Received by Melvin Gibson 1/9/90

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

JAN 4 1990

MEMORANDUM

Subject: Prodiamine, N3,N3-di-n-propyl-2,4-dinitro-6-trifluoromethyl)-m-phenylenediamine: ID Number 55947-UR; 55947-UE; 55947-UG
Tox Chem No. 727A
Project No. 9-0904
PESTICIDES AND

From: John H.S. Chen, D.V.M. John H.S. Chen 12/7/89
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

To: Lawrence J. Schnaubelt, PM 23
Herbicides-Fingicides Branch
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Thru: Yiannakis M. Ioannou, Ph.D., Section Head Y.M. Ioannou 12/11/89
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief Marcia Van Gemert 12/18/89
Toxicology Branch II
Health Effects Division (H7509C)

Petitioner: Sandoz Crop Protection Corporation
Des Plaines, Illinois 60018

Action Requested:

1. Review and assessment of the submitted toxicological studies with technical prodiamine.

2. Review of Registrant's responses to previous review comments concerning the toxicological studies with technical prodiamine described in the Toxicology Branch Memorandum of January 12, 1987, Winnie Teeters, Ph.D.

Recommendations:

I. The Registrant should be apprised of the deficiencies noted in the following studies (See also DERS).

1. Acute inhalation study with prodiamine 65 WDG in rats Huntington Research Center No. VCL 111/86831, November 7, 1986. Supplementary

2. Effect of prodiamine on reproductive function of two generations in the rat. Huntington Research Center No. VCL 73/871075, February 22, 1988. Parental Toxicity NOEL = 200 ppm; Parental Toxicity LEL = 2000 ppm; Reproductive Toxicity NOEL = 200 ppm; Reproductive Toxicity LEL = 2000 ppm. Supplementary (The purity of the test article was not reported in this study).

II. The following toxicological studies are found to be adequate and acceptable to support the Registrant's request:

1. Oncogenicity feeding study with prodiamine in mice. Huntington Research Center No. VCL 37/871188, April 12, 1988. Systemic Toxicity NOEL = 500 ppm; Systemic Toxicity LEL = 5000 ppm; Prodiamine was not oncogenic to mice at dietary levels of 50, 500, or 5000 ppm for 99 weeks. Core Minimum

3. Oncogenicity feeding study with prodiamine in rats. Huntington Research Center No. VCL 74/871495, January 29, 1989. Systemic Toxicity NOEL = 200 ppm; Systemic Toxicity LEL = 800 ppm; Prodiamine was oncogenic to male and female rats receiving 3200 ppm, inducing the formation of thyroid tumors. Core Guideline

III. Review of the Registrant's response to the previous Toxicology Branch review comments concerning the following toxicological studies with prodiamine (TB MEMO 1/12/87, Winnie Teeters):

1. Rabbit teratology study with prodiamine. Wil Research Labs No. WIL-15153, November 7, 1985. Registrant's response to the deficiency cited in the previous Toxicology Branch review of this study is considered adequate and acceptable. The study is upgraded from Core Minimum to Core Guideline. Maternal Toxicity NOEL = 100 mg/kg; Maternal Toxicity LEL = 300 mg/kg.

2. Rat teratology study with prodiamine. Wil Research Labs No. WIL-15150, November 11, 1985. Resgistrant's response to the deficiency cited in the previous Toxicology Branch review of this study is considered adequate and acceptable. The study is upgraded from Core Minimum to Core Guideline. Developmental Toxicity NOEL = 100 mg/kg; Developmental Toxicity LEL = 300 mg/kg.

3. Mouse lymphoma assay with prodiamine. Microbiological Associates No. T2840.701, May 20, 1985. Prodiamine induced thyroid tumors in both males and females and is structually similar to trifluralin which has been classified as a Category C (possible human) carcinogen (Trifluralin Registration Standard, August, 1986; TB Memo 1/12/87 Winnie Teeters). We disagree with the Registrant's position that this inconclusive study with a weakly positive response in the absence of metabolic activation should not be repeated (Dr. Irving Mauer concurs with our decision under such circumstance).

IV. The rat oncogenicity study provides evidence that prodiamine induces an increase in the incidence of follicular adenoma/carcinoma in the high-dose male and female groups. This study is being recommended to Health Effects Division Peer Review committee for final evaluation in a weight of the evidence determination of oncogenic potential. The toxicological studies submitted in support of Registrant's request for Non-Food Use are not considered adequate. Toxicology data gaps exist in the inhalation study (81-3), the rat reprodution study (83-4) and the mouse lymphoma assay (84-2) as described in the Sections I-1, I-2, and III-3. In addition, no acceptable acute dermal toxicity study is available for technical prodiamine. No data are available on the acute inhalation toxicity of prodiamine 65-WDG.

Tox Chem No. 727A

File Last Updated — —

Current Date 12/6/89

Prodiamine

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade Doc. No.
Reproduction - 2 generation Species: rat Huntington Res. Ctr.; VCL 73/871075 2/22/88	Prodiamine Tech - Batch No. C85177	405934-21 405934-22	Parental Toxicity NOEL = 200 ppm Parental Toxicity LEL = 2000 ppm (decreased body weight and increased adjusted liver weight) Reproductive Toxicity NOEL = 200 ppm Reproductive Toxicity LEL = 2000 ppm (reduced pup weight at day 21 in conjunction with the significant changes in adjusted liver weight) Levels tested: 0,50,200 & 2000 ppm		Supplementary
Feeding/oncogenic - 2 year; mouse; Huntington Res. Ctr.; VCL 37/871188; 4/12/89	Prodiamine Tech - Batch No. 84268; 91.3%	405897-01 405897-02 405897-03	Systemic Toxicity NOEL = 500 ppm Systemic Toxicity LEL = 5000 ppm (reduced body weight gains and increased liver weight) The significantly increased incidence of subcutaneous fibrosarcoma in high dose males was related to fighting activity caused by group caging. Levels tested:0,50,500 & 5000 ppm		Core Minimum
Feeding/oncogenic - 2 year; rat; Huntington Res. Ctr.; VCL 74/871495; 1/29/89	Prodiamine Rech -Batch No. C85177; 95%	409859-01 409859-02 409859-03	Systemic Toxicity NOEL = 200 ppm Systemic Toxicity LEL = 800 ppm (increased liver weight and minor biochemical disturbances) Significantly increased incidence of follicular adenoma/carcinoma was observed in both males and females receiving 3200 ppm. Levels tested: 0,50,200,800 & 3200 ppm		Core Guideline
Acute inhalation; rat; Huntington Res. Ctr.; VCL 111/86/831; 11/7/86	Prodiamine 65 WDG - Batch No. IL 9001; 65%	402293-06	LC_{50} > 1.81 mg/L (both sexes) (particle size was not less than 1 um; maximum attainable concentration was below 5 mg/L)		Supplementary

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Teratology Species: rabbit IBT 651-06116; 4/30/75	Rydex Tech.		IBT - Invalid. Clement Associates - Contract No. 68-01-5824-Accepted by EPA 12/14/81		002548
Reproduction-3 generation Species: rat IBT 623-06981; 6/14/79	Rydex tech.		IBT - Invalid, Dynamac Corporation. Accepted by EPA: 3/16/83.		003040
Teratology Species: rat Wil Research Lab Wil 15153; 11/7/85	Prodiamine Tech 91.3-96.3% pure	263739	Levels tested by gavage in New Zealand White strain from day 6-18 of gestation - 0, 100, 300 and 500 mg/kg ~~Maternal NOEL < 100 mg/kg (LDT). (decreased body wt. gain)~~ Developmental NOEL > 500 mg/kg(HDT). A/D ratio = <100/>500=<0.2		Minimum 005656
Teratology Species: rat Wil Research Lab Wil 15150; 11/11/85	Prodiamine Tech, batch 84268, 91.3% pure	263739	Levels tested by gavage in CRL:CD (SB)BR strain from day 6-15 of gestation-0,100,300 and 1000 mg/kg/day. Maternal NOEL=300 mg/kg Maternal LEL=1000 mg/kg (depressed body wt. gain) ~~Developmental NOEL < 100 mg/kg/day.~~ (increased incidence of ocular anomalies) A/D ratio=300/<100=>3		Minimum 005656
Teratology Species: rabbit Wil Research Lab WIL 15152; 7/22/85	Prodiamine Tech 96.3%	263739	Range finding maternal weight loss at 500 & 1000 mg/kg; no effects on other parameters for does or fetuses. Levels of 100, 300 & 500 mg/kg selected for main study.		Supplementary 005656
Teratology Species: rat Wil Research Lab 15144; 3/28/85	Prodiamine tech, batch 84268, 91.3% pure	263739	Range finding maternal body weight gain decreased at 1000 mg/kg. No effects on fetal parameters. Levels of 100, 300 & 1000 mg/kg selected for main study.		Supplementary 005656
Feeding/oncogenic-2 year Species: rat IBT 621-06644; 5/3/78	USB-3153 Tech.		IBT - Validated - supplementary. Experimental Pathology Labs.12/30/82 Levels tested: 100, 300, 600 ppm. Oncogenic NOEL> 600 ppm Systemic NOEL > 600 ppm (HLT). Maximum tolerated dose not used and histopathology was inadequate (10 animals/group in control and high levels for complete histopathology)		Supplementary 002618
Feeding/oncogenic-18 month Species: mice IBT 651-07145; 6/28/79	Rydex tech.		IBT - Invalid, Dynamac Corporation, Contract No. 68-01-6561. Accepted by EPA: 3/2/83.		003038

83-4 - Rat - Reproduction

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 12/6/89
Section I, Toxicology Branch - HFAS (H7509C)
Secondard reviewer: Yiannakis M. Ioannou, Ph.D. [illegible] 12/1/89
Section I, Toxicology Branch - HFAS (H7509C)

07660

DATA EVALUATION REPORT

Study Type: Rat Reproduction

Tox. Chem. No.: 727A

MRID No. 405934-21 & 405934-22

Accession No.:

EPA File Symbol: 55947-UR

Test Material: Prodiamine (Batch No. C85177)

Synonyms/CAS No.:

Study Number(s): VCL 73/871075

Sponsor: Sandoz Crop Protection Corporation
Des Plaines, IL 60018

Testing Facility: Huntington Research Center Ltd., Huntingdon, Cambridgeshire, England

Title of Report: Effect of Prodiamine on Reproductive Function of Two Generations in the Rat

Author(s): Cozens, David D., et al.

Report Issued: February 22, 1988

Conclusions:

Parental Toxicity NOEL = 200 ppm

Reproductive Toxicity NOEL= 200 ppm

Levels tested: 0, 50, 200, & 2000 ppm in the diet

Classification of Data: Supplementary

(Deficiency: lacking of test material purity information in this report)

I. Materials and Methods:

1. Test Material

A single batch of prodiamine (C85177) was used in this study. Diets containing 0, 50, 200, and 2000 ppm of technical prodiamine were prepared weekly in the Labsure Laboratory Animal Diet No. 2. Dietary analyses to examine the homogeneity of mixing and stability of the test diets were performed prior to the commencement of the study. During the course of the study, diets were analyzed for the intended concentrations on weeks 1, 11, 15, 20, 25, 34, 40, 43, 49 and 53. All mean results were within 11% of nominal values (See Addendum E - Table 1).

2. Animals and Experimental Design

Four-week old male and female Sprague Dawley Crl:COBS CD(SD)BR rats were obtained from Charles River (UK) Ltd., Margate, Kent. A total of 112 males and 112 females were chosen for the study following an acclimatization period of 7 days. Selected animals were randomized, assigned to 4 study groups and designated as the F_0 parental generation.

