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

10 JUN 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review Document on Prodiamine

FROM: Gary J. Burin, Ph.D., D.A.B.T.
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: JoAnn Miller
Product Manager #23
Registration Division (TS-767C)

The Health Effects Division Peer Review Committee (PRC) met on April 17, 1991 to discuss and evaluate the weight-of-the-evidence on Prodiamine with particular reference to its carcinogenic potential. This was the first evaluation of Prodiamine by the PRC. The PRC concluded that Prodiamine should be classified as Group C and that the RfD approach should be used for risk assessment.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp
William L. Burnam
Reto Engler
Karl Baetcke
Marcia Van Gemert
Esther Rinde
Marion Copley
Hugh Pettigrew

Robert Beliles

Julie Du

Yin-Tak Woo

Kerry Dearfield

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Bernice Fisher

John Chen

Yiannakis Ioannou

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Richard Hill

George Ghali

William Sette

John Quest

Jean Parker

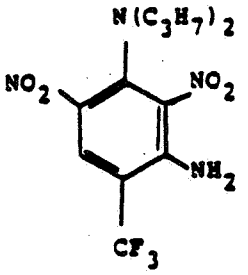
4. Other Attendees:

Gary Burin (HED), Bernice Fisher (HED), Carmine Pellosie (OPTS)

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. John Chen; Tables and statistical analysis by Ms. Bernice Fisher. The material reviewed is attached to the file copy of this report.

Structure:



C. Background Information:

Prodiamine is the common name for 2,4-dinitro-N^3,N^3-dipropyl-6-(trifluoromethyl)-1,3,-benzenediamine as recognized by the American National Standards Institute. The Chemical Abstracts Service Registry Number (CAS) is 29091-21-2. The Toxicology Chemical Number (Caswell) is 727A.

Prodiamine is currently registered for use on turf grasses only to control germinating weeds (nonfood use herbicide only). Previous applications of this product (Prodiamine 65 WDG) for all food uses (such as almonds, walnuts, cotton, soybeans and grapes) were withdrawn by the Sandoz Plant Protection Corporation.

D. Evaluation of Carcinogenicity Data for:

1. Two Year Rat Carcinogenicity Study

Reference: Powell, A.J., H.Peters, C. Copinath, S. Ames, A. Gibson, and L.D. Crook, 1989. Two Year Oncogenicity Feeding Study with Prodiamine in Rats. Study No. VCL 74/871495. Huntingdon Research Center, Ltd., Huntingdon, Cambridgeshire, England. MRID No. 409859-01-09.

Prodiamine was administered in the diet to groups of Sprague-Dawley male and female rats for 108 weeks at 50, 200, 800 or 3200 ppm; 0 ppm for concurrent controls. Prodiamine was associated with positive dose-trends in thyroid follicular cell adenomas and in combined follicular cell tumors (adenomas and carcinomas) in both males and females. A significant increase in combined (adenoma and carcinoma) follicular cell tumors at 3200 ppm (approximately 160 mg/kg/day) was found in both males and females when compared with concurrent controls. The incidences of thyroid follicular cell tumors in males are given in Table 1 and for females in Table 2. Slight increases in hyperplasia of the thyroid and pituitary were observed at the 3200 ppm dose level in both sexes. Neither thyroid hormones (T_3 and T_4) nor thyroid stimulating hormone (TSH) were measured.

Slight increases in the incidences of adenocarcinoma of the mammary gland and the islet cell adenoma of the pancreas were observed in females. The incidence of mammary gland tumor (adenoma, adenocarcinoma, fibroma and fibroadenoma, combined) was high in each group of females, including the control, with the greatest incidence at the 50 ppm dose level (25/70, 38/55, 37/55,

Table 1. Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Tests and Fisher's Exact Test Results in Male Rats

tumor	0	50	200	800	3200
adenomas p=	1/50 0.011*	4/49 0.175	0/49 0.505	3/49 0.301	6/49 0.053
carcinomas p=	1/50 0.165	0/49 0.505	1/49 0.747	3/49 0.301	2/49 0.492
both p=	2/50 0.007**	4/49 0.329	1/49 0.508	6/49 0.128	8/49 0.043*

[+] Number of tumor-bearing animals/number of animals examined, excluding those that died or were sacrificed before 53 weeks
* p<0.05
** p<0.01

Note: Significance of trend denoted at control. Significance of pair-wise comparison denoted at dose level.

