, and 7. The reviewers consider the changes in total protein, albumin, and globulin to be within the range of strain-matched historical controls. However, to confirm the compound related significance of these changes, the study author should provide historical control data on these indices from the study laboratory.

Based on changes in body weight, organ weights, cholesterol, and urinary protein, the LOEL is 4000 ppm and the NOEL is 1200 ppm prodiamine.