At study initiation, the F_0 animals were 6 weeks old. Throughout the course of this study, all animals were maintained in an environmentally controlled room and were provided tap water and the Labsure Laboratory Diet No.2 ad libitum. Parental animals of the same sex were group caged except during mating; females were individually housed following mating. After 70 days of treatment, females were paired with males from the same dose group for 20 days to produce the F_{1a} litters. Vaginal smears were taken daily throughout the mating period. On day 21 post partum, 24 male and 24 female pups per group were selected to form the basis of the F_{1a} generation. Shortly following (approximately 10 days) the weaning of the F_{1a} pups, F_0 males and females were remated employing alternative pairing and mating techniques. They were also mated for a period of 20 days during which daily vaginal smears were taken. At day 21 post partum, 24 male and 24 female pups per group were selected to form the basis of the F_{1b} generation.

After the F_{1b} litters had weaned, F_0 males and females were sacrificed and examined microscopically. Due to a relatively high incidence of total litter loss in F_{1a} control and test groups, F_{1b} offsprings were used as F_1 parental animals. F_{1a} pups were killed, necropsied and discarded. After 12 weeks of treatment, F_1 animals were paired as described to produce F_{2a} and F_{2b} litters. Pups from the F_{2b} litters (24 males and 24 females) were selected as F_2 parental animals. After the F_{2b} pups had weaned, the parental F_1 males and females were again sacrificed and examined microscopically.

3. Observations

Animals were examined daily for mortality and signs of toxicity. Detailed examinations were conducted weekly. All animals were weighed weekly. During the mating periods of each generation, all animals were weighed daily and daily weighing continued until parturition. Weights were also reported for days 0, 7, 14, 17,and 20 of pregnancy. Dams that littered were weighed on days 0, 7, 14

and 21 post partum. Food intake of rats was recorded weekly for each cage of animals during the premating and mating periods. Consumption by female rats was measured weekly during the first 3 weeks post partum commencing at the time of parturition. Water consumption was measured on a daily basis for each cage of animals during the initial two and final two weeks of the premating treatment period of each generation.

Gestation lengths were recorded and parturition was observed when possible. At parturition, the sex number of live and dead pups were recorded: pup survival was recorded daily. Pups were examined for external abnormalities and weighed on days 8, 12 and 21 post partum. Animals found dead or killed in a moribund state were subjected to a gross necropsy: all abnormal tissues were preserved in 1% formalin. Liver, kidneys, pituitary and gonads from the F_0 and F_{1b} adults in the control and high dose groups were weighed. Selected tissues were examined histologically. The routine stain used was hematoxylin and eosin..

4. Statistical Methods

Statistical analyses were performed routinely on litter data using the litter as the basic sample unit. Non-parametric tests (Kruskal-Wallis and Jonckheere) were generally used for values of litter size, pup mortality, and mean pup weights as these values rarely follow a normal distribution. Organ weights were analyzed by analysis of variance adjusting for final bodyweight as covariate provided that this was found to be a significant relationship ($P<0.01$; F test). Where appropriate, a long transformation failed to stablize the variance, the organ weights were analyzed by the Kruskal-Wallis test. Treatment means were compared with control values by Williams' test or its non-parametric equivalent

II. Reported Results:

1. Test Material Analysis

The stability of prodiamine in rodent diet, stored at room temperature, was confirmed for up to 18 days (Table 4.1). The variation in stability data observed for diets at 100 ppm may be attributed to the lack of homogeneity of the original preparation. Results of the chemical analyses indicated that the prodiamine concentrations in test diets were generally within 11% of norminal values. However, the difference in percent recovery (mean value) from top, middle or bottom samples did not vary by more than 2.4%.

2. Clinical Examination

Clinical examination of parental males and females indicated no treatment-related effects.

3. Mortality

Parent Generation	Dietary Level (ppm) Males 0	50	200	2000	Females 0	50	200	2000
Fo	0	1	0	0	3	2	2	2
F1a	1	1	0	0	0	0	0	0
F1b	0	0	1	0	0	1	2	3
Total	1	2	1	0	3	3	4	5

Results: None of the deaths appeared to be associated with treatment.

4. Body Weights (g)

Dose Level (ppm)		Treatment Week 4	10	14	27
Fo Males:	0	377	498	540	667
	50	379	503	543	668
	200	382	503	546	673
	2000	377	499	539	664
F1b Males:	0	110	412	503	641
	50	118	414	501	644
	200	112	403	488	610
	2000	111	404	494	612
Fo Females:	0	239	294	307	333
	50	239	291	315	328
	200	241	293	320	332
	2000	226	278	290	317
F1b Females:	0	99	245	281	343
	50	103	250	289	357
	200	101	248	292	359
	2000	99	234	266	323

Results: At week 27, the body weights of Fo and F1b males receiving 2000 ppm were 99.6% and 95.5% of the control mean, respectively. At the same week, the body weights of Fo and F1b females receiving 2000 ppm were 95.2% and 94.2% of the control mean, respectively. At 50 and 200 ppm, there was no consistent pattern of weight change during this study.

5. Water Consumption

Mean water consumption values were essentially similar in all groups.

6. Food Consumption (g/Week)

Dose Level (ppm)		Treatment Week 5	10	14	27
Fo Males:	0	191	182	182	190
	50	188	174	176	187
	200	191	178	178	185
	2000	189	173	175	184
Fla Males:	0	111	206	200	---
	50	119	202	196	---
	200	113	206	196	---
	2000	107	199	192	---
Flb Males	0	135	193	194	---
	50	133	189	190	---
	200	134	178	194	---
	2000	131	186	190	---
Fo Females:	0	140	127	---	---
	50	140	127	---	---
	200	142	128	---	---
	2000	141	128	---	---
Fla Females:	0	98	145	136	---
	50	97	145	137	---
	200	95	140	131	---
	2000	93	137	130	---
Flb Females	0	111	132	127	---
	50	114	133	134	---
	200	109	131	133	---
	2000	108	126	126	---

Results: Parental male and female food consumption data indicated no significant differences ($P>0.05$) among dose and its corresponding control groups. However, the mean achieved intakes of test substance were essentially proportional to the dietary concentrations. However, food conversion ratios of females at 2000 ppm indicated less efficient food utilization than in the controls.

7. Organ Weights [1]

Dose Level (ppm)		Bod	Absolute Weights Liv	Adr	Tes/Ova	Adjusted Weights Liv	Adr	Tes/Ova
Fo Males:	0	662	23.3	59.4	4.87	23.4	59.5	-
	50	661	22.0	56.5	4.93	22.0	56.6	-
	200	668	22.6	56.0	4.83	22.4	55.9*	-
	2000	662	25.7	53.0	5.06	25.8**	53.1**	-
Fo Females:	0	335	12.9	66.6	99.4	12.8	66.5	-
	50	337	13.1	69.8	88.0	13.0	69.6	-
	200	340	13.5	73.0	98.0	13.3	72.6	-
	2000	323	14.1	68.0	98.0	14.5**	68.8	-

7. Organ Weights

Dose Level (ppm)		Bod	Absolute Weights[1] Liv	Adr	Tes/Ova	Adjusted Weights Liv	Adr	Tes/Ova
F1b Males:	0	730	26.3	52.9	5.05	25.6	52.4	-
	50	733	27.0	54.9	5.09	26.2	54.3	-
	200	691	25.4	56.5	4.89	26.2	57.1	-
	2000	694	27.9	54.6	5.04	28.7**	55.1	-
F1b Females:	0	354	13.5	61.5	80.0	13.8	-	-
	50	382	14.1	66.7	71.5	13.7	-	-
	200	385	13.8	65.9	79.9	13.3	-	-
	2000	342	14.4	61.4	77.4	15.0**	-	-
F1a Males:	0	56	2.9	20.8	0.32	2.9	20.7	0.32
(Weanlings)	50	58	3.1	21.4	0.34	3.0	20.9	0.33
	200	56	2.9	21.6	0.32	2.9	21.4	0.32
	2000	53	2.9	21.5	0.31	3.1**	22.2	0.33
F1a Females:	0	52	2.6	21.0	19.3	2.6	20.6	19.3
(Weanlings)	50	55	2.8	21.0	18.3	2.7	19.7	17.3
	200	52	2.7	20.0	16.3	2.7	20.3	16.3
	2000	50	2.8	21.0	18.7	3.0**	21.8	19.6
F1b Males:	0	56	2.8	20.4	0.31	2.8	20.5	0.32
(Weanlings)	50	59	3.0	22.5	0.33	2.9	22.2	0.32
	200	57	3.0	22.8	0.31	2.9	22.7	0.31
	2000	55	3.1	21.4	0.33	3.3**	21.7	0.34**
F1b Females:	0	52	2.7	20.8	18.5	2.7	21.0	18.7
(Weanlings)	50	55	2.7	21.6	18.0	2.6	21.3	17.7
	200	54	2.9	22.2	20.5	2.8	22.1	20.4
	2000	52	3.1	21.0	18.1	3.1**	20.7	18.3
F2a Males:	0	61	3.2	20.9	0.35	3.1	20.2	0.34
(Weanlings)	50	59	3.1	22.3	0.34	3.0	21.5	0.34
	200	59	3.3	21.5	0.33	3.3**	21.1	0.36
	2000	56	3.4	19.5	0.34	3.6**	21.1	0.36
F2a Females:	0	58	3.1	20.5	18.4	2.9	19.7	17.8
(Weanlings)	50	57	3.0	19.1	17.6	3.0	18.8	17.4
	200	56	3.0	19.4	18.7	3.0	19.4	18.8
	2000	52	3.2	19.1	18.7	3.4**	20.2	19.4
F2b Males:	0	60	3.4	19.6	0.36	3.3	19.4	0.35
(Weanlings)	50	59	3.4	20.7	0.35	3.4	20.6	0.35
	200	61	3.5	23.4	0.35	3.3	23.0*	0.34
	2000	57	3.7	20.5	0.34	3.9**	21.0*	0.36
F2b Females:	0	57	3.2	19.3	15.6	3.1	18.9	15.1
(Weanlings)	50	56	3.2	20.4	17.7	3.2	20.4	17.1
	200	56	3.3	20.5	20.4	3.2	20.3	19.3*
	2000	53	3.4	19.4	17.2	3.6**	20.0	17.4*

* P < 0.05; ** P < 0.01 (different from control values statistically significant at William's test)
Bod = Body Weights; Liv = Liver; Adr = Adrenals; Tes = Testes; Ova = Ovary.
1 - Body Weight, Liver Weight and Testes Weight in grams; Adrenal Weight and Ovary Weight in mg.

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Results: As shown in this summarized table, the relative mean liver weights of adults and weanlings were significantly increased in the high dose male and female groups in all generationss when compared with that of the corresponding control groups. Other significant changes in the relative mean organ weights (adrenals, testes, ovary, and heart) were observed in a specific generation only.

8. Histopathological Examination of Parental Animals

	Incidence of Microscopic Findings							
	Fo Generation				Flb Generation			
	Males		Females		Males		Females	
Dose Level (ppm)	0	2000	0	2000	0	2000	0	2000
No. Examined	28	28	25	26	24	24	24	24
Liver								
Foci of mononuclear cells	2	5	3	2	2	1	2	3
Foci of hepatocyte necrosis & inflammation	1	0	1	0	0	0	0	0
Minimal Centrilobular hepatocyte vacuolation	10	8	0	0	11	4	0	0
Minimal periportal hepatocyte vacuolation	0	0	1	0	0	0	0	1
Area of dilated sinusoids	0	1	0	0	1	0	0	1
Testes								
Bilateral Testicular atrophy	1	0	-	-	2	1	-	-
Epididymides								
Spermatozoa absent	1	0	-	-	2	1	-	-
Ovaries								
Cysts	-	-	0	0	-	-	0	1
Corpora lutea absent	-	-	0	0	-	-	0	1

Results: Histopathological examination of Fo and Flb generation adults indicated that no evidence of a treatment-related effects on liver, testes, epididymides, and ovaries was observed.

9. Pregnancy Data

Litter Generation	Mating	Dose Level (ppm)	No. of Pair	Duration of Gestation (days)	Pregnant No.	Pregnant %	Median Pre-coital Time (days)
Fo	1	0	28	21.9	26	93	3.0
		50	27	22.2	26	96	4.0
		200	28	22.1	26	93	3.0
		2000	28	22.0	28	100	3.0
	2	0	27	22.1	27	100	3.0
		50	26	22.2	23	88	2.5
		200	27	22.3	26	96	3.0
		2000	28	22.4	28	100	2.5
F1b	1	0	24	22.7	23	96	2.5
		50	24	22.3	21	88	3.0
		200	24	22.3	20	83	2.5
		2000	24	22.3	23	96	3.0
	2	0	24	22.7	22	92	3.5
		50	24	22.5	21	88	3.0
		200	24	22.4	18	75	2.5
		2000	24	22.5	23	96	3.0

Results: The percentage of females mating and bearing live pups were generally comparable in all groups for the Fo and F1b litters. Although a slightly lower pregnancy was observed at the 200 ppm dosed group in the second mating of the F1b generation, this was no biological significance in this incidence. Similarly, median pre-coital times and gestation lengths for all groups of all generations were comparable.

10. Pup Weights, Litter Size and Sex Ratios

Litter Generation	Mating	Dose Level (ppm)	Group Mean Pup Wt.(g) 4-day	12-day	21-day	Litter Size	% Males at 21-day
Fo	1	0	9.3	26.5	51.9	7.4	52
		50	9.4	27.3	54.3	7.6	47
		200	8.6	25.5	51.4	7.4	50
		2000	8.4	24.8	49.2	7.5	49
	2	0	9.1	27.0	52.2	7.8	48
		50	9.6	28.3	54.6	7.8	52
		200	9.1	26.9	52.4	7.8	52
		2000	9.1	26.6	51.0	7.9	51

10. Pup Weights, Litter Size and Sex Ratios

Litter Generation	Mating	Dose Level (ppm)	Group Mean Pup Wt.(g) 4-day	12-day	21-day	Litter Size	% Males at 21-day
F1b	1	0	10.2	28.2	56.2	7.0	50
		50	9.4	27.9	54.6	7.6	49
		200	9.2	27.4	54.0	7.5	48
		2000	9.6	26.2	51.3*	7.8	58
	2	0	10.9	27.5	53.5	7.0	48
		50	10.4	-	53.5	7.8	46
		200	10.0	28.5	54.7	7.8	49
		2000	10.5	27.0	51.7	7.4	47

*Significantly different from the control $P < 0.05$

Results: (a) The mean pup weights in the high dose group (2000 ppm) at day 21 were consistently slightly lower than the corresponding control value in the Fo and F1b litters. This slight reduction of mean pup weights was considered to be an effect of treatment although this was probably related to systemic toxicity; (b) Sex ratios were unaffected by the treatment of prodiamine in the Fo and F1b litters; and (c) No significant difference in the mean group values of live litter size and the pup mortality was found between the dosed groups and their corresponding control groups in the Fo and F1b litters.

11. Pup Development

Litter Generation	Dose Level (ppm)	Gestation Period (day)	Mean age (days post coitum) for attaining Surface righting	Startle response	Air righting	Pupil reflex (day 20) % successful
Fo - 1st mating	0	22.0	23.8	35.0	37.9	100
	50	22.1	23.5	34.7	37.8	100
	200	22.1	24.2	35.5*	38.4	100
	2000	22.0	24.2	35.3	38.2	100
- 2nd mating	0	22.0	23.6	35.3	37.5	100
	50	22.2	24.1*	35.3	37.4	100
	200	22.3	23.8	35.4	37.8	100
	2000	22.4	24.1*	35.3	37.7	100
F1b - 1st mating	0	22.6	24.3	35.2	37.7	100
	50	22.3	24.1	35.3	37.6	100
	200	22.2	24.1	34.9	37.5	100
	2000	22.2	24.4	35.0	37.4	100
- 2nd mating	0	22.8	24.6	34.5	37.4	100
	50	22.5	24.8	34.5	37.4	100
	200	22.4	24.6	34.3	37.1	100
	2000	22.5	24.7	34.2	37.3	100

*Significantly different from control $P < 0.05$

Results: Although some statistically significant differences in the mean ages for the attainment of developmental landmarks (i.e., surface righting or startle response) were observed in the Fo litters, these were associated with the slightly lower pup weights (See Reported Results No. 10) rather than an effect of treatment.

II. Study Author's Conclusions

The study author concluded that "The administration of prodiamine in the diet at a concentration of 2000 ppm through two generations of rats was associated with retardation of body weight gain of females, with gain of F1 males slightly retarded. Mean weight gain of females during lactation was generally greater than the control value. Food conversion ratios of females during the pre-mating period indicated less efficient food utilization. Mating performance was unimpaired, but mean pup weight at day 21 post partum was slightly lower than the control value. Mean liver weights were consistently greater than the control value. At 200 ppm, growth and reproductive performance were essentially similar to that of control animals. At 50 ppm, the performance was similar to that of control animals. Within the context of this study, 200 ppm is considered to represent a no-effect level."

III. Reviewers' Discussion and Conclusion

1. Test Material Analysis: The results of chemical analysis indicated that the test diets containing 50, 200 and 2000 ppm were properly prepared and homogeneously distributed in the Labsure laboratory animal diet No. 2. used in this study. Although the preparation of the 2000 ppm diet at the 15th week was lower than the nominal concentration, the overall results demonstrate that the high dose diet mixtures were properly prepared. Findings from the stability studies showed that homogeneity of 100 ppm test diet was not as uniform as the higher doses. Since no toxicological effects were noted at this low dose, the lack of uniform mixing did not compromise the study.

2. Parental Data: We assess that the reduction in body weights and the increase in relative liver weights to be indicative of parental toxicity at 2000 ppm for males and females of all generations. Since liver abnormalities were not observed in high-dose males and females of Fo and F1b generations, the findings are suggestive of adaptive changes rather than overt toxicological effects.

3. Reproductive Data: The test material had no effect on mating or pregnancy rates. In the absence of significant changes in absolute organ weights, we do not regard the sporadic occurrence of significantly increased relative testicular or ovarian weights to be compound-related. We conclude, in agreement with the study author, that litter size, sex ratios, and development of pups were unaffected by exposure to the test material. However, the reduced pup weights at day 21 post partum in conjunction with the significant changes in relative liver weights for F1b male and female weanlings are considered to be indicative of reproductive toxicity at 2000 ppm. Histopathological changes in the liver of high-dose weanlings were similar to those seen in high dose parental animals. No adverse effects were observed in the mid- or low-dose groups.

4. Classification of Data: Supplementary

The study is currently classified as Supplementary because of lacking test material (Batch No. C85177) purity information in this report. The study may be upgraded on resolution of the reported deficiency.

Parental Toxicity NOEL = 200 ppm

Reproductive Toxicity NOEL = 200 ppm

EPA No.: 68D80056
DYNAMAC No.: 172-B
TASK No.: 1-72B
September 12, 1989

307560

DATA EVALUATION RECORD

PRODIAMINE

Chronic Toxicity/Oncogenicity Feeding Study in Rats

APPROVED BY:

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

Signature: Roman J Prieto for

Date: 9-12-89

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EPA No.: 68D80056
DYNAMAC No.: 172-B
TASK No.: 1-72B
September 12, 1989

DATA EVALUATION RECORD

PRODIAMINE

Chronic Toxicity/Oncogenicity Feeding Study in Rats

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DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats. GUIDELINE § 83-5

MRID NUMBER: 409859-01-09.

TEST MATERIAL: Prodiamine.

SYNONYM(S): N/A

STUDY NUMBER(S): HRC Report No. VCL 74/871495.

SPONSOR: Sandoz Crop Protection Corporation, Des Plaines, IL.

TESTING FACILITY: Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, England.

TITLE OF REPORT: Prodiamine, Potential Tumorigenic and Toxic Effects in Prolonged Dietary Administration to Rats.

AUTHOR(S): A. J. Powell, H. Peters, C. Gopinath, S. Ames, A. Gibson, and L. D. Crook.

REPORT ISSUED: January 29, 1989.

CONCLUSIONS:

Prodiamine was fed to male and female rats for 25 months at dietary levels of 0, 50, 200, 800, or 3200 ppm. There was a significant increase ($p=0.019$) in follicular adenomata of the thyroid in females receiving 3200 ppm. This incidence was 12% compared to 0% in concurrent control females and 2.1% in historical controls. There was a significant increase ($p=0.039$) in males receiving 3200 ppm, 12%, compared to 2% for concurrent controls and 7.8% for historical controls. No increases in malignant thyroid tumors were observed; however, the incidence of follicular adenoma/carcinoma in both males (16%) and females (12%) was significantly increased ($p < 0.05$) when compared to control males (4%) and females (0%). It is considered that prodiamine is a potential carcinogen in male and female rats and should be evaluated by the HED review committee. There were not any increases in tumors at other sites that were considered related to dosing. Body weight gains were slightly decreased in both sexes throughout the study; from initiation to week 78, gains were 10 and 5.6% lower than controls for high-dose males and females, respectively. There were increases in liver weights in both males and females at 800 and 3200 ppm. The increases were significant ($p \leq 0.01$) in males at both doses at the terminal sacrifice and in females at both doses at the interim sacrifice and at 3200 ppm at the final sacrifice. There were no correlating histologic liver changes. There were no biologically important changes in hematology parameters and only minor changes in clinical chemistry parameters. Serum cholesterol levels were slightly increased in females receiving 3200 ppm at weeks 26, 52, and 78. There were decreases in alanine aminotransferase (SGPT) and aspartic aminotransferase (SGOT) activities and lactic acid dehydrogenase (LDH) activities in dosed groups in the first year of the study, but these did not persist and the changes were not considered of toxicologic importance. The LOEL for chronic toxicity is 800 ppm and the NOEL 200 ppm.

Classification: CORE Guideline.

A. MATERIALS:

1. Test Compound: Prodiamine; description: yellow powder; batch No.: Lot C 85177; purity: 95%.

2. Test Animals: Species: rat; strain: Sprague-Dawley; age: approximately 6 weeks old at study initiation; weight: mean body weights at initiation were 182 g for males and 138 g for females, individual animal weights were within a range of 10 g for each sex; source: Charles River Breeding Laboratories, Portage, MI.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 11 days and were assigned randomly by sex to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study (109 weeks)		Satellite Group (52 weeks)	
		Male	Female	Male	Female
1	Control 0	50	50	20	20
2	50	50	50	20	20
3	200	50	50	20	20
4	800	50	50	20	20
5	3200	50	50	20	20

Rats were housed five to a cage in a room with temperature and humidity controls set at 21°C and 50%, respectively, with a 12-hour light/dark cycle. The animals were examined to ensure they were in good health and quarantined 7 days between randomization and study start.

2. Diet Preparation: Premixes were prepared each week by grinding prodiamine directly into Labsure Laboratory Animal Diet No. 2 and mixing in an inflated polyethylene bag for a minimum of 3 minutes. The premixes were diluted with the appropriate amount of untreated diets to give the required concentration and mixed in a double-cone blender for 7 minutes. Homogeneity was analyzed on duplicate samples from the top, middle, and bottom of the blender on representative diets prior to study initiation. Stability was determined over 9 and 18 days at room temperature. Diets were analyzed at 3-month intervals and at 110 weeks for test compound concentration.

Results: Homogeneity was acceptable; the relative standard deviation for the 50-ppm diets was 4.4%. The test compound was stable in diets over 18 days. All mean analyzed values for test compound were within 10% of nominal concentration. The mean concentrations for 10 intervals of analysis were 0, 50.7, 119.7, 799, and 3200 ppm prodiamine.

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

4. Statistics: Food consumption data were analyzed on a cage basis using cumulative totals, and body weight data were analyzed using weight gains. For these and other non-incidence data, Bartlett's test was used to assess homogeneity of variance, and heterogeneous data were log transformed. One-way analysis of variance was used and if data were heterogenous after transformation, the Kruskal-Wallis rank analysis was utilized. For organ weight data, analysis of covariance was carried out with the final body weight as the covariate. For selected tumors, the IARC recommended statistical analyses were performed.

5. Quality Assurance: A quality assurance statement was signed and dated January 23, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and moribundity. Rats were examined daily on weekdays for all signs of ill health, behavioral changes, and reaction to treatment for the first 4 weeks of the study. Thereafter, these examinations were made weekly. From week 27 on, the examinations were made twice weekly and all rats were examined for palpable masses. Observed masses were followed every 2 weeks.

   Results: There was yellow fur staining in the 3200-ppm dose group. This was considered to have come from the test compound, a yellow powder. There were no other clinical findings suggesting a compound-related effect.

   Cumulative mortality and survival are summarized Table 1. There was no effect of dosing on survival. In the satellite groups (20/sex), one male receiving 800 ppm died as well as one control female, one female receiving 50 ppm, and two females receiving 200 ppm.

   There were no increases in palpable masses related to dosing. In male groups of the main study, the incidence varied from 54 to 74% and in female groups from 72 to 78% with no apparent dose trend.

TABLE 1. Cumulative Mortality and Percent Survival in Rats Fed Prodiamine for 108 Weeks[a]

Dose Group (ppm)	Mortality (Percent Survival) at Week			
	Satellite Groups	Main Groups		
	52	52	78	Termination
		Males		
0	0 (100)	0 (100)	9 (82)	31 (38)
50	0 (100)	1 (98)	4 (92)	22 (56)
200	0 (100)	1 (98)	5 (90)	30 (40)
800	1 (95)	1 (98)	9 (82)	31 (38)
3200	0 (100)	1 (98)	14 (72)	33 (34)
		Females		
0	1 (95)	1 (98)	9 (82)	31 (38)
50	1 (95)	1 (98)	7 (86)	27 (46)
200	2 (90)	0 (100)	4 (92)	28 (44)
800	0 (100)	3 (94)	7 (86)	21 (58)
3200	0 (100)	2 (96)	7 (86)	29 (42)

[a]Percent survival was based on 50 rats/sex/dose of the main group and 20/sex/dose in the satellite groups.

2. Body Weights: Rats were weighed at the time of randomization to test groups, on the first day of treatment, and once a week thereafter.

Results: Representative data on mean body weight gains are summarized in Table 2. In the first 78 weeks of the study, reduced body weight gains were reported in the 3200-ppm males. In females in the 3200-ppm group, there were also reduced body weight gains between weeks 53 and 78, but gains were not statistically significant from weeks 0 to 78. During the second year of treatment, intergroup values varied considerably. It was reported that no clear treatment-related effect on body weights was apparent among other treated groups. At 78 weeks, the mean body weights were 10 and 5.6% lower in high-dose males and females, respectively, than in controls; at 108 weeks, mean weights were 5.7 and 1.2% lower than in controls for high-dose males and females, respectively.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated on a weekly basis. Efficiency and compound intake were calculated from food consumption and body weight gain data. Water consumption was measured over daily periods for 1 week in each month for all cages in all groups.

Results: A slight but significant ($p \leq 0.05$) reduction in food consumption was seen in males receiving 3200 ppm when compared to controls. Over the entire study, food consumption in high-dose males and females was 96 and 98% of control consumption, respectively. Decreased values were seen for the first 78 weeks in males and between weeks 53 and 78 in females. Food conversion over the first 26 weeks, measured as grams of food consumed divided by body weight gain, was not markedly affected by dosing. Water consumption was similar in all groups. Mean compound intake values for 109 weeks of the study were 1.8, 7.2, 29.4, and 720 mg/kg/day for males and 2.3, 9.1, 37.0, and 151 mg/kg/day for females receiving 50, 200, 800, or 3200 ppm, respectively.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on all animals in group 1 (control) and group 5 (3200 ppm) prior to initiation, at week 52, and prior to termination (week 104). The animals' pupils were dilated using Tropicamide ophthalmic solution prior to examinations.

TABLE 2. Representative Results of Mean Body Weight Gains (±S.D.) of Rats Fed Prodiamine for 108 Weeks

Dose Group (ppm)	Mean Body Weight Gain (g/rat) at Weeks				
	0-26	26-52	53-78	0-78	0-108
			Males		
0	417 ± 65	118 ± 76	75 ± 78	600 ± 106	501 ± 126
50	392 ± 59*	116 ± 43	83 ± 42	584 ± 103	538 ± 150
200	395 ± 56*	118 ± 34	69 ± 62	581 ± 108	523 ± 123
800	395 ± 61*	112 ± 43	56 ± 76	562 ± 117	513 ± 122
3200	376 ± 63	88 ± 47**	43 ± 49**	514 ± 111**	463 ± 79
			Females		
0	165 ± 42	79 ± 35	89 ± 41	335 ± 93	341 ± 84
50	180 ± 41	89 ± 42	70 ± 55	329 ± 96	360 ± 117
200	184 ± 45	89 ± 37	93 ± 44*	369 ± 92	376 ± 114
800	168 ± 34	86 ± 38	71 ± 35	329 ± 86	331 ± 100
3200	156 ± 34	75 ± 30	66 ± 34*	307 ± 73	329 ± 107

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

Results: There were no ocular lesions related to dosing with prodiamine. The abnormalities noted were low in incidence, not increased in high-dose groups, and were common for the age and strain of rat studied.

5. Hematology and Clinical Chemistry: Blood was collected from the orbital sinus at weeks 26, 52, 78, and 104 for hematology and clinical analyses from 10 male and 10 female rats from each surviving group. These samples were taken from satellite-group rats at weeks 26 and 52 and from main group rats at weeks 78 and 104. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)[+]
X Hemoglobin (HGB)[+]
X Leukocyte count (WBC)[+]
X Erythrocyte count (RBC)[+]
X Platelet count[+]
Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count
Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation:thromboplastin time (PT)[++]

Blood smears were prepared for all animals sacrificed during the study prior to termination and at the interim sacrifice. These slides were fixed, stained, and examined if the pathologist considered it necessary.

Results: There were no effects of biological importance on hematology parameters in rats dosed with prodiamine. All mean values at all intervals were within the normal range. Significant increases ($p \leq 0.05$ or 0.01) were found for MCHC values in males receiving 200, 800, and 3200 ppm at weeks 26 and 52; however, the changes were slight. Significant decreases in the same groups were observed at week 78, and there were no changes at week 104. In addition, there were no correlating change in HCT or HGB. Slight but significant increases in MCHC were also observed at 78 weeks in females receiving 200, 300, and 3200 ppm but there was no consistent pattern of changes with time or dose.

[+]Recommended by Subdivision F (October 1982) Guidelines.
[++]Not performed on predosed animals.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium[+]	X	Albumin[+]
X	Chloride[+]		Albumin/globulin ratio
	Magnesium[+]	X	Blood creatinine[+]
X	Phosphorus[+]	X	Blood urea nitrogen[+]
X	Potassium[+]	X	Cholesterol[+]
X	Sodium[+]	X	Globulins
		X	Glucose[+]
	Enzymes	X	Total bilirubin[+]
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein[+]
	Creatinine phosphokinase[+]		Triglycerides
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (SGPT)[+]		
X	Serum aspartate aminotransferase (SGOT)[+]		
	Gamma glutamyltransferase (GGT)		

Results: There were no effects of toxicologic importance on serum enzyme activities. Mean activities of SGPT and SGOT were similar in all groups of males and females at weeks 78 and 104 and activities of LDH were similar in all groups of males and females at weeks 52, 78, and 104. Activities of SGPT and SGOT tended to be lower in dosed animals than in controls at week 26 and 52 and the activity of LDH tended to be decreased compared to controls in both dosed males and females at 26 weeks. The authors reported that the decreases were not due to cofactor depletion (for SGOT and SGPT) and that the LDH values in controls at week 26 were abnormally high. Data for SGPT, SGOT, and LDH are summarized in Table 3.

Other intergroup differences in clinical chemistry parameters that reached a level of statistical significance were considered unrelated to dosing by the study authors since no consistent pattern was apparent. Decreased serum alkaline phosphatase activity was reported for males receiving 3200 ppm at 52 weeks and for both sexes in this group at weeks 78 and 104. Cholesterol was significantly increased in females receiving 3200 ppm at weeks 26, 52, and 78 but values were within the normal range.

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 3. Selected Clinical Chemistry Results (Mean ± S.D.) in Rats Fed Prodiamine for 108 Weeks[a]

Parameter/ Week	Dose Level (ppm)									
	Males					Females				
	0	50	200	800	3200	0	50	200	800	3200
SGPT (mU/mL)										
26	27 ± 4.2	35 ± 34.9	24 ± 5.4	20 ± 2.4**	18 ± 4.0**	25 ± 6.6	20 ± 2.5	19 ± 4.1*	19 ± 2.2*	19 ± 6.8*
52	24 ± 6.4	24 ± 4.2	28 ± 13.7	20 ± 7.0	16 ± 3.7**	28 ± 8.7	20 ± 2.6	29 ± 23.2	26 ± 15.5	16 ± 1.8**
78	36 ± 43.1	29 ± 8.3	24 ± 6.5	23 ± 7.5	21 ± 4.8	25 ± 8.7	27 ± 6.7	32 ± 27.8	24 ± 7.0	21 ± 4.1
104	22 ± 7.2	28 ± 13.8	25 ± 12.1	21 ± 6.4	20 ± 6.6	27 ± 11.9	26 ± 13.9	28 ± 24.3	27 ± 11.1	27 ± 20.0
SGOT (mU/mL)										
26	56 ± 8.0	62 ± 30.5	51 ± 9.6	50 ± 6.0	42 ± 6.5**	61 ± 11.9	49 ± 6.0**	49 ± 8.7**	44 ± 7.3*	45 ± 12.3**
52	46 ± 7.9	44 ± 7.2	48 ± 9.4	45 ± 10.6	41 ± 7.4	60 ± 14.1	43 ± 4.7*	53 ± 32.5*	69 ± 82.9*	38 ± 4.8**
78	56 ± 36.3	51 ± 10.3	48 ± 9.7	41 ± 6.9	44 ± 13.2	50 ± 6.2	52 ± 13.9	49 ± 19.3	46 ± 10.3	47 ± 13.0
104	53 ± 12.6	56 ± 14.5	47 ± 10.4	48 ± 15.2	44 ± 12.1	48 ± 14.5	53 ± 22.2	57 ± 26.0	66 ± 31.1	48 ± 13.1
LDH (mU/mL)										
26	894 ± 530.8	504 ± 321.0	558 ± 420.5*	434 ± 286.8**	259 ± 168.5**	664 ± 371.0	438 ± 198.5	441 ± 232.1	233 ± 19.1**	320 ± 295.4**
52	257 ± 133.6	177 ± 27.0	203 ± 54.5	309 ± 301.4	169 ± 31.6	335 ± 235.8	226 ± 76.8	229 ± 85.8	383 ± 561.2	213 ± 116.8
78	175 ± 52.4	205 ± 97.8	175 ± 49.4	190 ± 51.3	232 ± 199.9	211 ± 153.7	198 ± 78.0	180 ± 49.8	307 ± 174.4	200 ± 114.3
104	180 ± 61.9	196 ± 73.9	160 ± 47.3	189 ± 94.2	208 ± 94.2	219 ± 136.4	324 ± 407.3	282 ± 245.1	331 ± 264.8	173 ± 52.6

[a]Based on 10 rats/sex/group.
*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.01$).

6. Urinalysis: Urine was collected from 10 male and 10 female rats from each surviving group at weeks 86, 50, 78 and 102. The CHECKED (X) parameters were examined:

X	Appearance[+]	X	Glucose[+]
X	Volume[+]	X	Ketones
X	Specific Gravity[+]		Bilirubin[+]
X	pH		Blood[+]
	Sediment (microscopic)[+]		Nitrate
X	Protein[+]	X	Urobilinogen

Results: There were no toxicologically important effects of dosing on urinary parameters.

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

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	X Aorta[+]	XX Brain[+]
X Salivary glands[+]	XX Heart[+]	X Peripheral nerve (sciatic nerve)[+]
X Esophagus[+]	X Bone marrow[+]	X Spinal cord (3 levels)
X Stomach[+]	X Lymph nodes[+]	XX Pituitary[+]
X Duodenum[+]	XX Spleen[+]	X Eyes (optic nerve)[+]
X Jejunum[+]	XX Thymus[+]	
X Ileum[+]		
X Cecum[+]		
X Colon[+]		
X Rectum	Urogenital	Glandular
XX Liver[+]	XX Kidneys[+]	XX Adrenals[+]
Gallbladder[+]	X Urinary bladder[+]	Lacrimal gland
X Pancreas[+]	XX Testes[+]	X Mammary gland[+]
	X Epididymides	XX Thyroids[+]
	Prostate	XX Parathyroids[+]
	X Seminal vesicle	Harderian glands
Respiratory	XX Ovaries	
X Trachea[+]	XX Uterus[+]	
XX Lung[+]		

Other
X Bone (sternum and femur)[+]
X Skeletal muscle[+]
X Skin
X All gross lesions and masses

[+]Recommended by Subdivision F (October 1982) Guidelines.

Results: A complete inventory of tissues was examined for all rats in the control and high-dose groups and for all rats in other groups that died during the study. Lungs, liver, kidneys, thyroids, and gross lesions were examined for all rats in the low- and intermediate-dose groups as well as any tissue showing a compound-related change at the high dose.

a. Organ Weights: At the interim sacrifice, the mean liver weights were significantly increased in females receiving 800 and 3200 ppm; at the terminal sacrifice, liver weights were increased in males receiving 800 and 3200 ppm and in females receiving 3200 ppm. The study authors considered slight weight changes in other organs to be incidental and not related to dosing. Table 4 presents liver weight data. The statistical treatment by the authors was performed by analysis of covariance with body weight as the covariate. The reviewers calculated liver-to-body weight ratio data at study termination and performed statistical analysis of the ratio data.

b. Gross Pathology: Table 5 summarizes the incidence of frequently observed gross lesions in rats that died in the main study were sacrificed in extremis, or sacrificed at termination. The increase in liver masses in high-dose females did not correlate with any histologic findings of toxicologic importance nor was the increased incidence of pale foci of the lungs in high-dose males and females of any toxicological importance. Most of the findings were common to aging rats and were within the expected background range. None of the findings were considered of toxicologic importance by the study authors.

c. Microscopic Pathology:

1) Nonneoplastic: Table 6 summarizes the incidence of frequently occurring nonneoplastic findings. It was reported that the only evidence of a compound-related effect was an increase in the incidence of ballooned cells in the livers of rats that died. This was seen predominantly in males where the incidence was 5/30, 9/22, 4/30, 12/31, and 17/33 in rats that died after receiving 0, 50, 200, 800, or 3200 ppm, respectively. Most of the other changes were age related and within the normal background range. An increase in medullary hyperplasia of the adrenal was noted in males receiving ≥200 ppm and hyperplasia was evident in the pituitary of high-dose males and of females receiving ≥200 ppm. Cystic follicular hyperplasia was slightly increased in high-dose males and females but there was no clear dose-related trend.

TABLE 4. Mean Liver Weights (±S.D.) and Liver-to-Body Weight Ratios in Rats Fed Prodiamine for 108 Weeks

Dietary Level (ppm)	Interim Sacrifice (Week 52) Males (g)	Females (g)
0	31.1 ± 4.3 (29.9)[a]	13.2 ± 2.7 (13.4)
50	29.0 ± 5.5 (28.9)	14.5 ± 3.0 (13.5)
200	28.5 ± 5.4 (28.1)	14.9 ± 4.0 (13.9)
800	29.4 ± 4.4 (28.9)	15.9 ± 3.1 (16.1)**[b]
3200	30.2 ± 3.3 (32.5)	15.2 ± 2.2 (16.6)**[b]

	Terminal Sacrifice (Week 108) Males		Females	
	(g)	(%)[c]	(g)	(%)[c]
0	23.6 ± 4.8	3.30 ± 0.48[T]	19.4 ± 5.1	3.95 ± 0.94[T]
50	24.8 ± 4.1	3.37 ± 0.64	20.5 ± 5.1	4.07 ± 0.77
200	25.6 ± 4.7	3.49 ± 0.48	19.6 ± 4.3	3.83 ± 0.56
800	27.3 ± 5.0**[c]	3.75 ± 0.56*	20.3 ± 4.1	4.34 ± 0.79
3200	28.1 ± 3.4**[c]	4.14 ± 0.58**	23.6 ± 5.2**[c]	4.97 ± 0.88**

[a]The values in parentheses are adjusted values for body weight, which was used as the covariate; reported by the study authors.

[b]Statistical analysis by study authors using analysis of covariance; body weight was used as the covariate.

[c]Percent body weight, calculated by our reviewers; statistical analysis by ANOVA.

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

[T]Significant trend ($p < 0.01$), by linear regression.

TABLE 5. Representative Gross Findings in Rats Fed Prodiamine for 108 Weeks[a]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	50	200	800	3200	0	50	200	800	3200
Thyroid										
Mass	1	2	1	4	0	1	1	1	2	2
Enlarged	1	3	2	0	2	1	0	0	1	2
Adrenals										
Enlarged	11	11	20	16	13	23	25	21	15	19
Pituitary										
Enlarged	11	24	17	20	9	37	42	37	35	28
Hemorrhagic	7	17	13	18	5	29	37	29	27	19
Liver										
Masses	1	1	0	0	3	1	0	1	0	8
Enlarged	4	2	4	5	2	2	3	4	7	5
Lungs										
Pale foci	11	15	9	12	18	6	7	5	9	14
Pancreas										
Masses	10	11	10	7	7	4	7	6	3	10
Skin										
Mass	3	7	6	5	2	1	0	1	2	4
Mammary glands										
Cysts						21	22	21	19	23

[a]Includes all animals in main groups (50/sex/group), but not interim-sacrifice animals.

TABLE 6. Representative Nonneoplastic Findings in Rats Fed Prodiamine for 108 Weeks[a]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	50	200	800	3200	0	50	200	800	3200
Lungs	(50)[a]	(50)	(50)	(49)	(50)	(50)	(50)	(50)	(50)	(49)
Alveolar macrophages	9	17	18	16	18	14	8	7	8	14
Interstitial pneumonitis	0	2	4	9	4	0	1	4	1	2
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Ballooned cells	16	23	27	21	30	3	5	5	8	8
Vacuolation, periportal	17	26	20	17	21	29	24	28	24	21
Vacuolation, centrilobular	17	7	10	7	3	12	8	14	9	13
Focal sinusoidal dilation	11	19	16	20	16	29	23	34	37	34
Kidneys	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Progressive glomerulonephritis	39	44	44	39	41	23	30	26	22	26
Cortical cysts	3	3	8	5	3	0	2	1	0	0
Pyelitis	1	1	1	4	1	2	0	2	1	6
Spleen	(50)	(27)	(39)	(40)	(50)	(50)	(29)	(30)	(22)	(49)
Extramedullar hemopoiesis	6	6	6	15	10	18	7	11	3	21
Hemosiderosis	6	11	10	9	10	23	20	13	14	22
Thyroid glands	(50)	(50)	(50)	(50)	(50)	(50)	(48)	(50)	(50)	(50)
Cystic follicular hyperplasia	4	1	2	1	7	0	0	1	1	4
Parafollicular hyperplasia	1	6	2	4	1	2	7	4	8	3
Adrenal glands	(50)	(47)	(47)	(50)	(50)	(50)	(49)	(49)	(48)	(49)
Medullary hyperplasia	6	2	9	9	10	3	1	2	1	0
Enlarged cortical cells	15	23	12	21	8	17	16	21	20	18
Pituitary gland	(50)	(34)	(39)	(34)	(50)	(50)	(45)	(43)	(43)	(50)
Hyperplasia	6	2	3	3	15	3	2	7	6	9

[a]The numbers in parentheses are the number of animals with tissues examined; includes animals in main groups but not in satellite groups.

Histologic examination of animals in the satellite groups (12 months) did not indicate any increases in nonneoplastic findings related to dosing. Minimal centrilobular vacuolation in the livers was frequent in satellite males (17/20 in controls and 12/20 in the high dose). Minimal glomerulonephrosis in the kidneys was seen in all groups of males (25-45%), and minimal hemosiderosis in the spleen was noted in 6/20 control females and 8/20 high-dose females. Other findings were less frequent and not dose related.

2) Neoplastic: Table 7 summarizes neoplastic findings in rats. There was an increase in the incidence of follicular tumors of the thyroid in males and females receiving 3200 ppm prodiamine. The increase in follicular adenomata in high-dose females was significant ($p < 0.019$) and there was a positive dose-trend ($p < 0.001$); the laboratory control incidence in females was 15/724 (2.1%; range, 0 to 6%). The incidence of follicular tumors (adenoma and/or carcinoma) in high-dose males (16%) was increased significantly ($p=0.039$; positive trend, $p \leq 0.002$) when compared to concurrent controls; however, the incidence was within the laboratory control range. The historical value for untreated males was 56/722 adenomata (7.8%) and the range was 4 to 12%. The increase in males was not considered by the authors to be of toxicological importance. There was a slight increase in the incidence of mammary epithelial tumors in dosed females when compared to controls, but the values were reported to be within the laboratory historical range. The laboratory background incidence for adenoma was 8.2% (range, 0-28%) and for adeno-carcinoma was 18% (range, 0-28%) based on 12 studies with a total of 726 rats.

There were no other increases in neoplasms that were considered to be related to dosing.

D. STUDY AUTHORS' CONCLUSIONS:

There was a marginal increase in benign follicular adenomas in both males and females fed prodiamine at dietary levels of 3200 ppm for lifetime. The increases in males were within the normal laboratory range. No increases in malignant tumors were

TABLE 7. Neoplastic Findings in Rats Fed Prodiamine for 108 Weeks[a]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	50	200	800	3200	0	50	200	800	3200
Thyroid gland	(50)[b]	(50)	(50)	(50)	(50)	(50)	(48)	(50)	(50)	(50)
Follicular adenoma	1	4	0	3	6	0	2	0	0	6*
Follicular carcinoma	1	0	1	3	2	0	0	0	2	0
Follicular adenoma/ carcinoma	2	4	1	6	8*	0	2	0	2	6*
C-cell carcinoma	8	2	4	3	0	5	4	6	5	4
Mammary gland	(50)	(28)	(33)	(50)	(50)	(50)	(48)	(46)	(39)	(50)
Adenocarcinoma	1	2	0	2	0	6	10	14	13	14
Adenoma	0	0	1	1	0	0	0	4	0	2
Fibroadenoma	0	1	2	2	0	23	25	30	25	31
Fibroma	0	0	0	0	0	0	3	0	4	3
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(49)
Hemangiosarcoma	1	0	0	0	2	0	0	0	0	3
Pancreas	(50)	(31)	(35)	(33)	(49)	(50)	(31)	(32)	(24)	(49)
Islet cell adenoma	4	6	7	3	4	2	3	1	2	7
Islet cell carcinoma	4	1	2	2	3	3	3	3	0	1
Pituitary	(50)	(34)	(39)	(39)	(50)	(50)	(45)	(43)	(43)	(50)
Adenoma	18	24	21	24	9	34	36	28	27	29
Carcinoma	0	0	2	0	0	1	3	5	4	1
Testes	(50)	(31)	(36)	(37)	(50)					
Interstitial cell tumor	2	4	2	4	5					
Adrenal	(50)	(47)	(47)	(50)	(50)	(50)	(49)	(49)	(48)	(49)
Pheochromocytoma	2	2	3	2	2	0	0	0	0	0
Skin	(50)	(37)	(41)	(38)	(50)	(50)	(30)	(28)	(26)	(50)
Squamous papilloma	0	3	3	4	3	0	0	0	0	0
Dermal fibroma	0	2	5	3	2	0	0	0	1	2

(Continued)

TABLE 7. Neoplastic Findings in Rats Fed Prodiamine for 108 Weeks[a] (continued)

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	50	200	800	3200	0	50	200	800	3200
Subcutis	(11)	(14)	(14)	(9)	(8)	(5)	(9)	(6)	(3)	(7)
Fibrosarcoma	2	0	2	1	1	0	1	1	0	3
Fibroma	6	5	3	3	4	4	3	1	1	3
Lipoma	4	8	10	2	2	1	3	2	1	0

[a]Includes animals in main study that died, were sacrificed moribund, or sacrificed at study termination.

[b]The numbers in parentheses are the number of animals with the specific tissue examined histologically.

*Significantly different from control incidence ($p \leq 0.05$).

observed. It is questionable if the increase in benign thyroid tumors is indicative of an oncogenic response. The maximum tolerated dose (MTD) was demonstrated to be 3200 ppm based on decreased body weight gains in both sexes. There was no effect of dosing on mortality, clinical signs, or hematology parameters. There were slight increases in food consumption, increased liver weights, and minor biochemical disturbances at 3200 ppm. Serum cholesterol levels were increased in females receiving 3200 ppm at weeks 26, 52, and 78. There were no histopathologic changes in the liver that correlated with the biochemical changes. Females receiving 800 ppm had increased liver weights at interim sacrifice, and males at the same dose had increased liver weights at terminal sacrifice. The NOEL was 200 ppm and the LOEL was 800 ppm, based on minor biochemical disturbances and liver weight changes.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were acceptable. Histopathologic examination was adequate and laboratory historical data for relevant tumors were presented for reference. Summary data were supported by individual animal data and most mean values that were validated agreed with the authors' values, with the exception of some differences in rounding the last digit. We agree with the authors' assessment that the slight increase in mammary tumors in dosed females was not indicative of an oncogenic effect. There was a definite increase in follicular adenomas in thyroids of high-dose females when compared to concurrent controls or laboratory historical incidence; there were no increases in malignant thyroid tumors. Since the increase in combined thyroid tumors in males (follicular adenomas and carcinomas) receiving 3200 ppm was also significant ($p < 0.05$) in both males and females, it appears that prodiamine is a potential carcinogen and should be evaluated by the HED review committee.

Although there was a slight increase in cystic follicular hyperplasia of the thyroid and an increase in hyperplasia of the pituitary in high-dose males and females, there were no effects on weights of endocrine organs and the biological importance of the hyperplasia cannot be adequately assessed without clinical chemistry data on thyroid hormones and thyroid stimulating hormone. The lack of these data do not affect the classification of the study. However, if frozen blood samples are still available and can be analyzed, the submission of these data by the sponsor might help in interpretation of the biological importance of the histologic thyroid findings.

We agree with the study authors that an MTD was approached based on slight effects on body weight gains and increased liver weights; however, the rats may have tolerated a higher dose. The effects on hematology were minimal and probably not

related to dosing. The decreases in activities of SGPT, SGOT, and LDH in dosed animals remain unexplained. They may relate to dosing; however, increases, not decreases, are considered a toxicologic indicator and the observed changes were not consistent throughout the entire study. There were no histologic changes in the liver that correlated with the biochemical disturbances or liver weight increases. The increased liver weights may be due to enzyme induction and an adaptive effect rather than a toxicologically adverse effect.

Based on all parameters, we assess that the NOEL for systemic toxicity is 200 ppm and the LOEL is 800 ppm.

EPA No.: 68D80056
DYNAMAC No.: 172-A
TASK No.: 1-72A
September 8, 1989

007660

DATA EVALUATION RECORD

PRODIAMINE

Oncogenicity Feeding Study in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
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Dynamac Corporation

Signature: Roman J Prenta for

Date: 9-8-89

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EPA No.: 68D80056
DYNAMAC No.: 172-A
TASK No.: 1-72A
September 8, 1989

DATA EVALUATION RECORD

PRODIAMINE

Oncogenicity Feeding Study in Mice

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DATA EVALUATION RECORD

GUIDELINE §83-2

STUDY TYPE: Oncogenicity feeding study in mice.

MRID NUMBER: 405897-02.

TEST MATERIAL: Prodiamine.

SYNONYM(S): Endurance.

STUDY NUMBER(S): VCL 37/871188.

SPONSOR: Sandoz Crop Protection Corporation, 1300 East Touhy Avenue, Des Plaines, IL.

TESTING FACILITY: Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, England.

TITLE OF REPORT: Prodiamine Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice.

AUTHOR(S): Powell, L. A. J., et al.

REPORT ISSUED: April 12, 1988.

CONCLUSIONS:

Under the conditions of the study, prodiamine was not oncogenic to CD-1 mice at dietary levels of 0, 50, 500, or 5000 ppm for 99 weeks. There was a significant increase in the incidence of subcutaneous fibrosarcoma in the high-dose males (8/52 compared to 1/52 for controls, $p<0.03$); however, this was not considered to be of toxicological importance because it was related to fighting activity caused by group caging.

Males and females receiving 5000 ppm were observed with yellow fur staining and there was an increased incidence of apparent fighting injuries (skin scabs and ulcerations). Palpable masses were increased in dosed males and females but there were no dose-related patterns. Mortality incidence in males and females receiving 5000 ppm was slightly increased especially after 78 weeks of study; the combined incidence of males and females at 5000 ppm was statistically significant ($p <0.01$) when compared with controls. Overall body weight gains were significantly ($p <0.01$) lower than controls in males receiving 500 or 5000 ppm but there was no corresponding effect in females. Neutrophils were significantly ($p <0.01$) increased and lymphocytes were significantly decreased ($p <0.01$) in high-dose males (at weeks 78 and 99) and females (at week 52) when compared to controls. Absolute and relative liver weights were slightly increased in high-dose males and females; the increase was statistically significant ($p <0.01$) in females. Absolute kidney weights were significantly decreased in females of mid- ($p <0.05$) and high- ($p <0.01$) dose groups; relative kidney weights were also significantly ($p <0.01$) decreased when compared to controls for both dose groups.

Gross pathological examinations disclosed an increased incidence of subcutaneous masses in high-dose males with no abnormalities observed in animals receiving 50 or 500 ppm. Histopathological findings were characterized by a marginal increase in the incidence of prominent dermal collagen at various skin sites in males of the high-dose group. Other nonneoplastic lesions were considered to be of no toxicological importance. Neoplastic lesions included a statistically significant ($p <0.03$) increased incidence of subcutaneous fibrosarcoma in males receiving 5000 ppm when compared with controls.

It is concluded that the LOEL for systemic toxicity is 5000 ppm based on mortality, reduced body weight gains and increased liver weights and the NOEL is 500 ppm for prodiamine fed orally to CD-1 mice for 99 weeks.

Classification: CORE Minimum because of multiple housing of mice which may have contributed to relatively high incidence of fibrosarcomas in high-dose males.

A. MATERIALS:

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

2. Test Animals: Species: mice; strain: CD-1; age: 28 days old; weight: males--25-26 g, females--20 g (week 1) with a range of 3 g for each sex; source: Charles River Breeding Laboratories, Manston, Kent, U.K.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions and their health was observed. Males and females free of abnormal signs were assigned to one of the following three test groups or a control group using a computer randomization method with consideration for approximate homogenous weight:

Test Group	Dose in Diet (ppm)	Number of Animals	
		Males	Females
1 Control	0	52	52
2 Low (LDT)	50	52	52
3 Mid (MDT)	500	52	52
4 High (HDT)	5000	52	52

Animals were housed four to a cage in a temperature- and humidity-controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: Diets containing the compound were prepared each week. At each preparation, basal diet and compound were weighed, uniformly mixed, blended, and stored at ambient temperature. Samples of the diet containing compound were analyzed for stability, homogeneity, and concentration.

Results: Table 1 represents the mean concentrations of prodiamine in test diets. Diets were reported to be homogeneous and prodiamine was demonstrated to be stable. Mean results of concentration were within ±8% of nominal values, with the exception of one result of 5000 ppm at week 39, which was 13.6% above the nominal concentration.

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

TABLE 1. Mean Concentrations of Prodiamine in Test Diets Analyzed During the Chronic Study in Mice[a]

Week of Study	Nominal Inclusion (ppm)	Mean Analyzed Concentration (ppm)	Deviation from Nominal (%)
1	50	50.2	+0.4
	500	494	-1.2
	5000	4950	-1.0
13	50	50.2	+0.4
	500	513	+2.6
	5000	5330	+6.6
26	50	53.5	+7.0
	500	503	+0.6
	5000	4930	-1.4
39	50	50.9	+1.8
	500	514	+2.8
	5000	5680	+13.6
44	50	47.7	-4.6
	500	515	+3.0
	5000	5210	+4.2
52	50	53.7	+7.4
	500	504	+0.8
	5000	4980	-0.4
65	50	47.3	-5.4
	500	468	-6.4
	5000	4920	-1.6
78	50	46.5	-7.0
	500	490	-2.0
	5000	4810	-3.8
91	50	52.4	+4.8
	500	501	+0.2
	5000	5020	+0.4
100	50	48.1	-3.8
	500	516	+3.2
	5000	5100	+2.0

[a]Data extracted from study No. 405897, Table 1, Addendum 4.

4. Statistics: Food consumption, water consumption, body weight, organ weight, and clinical pathology data were analyzed by frequency analysis if data consisted predominately of one particular value, otherwise Bartlett's test was applied to test for heterogeneity of variance between treated groups. If no significant heterogeneitywas detected, a one-way analysis of variance (ANOVA) was carried out; any significant differences were further examined using the Kruskal-Wallis analysis. For a dose-related response, analyses of variance were followed by Student's t test and Williams' test and the Kruskal-Wallis analyses were followed by Shirley's test.

   Analyses of covariance were used in place of analysis of variance for organ weight data. This is used when the relationship between organ weight and body weight was significant at the 10% level.

   Mortality data were analyzed using log-ranks method, and analyses of tumor incidence data, when considered necessary, were performed using the guidelines of the International Agency for Research on Cancer (IARC).

5. Quality Assurance: A quality assurance statement was signed and dated January 3, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and moribundity. Animals received detailed examinations every week day for the first 4 weeks of the study, once a week until week 68, and twice a week thereafter. A detailed examination of individual mice was conducted for signs of toxicity, behavioral effects, and physical changes, and animals were palpated for tissue masses weekly.

   Results: Table 2 summarizes mortality incidences in mice fed prodiamine for 99 weeks. Mortality incidence in males and females receiving 5000 ppm was slightly higher relative to controls, but, when male and female data were pooled together, there was a significant difference ($p < 0.01$) when compared with the control group (60% mortality at 5000 ppm and 39% in controls).

   Males and females of dosed groups were observed with increased number of masses in comparison with controls. The highest incidence was observed in males receiving 50 ppm; there was no dose-related pattern. Palpable masses observed during the study are shown in Table 3.

TABLE 2. Cumulative Mortality Incidences and Percent Survival in Mice Fed Prodiamine for 99 Weeks[a,b]

Dietary Level (ppm)	Number of Mortalities and Percent Survival at Study Week			
	26	52	78	99
		Males		
0	4 (92)	8 (84)	15 (71)	24 (54)
50	2 (96)	4 (92)	10 (79)	29 (45)
500	1 (98)	3 (94)	5 (90)	23 (56)
5000	1 (98)	2 (96)	2 (77)	36 (31)
		Females		
0	0 (100)	1 (98)	6 (89)	15 (62)
50	0 (100)	2 (96)	4 (92)	14 (73)
500	0 (100)	1 (98)	5 (90)	18 (65)
5000	0 (100)	0 (100)	6 (89)	24 (54)

[a]Data extracted from study No. 405897, p. 26, and Appendix 5.

[b]Values based on 52 animals/group/sex; percent survival is in parentheses.

TABLE 3. Incidences of Palpable Masses Observed in Mice Fed Prodiamine for 99 Weeks[a]

Description	Palpable Masses at Dietary Level (ppm)							
	Males				Females			
	Control	50	500	5000	Control	50	500	5000
No. of affected animals	14	30	20	26	7	9	9	10
Total number of masses	22	43	29	38	7	11	9	12

[a]Data extracted from study No. 405897, p. 27.

Frequent clinical signs are summarized in Table 4. An increase in the occurrence of yellow staining of fur around the urogenital/ventral region and at general sites was observed in males and females dosed 5000 ppm (n=44 and 9, respectively). This yellow staining was due to prodiamine in the urine. No increase in fur staining was observed in animals dosed 50 or 500 ppm. Skin scabs in males at 500 and 5000 ppm were observed at higher incidences than controls; some males were also isolated in these two groups due to fighting or aggressive behavior. Other signs observed were common in CD-1 mice of this age with no apparent dose-response relationship.

2. Body Weight: Body weights were recorded at the time of allocation of animals to groups, at study initiation, and every week thereafter.

   Results: Table 5 summarizes the mean body weight gains in mice at specific intervals. Body weight gains tended to be lower in both males and females receiving 5000 ppm when compared to controls. Significant decreases were seen in high-dose males (5000 ppm) when compared to controls between weeks 0-26 and 27-52 ($p < 0.01$) and in females between weeks 27-52 and 53-78 ($p < 0.01$) and 79-99 ($p < 0.05$).

   Overall weight gains (weeks 0-99) were significantly lower ($p < 0.01$) than control in males receiving 500 or 5000 ppm, but there was no significant effect in females.

3. Food and Water Consumption and Compound Intake: Food consumption was determined and mean daily diet consumption was calculated weekly. Water consumption (g/mouse) was measured over daily periods for 1 week in each month for all groups. Compound intake was determined from the consumption and body weight gain data.

   Results: There were no apparent dose-related effects on food and water consumptions among males or females at 50, 500, or 5000 ppm, although males at 5000 ppm showed a tendency for slightly higher (nonsignificant) mean water consumption when compared with controls during the last 20 weeks of study.

   The mean compound intakes were proportionate to the concentrations of the compound in the diet. The calculated mean daily compound intake for the entire study was 6.2, 59.4, or 606.6 mg/kg/day for males receiving dietary levels of 50, 500, or 5000 ppm, respectively, and 6.8, 64.6, or 646.2 mg/kg/day for females receiving the same dietary levels of prodiamine.

TABLE 4. Selected Incidences of Clinical Signs Observed in Mice Fed Prodiamine for 99 Weeks[a]

Clinical signs	No. of Affected Mice at Dietary Level (ppm)							
	Males				Females			
	Control	50	500	5000	Control	50	500	5000
Yellow fur staining:								
Urogenital/ ventral region	20	28	20	33	1	1	3	7
General/other sites	0	0	0	11	0	0	0	2
Total:	20	28	20	44	1	1	3	9
Skin scabs[b]	15	16	25	29	2	2	4	4
Skin ulcerations[c]	11	13	8	18	1	2	3	3

[a]Data extracted from study No. 405897, p. 27, and Appendix 5.

[b,c]Skin changes on masses and on tip of tail were not included.

TABLE 5. Body Weight Gains at Specific Intervals in Mice Fed Prodiamine for 99 Weeks[a]

Dietary Level (ppm)	Mean (g/mouse) ± Standard Deviation at Interval (weeks): 0-26	27-52	53-78	79-99	0-99
			Males		
0	14.5 ± 3.7	2.3 ± 2.4	1.4 ± 3.0	-2.6 ± 4.3	16.6 ± 4.1
50	14.3 ± 4.8	0.7 ± 2.5*	1.9 ± 3.6	-2.5 ± 3.5	16.0 ± 5.5
500	13.8 ± 3.8	1.3 ± 3.3*	0.7 ± 3.4	-3.0 ± 5.9	13.1 ± 4.6**
5000	11.6 ± 4.1**	0.0 ± 2.2**	0.5 ± 3.2	-1.6 ± 2.7	11.4 ± 4.2**
			Females		
0	10.2 ± 3.1	2.6 ± 2.4	2.5 ± 3.0	-2.8 ± 2.5	11.9 ± 4.7
50	9.6 ± 2.8	2.4 ± 2.5	2.5 ± 2.9	-2.5 ± 3.6	12.3 ± 4.2
500	9.6 ± 2.6	1.9 ± 2.4	2.2 ± 2.2	-2.1 ± 2.4	11.6 ± 4.3
5000	9.8 ± 2.7	1.2 ± 2.2**	0.8 ± 2.8**	-1.0 ± 3.4*	10.6 ± 2.6

[a]Data extracted from study No. 405897, Table 3, and Appendix 3.

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

4. Ophthalmological Examinations: No ophthalmological examinations were performed.

5. Hematology and Clinical Chemistry: Blood was collected by orbital sinus puncture and blood smears were prepared from all mice that died or killed during the study and from all surviving mice at weeks 52, 78, and 99. Evaluation of differential leukocyte count was performed on animals of control and high-dose groups with a subsequent inclusion of low- and mid-dose males at weeks 78 and 99. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)[+]	X	Leukocyte differential count
	Hemoglobin (HGB)[+]		Mean corpuscular HGB (MCH)
	Leukocyte count (WBC)[+]		Mean corpuscular HGB concentration (MCHC)
	Erythrocyte count (RBC)[+]		Mean corpuscular volume (MCV)
	Platelet count[+]		Coagulation:thromboplastin time (PT)
	Reticulocyte count (RETIC)		
	Red cell morphology		

Results: Table 6 presents data for differential leukocyte counts (neutrophils and lymphocytes) in mice fed prodiamine for 99 weeks. At week 52, females receiving 5000 ppm had significantly increased ($p < 0.01$) neutrophil counts and significantly decreased ($p < 0.01$) lymphocyte counts when compared to controls; there was no effect in males. At weeks 78 and 99, neutrophils were significantly increased ($p < 0.01$) and lymphocytes were decreased ($p < 0.01$) when compared to control in high-dose males, but there were no corresponding effects in females.

b. Clinical Chemistry: Evaluation of clinical chemistry parameters was not performed.

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 6. Mean Neutrophil and Lymphocyte Values in Mice Fed Prodiamine for 99 Weeks[a]

Dietary Level (ppm)	Neutrophils (10^3/mm^3)			Lymphocytes (10^3/mm^3)		
	Study Week					
	52	78	99	52	78	99
			Males			
0	43.23	43.86	34.46	55.95	52.97	60.75
50	---	49.93	41.43	---	46.88	55.78
500	---	46.87	35.34	---	49.81	59.76
5000	43.28	57.37**	51.19**	55.52	40.17**	44.44**
			Females			
0	21.16	32.98	34.81	78.73	65.60	63.35
5000	31.44**	37.64	38.34	68.02**	60.30	59.21

[a]Data extracted from study No. 405897, Table 6, and Appendix 4.

**Significantly different from control values ($p < 0.01$).

6. Urinalysis: Urinalysis was not performed.

7. Sacrifice and Pathology: All animals that died or 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:

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

[+]Recommended by Subdivision F (October 1982) Guidelines.

Results:

a. Organ Weights: Table 7 presents mean body and organ (liver and kidney) weights of mice at termination of the study. There were no significant effects in males although the liver weights were slightly increased in the 5000-ppm group when compared to controls. Females receiving 5000 ppm had significantly increased ($p < 0.01$) mean liver weight when compared to controls. Mean kidney weights were significantly decreased ($p < 0.05$) in females receiving 500 and 5000 ppm when compared to controls. The reviewers analyzed organ-to-body weight ratios using covariance analyses and found the same levels of significance as those reported by the authors; the data are presented in Table 8. No notable effect was observed in organ weight data of animals that died prior to terminal sacrifice.

b. Gross Pathology: Table 9 summarizes selected gross pathology findings in mice fed prodiamine for 99 weeks. The most common gross pathological findings included increased incidence of subcutaneous masses in males receiving 5000 ppm and dose-related increased incidences of fur staining in males and females receiving 5000 ppm. Other gross observations were considered to be incidental in nature and unrelated to treatment.

c. Microscopic Pathology:

1) Nonneoplastic: Table 10 summarizes selected nonneoplastic lesions in mice fed prodiamine for 99 weeks. Males receiving 5000 ppm were noted with possibly dose-related increased incidences of prominent dermal collagen; although these increases showed a statistically significant trend ($p=0.006$), they were not significant in pairwise comparison ($p=0.09$). A variety of other nonneoplastic histopathologic findings in liver and other organs were considered to be incidental and commonly observed in CD-1 mice of this age with no toxicological importance.

TABLE 7. Body and Organ (Liver and Kidneys) Weights of Mice Fed Prodiamine for 99 Weeks[a,b]

Dietary Level (ppm)	Weights (mean g/g)		
	Body Weight	Liver	Kidneys
		Males	
0	43	2.554 2.63	0.8135 0.822
50	42	2.623 2.66	0.8801 0.884
500	40	2.762 2.69	0.8152 0.806
5000	41	3.057 3.01	0.8094 0.803
		Females	
0	33	1.811 1.81	0.4950 0.496
50	33	1.823 1.85	0.4856 0.491
500	33	1.750 1.76	0.4614* 0.463
5000	31	2.100** 2.04	0.4492** 0.439

[a]Data extracted from study No. 405897, Table 7, and Appendix 5.

[b]Where values have been adjusted for final body weight (as the covariate) during statistical analysis, the adjusted values are given first.

*Significantly different from control values ($p \leq 0.05$).

**Significantly different from control values ($p \leq 0.01$).

TABLE 8. Mean (± S.D.) Liver- and Kidney-to-Body Weight Ratios in Mice Fed Prodiamine for 99 Weeks[a]

Dietary Level (ppm)	Liver-to-Body Weight Ratio (g/100 g)	Kidney-to-Body Weight Ratio (g/100 g)
	Males	
0	0.0620 ± 0.0220	0.0194 ± 0.0030
50	0.0637 ± 0.0189	0.0214 ± 0.0044
500	0.0664 ± 0.0204	0.0203 ± 0.0046
5000	0.0740 ± 0.0195	0.0199 ± 0.0021
	Females	
0	0.0556 ± 0.0063	0.0153 ± 0.0021
50	0.0559 ± 0.0133	0.0148 ± 0.0019
500	0.0540 ± 0.0107	0.0142 ± 0.0020*
5000	0.0657 ± 0.0152**	0.0141 ± 0.0018*

[a]Organ-to-body weight ratios are calculated by reviewers and evaluated by ANOVA.

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

TABLE 9. Selected Gross Pathological Findings in Mice Fed Prodiamine for 99 Weeks[a]

Gross Pathological Findings	Dietary Level (ppm)[b]							
	Males				Females			
	0	50	500	5000	0	50	500	5000
No. of mice with subcutaneous mass/masses	1	4	3	10	6	4	3	6
No. of mice with yellow/brown stained fur	11	16	12	29	1	1	5	11
No. of mice with liver mass/masses	14	16	23	18	8	6	3	1

[a]Data extracted from study No. 405897, Table 8, and Appendix 5.

[b]Fifty-two mice/sex were examined at each dietary level.

TABLE 10. Selected Nonneoplastic Lesions in Mice Fed Prodiamine for 99 Weeks[a]

Organ/Diagnosis	Dietary Level (ppm)							
	Males				Females			
	0	50	500	5000	0	50	500	5000
Skin	(52)[b]	(32)	(26)	(52)	(52)	(14)	(18)	(52)
Scabs	0	0	2	3	0	0	0	2
Ulcerations	10	10	8	11	1	0	2	4
Focal dermal inflammation	2	0	3	4	1	2	2	3
Prominent dermal collagen	1	0	3	5*	0	0	0	0
Acanthosis (including focal)	5	3	3	3	0	0	2	1
Hyperkeratosis (including focal)	2	0	2	2	0	0	0	1
Liver	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Area of basophilic hepatocytes	0	3	3	2	0	1	1	0
Hepatocyte enlargement, generalized (minimal)	2	6	3	5	0	1	0	0
Granulomatous inflammation (minimal)	0	10	6	10	1	1	5	2

[a]Data extracted from study No. 405897, Table 10, and Appendix 5.

[b]The numbers in parentheses are the number of animals with tissues examined microscopically.

*Although there was a statistically significant positive trend (p = 0.006), it was not significant in pairwise comparison (p = 0.09) according to results from IARC analysis.

2) Neoplastic: Table 11 presents selected neoplastic lesions in mice fed prodiamine for 99 weeks. Males receiving 5000 ppm were noted with significantly increased incidences of subcutaneous fibrosarcomas ($p < 0.03$) when compared to control group; these incidences were considered to be dose related. Males receiving treatment were also observed with increased incidences of liver cell tumors but were not statistically significant. A variety of other neoplasms were observed in various organs and tissues; however, their incidence was low, they were not increased by dosing, and they were considered to be sporadic with no toxicologic importance.

D. STUDY AUTHORS' CONCLUSIONS:

The study authors concluded that toxicological responses were observed in animals at the highest feeding level of 5000 ppm. This was evidenced by persistent decrements in body weight gains in males and females and overall increased incidence of fighting injuries in males. Other dose-related effects at 5000 ppm included a change in leukocyte differential count with increased neutrophils and decreased lymphocytes in males and females; increased liver weights and decreased kidney weights were observed in females. An increased incidence of subcutaneous fibrosarcoma was observed in males receiving 5000 ppm.

It was concluded that the NOEL is 500 ppm and the LOEL is 5000 ppm for mice when prodiamine was fed for 99 weeks.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

Prodiamine was fed to CD-1 mice for 99 weeks at dietary levels of 0, 50, 500, and 5000 ppm to assess its tumorigenic dose responses.

The study protocol was complete and conduct of the study was adequate. Hematology evaluation was limited to differential leukocyte counts comprising neutrophils and lymphocytes. Data for ophthalmological examinations, clinical chemistry determinations and urinalyses were not reported; however, for the oncogenicity study, these data are not required by the guidelines. In-life parameters were completely reported; results obtained during the study were summarized and supported by individual animal data.

We agree with the study authors that there were no dose-related effects in males and females receiving 50 or 500 ppm on body

TABLE 11. Selected Neoplastic Lesions in Mice Fed Prodiamine for 99 Weeks[a]

Organ/Diagnosis	Dietary Level (ppm)							
	Males				Females			
	0	50	500	5000	0	50	500	5000
Total tumor-bearing mice	21	26	26	31	25	32	33	22
Subcutaneous mass:	(1)[b]	(4)	(3)	(10)	(5)	(4)	(4)	(6)
Fibrosarcoma	1	3	2	8*	1	-	-	1
Fibroma	-	2	-	-	-	-	-	-
Hemangioma	-	-	-	1	-	-	1	-
Liver	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Malignant liver cell tumor	3	8	6	7	-	-	-	-
Multiple malignant liver cell tumor	2	1	-	1	-	-	-	-
Benign liver cell tumor	5	7	10	6	4	1	1	-
Multiple benign liver cell tumor	1	1	3	3	-	1	-	-
Hemangioma	1	-	-	1	3	2	-	-
Multiple hemangiomata	-	-	1	-	1	-	-	-

[a]Data extracted from study No. 405897, Tables 9 and 11, and Appendix 5.

[b]The numbers in parentheses are the number of animals with tissues examined microscopically.

*Significantly different from control values ($p < 0.03$).

weight gains, life span, hematology evaluation, or on incidences of histopathological findings. In addition, there were no dose-related effects observed on food and water consumption during treatment.

We further agree with the study authors that dose-related toxic effects were observed in both males and females at the highest dose of 5000 ppm.

Slightly lower mean body weight gains in males and females receiving 5000 ppm are considered to be due to reduction in submissive behavior. Decreased terminal kidney weights in high-dose females of this study are of doubtful toxicological importance. Increased terminal liver weights in high-dose females were noted with a statistical significance; liver weights in high-males were also increased. Increased liver weights, though, did not induce the histomorphologic changes, we assess that these increases are of toxicological importance. The increased occurrence of yellow fur staining in high-dose males is due to coloration of urine by prodiamine. Fibrosarcoma was a contributory factor of increased mortality incidences in high-dose males (36/52). The changes in leukocyte differential count including increased neutrophils and decreased lymphocytes in males and females were within the normal reference range and we do not consider these changes dose related. Aggressive behavior in males inflicted continuing wounds that correlated with skin scabs, ulcerations, and dose-related increased incidence of "prominent dermal collagen" in high-dose males. The finding of "prominent dermal collagen" (control, 1/52; 50 ppm, 0/32; 500 ppm, 3/26; and 5000 ppm, 5/52) is a chronic sequel to dermal injury induced by fighting and the statistical significance of this lesion by the trend test was $p = 0.006$ and was not significant in pairwise comparison ($p = 0.09$).

The biological importance of the increased incidence of subcutaneous fibrosarcomas in males receiving 5000 ppm was considered equivocal by the study authors (control, 1/52; 50 ppm 3/52; 500 ppm, 2/52; and 5000 ppm, 8/52). The increased incidence of subcutaneous fibrosarcomas in high-dose males (8/52, 15.4%) exceeded the highest historical control incidence data (3/52, 5.8%) provided by the study laboratory. "Cage effect" played an important role; four mice were housed per cage and, in one cage, three mice were noted with fibrosarcoma. All high-dose males with fibrosarcomas were in cages where some fighting activity was evidenced. The first fibrosarcoma was palpated during week 70 and these tumors were prevalent in the last third of the study. Statistical significance of these tumors is only $p = 0.03$; other dietary levels of 50 and 500 ppm did not show any effect and the possible dose-related effect is limited to male mice. In the absence of these tumors

in females and that invasiveness or metastases were not determined, we do not consider these neoplasms to be of biological significance but believed to be a sequel to dose-related induction of aggressiveness.

Under the conditions and results of this study, the LOEL for systemic toxicity is determined to be 5000 ppm based primarily on mortality, reduced body weight gains and increased liver weights; the NOEL is 500 ppm when prodiamine is administered orally to CD-1 mice for 99 weeks.

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 11/16/89
Section I, Toxicology Branch - HFAS (TS-769C)
Secondard reviewer: Yiannakis M. Ioannou, Ph.D. 11/20/89
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DATA EVALUATION REPORT

Study Type: Acute Rat Inhalation Toxicity (81-3) **Tox. Chem. No.:** 727A

MRID No.: 42293-06
Accession No.: **EPA File Symbol:** 55947-UR

Test Material: Prodiamine 65 WDG (batch IL 9001; 65%)

Synonyms/CAS No.:

Study Number(s): VCL 111/86831

Sponsor: Sandoz Crop Protection Corporation, Des Plaines, IL 60018

Testing Facility: Huntington Research Center Ltd., Huntington, Cambridgeshire, England

Title of Report: Acute Inhalation Toxicity in Rats

Author(s): Colin Hardy et al.

Report Issued: November 7, 1986

Conclusions:

LC_{50} > 1.81 mg/L (both sexes)

Level tested: 1.81 mg/L (total dust of 1.81 mg/L by gravimetric analysis; total prodiamine of 1.51 mg/L by chemical analysis)

Toxicity Category: Undeterminable

Classification of Data: Supplementary

(Deficiencies: Particle size was not less than 1 um; Maximum attainable concentration was below 5 mg/L)

Procedures:

1. Five male and 5 female Albino (Wistar) rats (7-9 weeks old; weighing 122-166 g) were exposed to a test atmosphere containing dust from Prodiamine 65 WDG for 4 hours. A Wright Dust Generator (J. Scient. Instruments, 27(1) 1950, P12) was used to produce a test atmosphere containing the dust of the test material. Chamber atmosphere contained an average total dust of 1.81 mg/L by gravimetric analysis and an average total prodiamine of 1.53 mg/L by chemical analysis.

2. All animals were observed for adverse effects daily following this exposure. The body weights, and food and water consumption of each animal were recorded daily. A gross pathological examination of the major tissues and organs was also conducted on all animals.

Results:

1. All rats survived during the 14-day observation period.

   LC50 > 1.81 mg/L (both sexes)

   Level tested: 1.81 mg/L (highest attainable concentration with the equipment described in this report)

2. Pharmacotoxic Signs: Closing or partial closing of the eyes, slow, shallow respiratory pattern, pilo-erection and brown staining around the snout and jaws were observed in the prodiamine 65 WDG-treated rats.

3. The weight gain and the food and water consumption for exposed rats were similar to that of the corresponding control rats.

4. No exposure related histologic changes were observed in the lungs, liver or kidneys of exposed animals. The only finding related to exposure was yellow staining of the tail in all exposed animals.

5. Based on the chemical analysis, approximately 85% of the total dust collected was prodiamine (Total dust in air, 1.81 mg/L; Prodiamine in air, 1.53 mg/L). The concentration of Prodiamine 65 WDG was taken as the result of the gravimetric analysis (1.81 mg/L). The results obtained from the determination of particle size distribution indicated that 45% of the total dust and 46% of the prodiamine was 5.5 um or less in aerodynamic diameter.

Classification of Data: Supplementary

(Deficiencies: Particle size was not less than 1 um - at least 25% of the particles should be 1 um or less; The maximum attainable concentration used was below 5 mg/L - See Comments on Standard Evaluation Procedure for Inhalation Toxicity Testing, Toxicology Branch Memo 4/18/89, Stanley B. Gross, Ph.D.)