Table 2. Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results in Female Rats

tumor	0 ppm	50	200	800	3200
adenomas p=	0/49 0.000**	2/47 0.237	0/50 1.000	0/48 1.000	6/48 0.012*
carcinomas p=	0/49 0.456	0/47 1.000	0/50 1.000	2/48 0.242	0/48 1.000
both p=	0/49 0.000**	2/47 0.237	0/50 1.000	2/48 0.242	6/48 0.012*

[+] Number of tumor-bearing animals/number of animals examined, excluding those that died or were sacrificed before 53 weeks
* p<0.05
** p<0.01

Note: Significance of trend denoted at control. Significance of pair-wise comparison denoted at the dose level.

35/56 and 38/68 for the 0, 50, 200, 800 and 3200 ppm groups, respectively). The increase in mammary gland tumors noted at all dose levels in females was not considered to be compound-related based on the absence of a dose-response relationship and comparison to the historical control data for this tumor type. The dose-trend for islet cell adenomas of the pancreas was significant in females only ($p<0.01$)(2/49, 2/48, 1/49, 2/48 and 7/48 for the 0, 50, 200, 800 and 3200 ppm groups, respectively). The dose-trend was not significant when adenomas and carcinomas of the pancreatic islet cell were combined.

The systemic NOEL was 200 ppm and the systemic LOEL was 800 ppm based on increased liver weight in females and clinical chemistry changes. Significantly reduced body weight gain was found in high dose male rats during the first 78 weeks of the study and in high dose female rats between weeks 53 and 78. The high dose level was considered to be adequate to assess the carcinogenic potential of prodiamine based upon the aforementioned body weight changes in both sexes (a 14.3% decrement in high dose males and 8.4% in high dose females).

2. Two year mouse carcinogenicity study.

Reference: Powell, L.A.J. et al. Oncogenicity Feeding Study with Prodiamine in Mice. Study VCL 37/871188. Huntingdon Research Center, Ltd., Huntingdon, Cambridgeshire, England. MRID No. 405897-02.

Four groups of CD-1 mice (52 males and 52 females per dose group) were fed 0, 50, 500 and 5000 ppm of prodiamine in the diet for 99 weeks. The results of this study showed a significant increase ($p<0.03$) in the incidence of subcutaneous fibrosarcomas in the high dose males when compared to concurrent controls (the incidences were 1/52, 3/52/ 2/52 and 8/52 for the 0, 50, 500 and 5000 ppm groups, respectively). A dose-related increase in the incidence of "prominent dermal collagen" was also noted in high dose males. Animals were gang-caged in this study and both of the above findings were considered to be secondary to fighting in cages. No other neoplastic or nonneoplastic histological findings were considered to be related to treatment. The LOEL for systemic toxicity was 5000 ppm and the NOEL was 500 ppm based on increased mortality, reduced body weight gain and increased liver weights. Cumulative body weight gain in males receiving 5000 ppm was 31.3% less than control males and cumulative mortality was 60% in the high dose animals (males and females combined) compared

to 39% in control animals. The high dose level (5000 ppm) was considered to be an adequate dose for assessing the carcinogenic potential of prodiamine based upon these effects on mortality and body weight gain.

E. Additional Toxicology Data on:

1. Mutagenicity

Prodiamine was tested for potential mutagenic activity in a number of tests which were considered acceptable e.g. the Salmonella assay, the gene mutation assay in cultured mouse lymphoma cells, an in vitro cytogenetic assay in cultured chinese hamster ovary cells, and the unscheduled DNA synthesis test in primary rat hepatocytes. These tests were generally negative, although there was a suggestive effect in Salmonella strain TA 1538 without activiation (but not reproducible) and in mouse lymphoma cells without activation (at levels inducing toxicity).

2. Metabolism

A metabolism study was conducted in Sprague-Dawley rats with either a single oral dose of 10 or 400 mg/kg of ^{14}C-prodiamine or a single oral dose of 10 mg/kg ^{14}C-prodiamine after 14 days of administration of 10 mg unlabelled prodiamine/kg/day (Sandoz Crop Protection Corp. No. 480425-5). Animals given either a single or repeated dose of 10 mg/kg were found to excrete 9 to 15.5% of the radiolabel in the first 7 hours after compound administration; in contrast, animals receiving 400 mg/kg eliminated <1%. Within 24 hours after dosing, all but the high-dose female rats had eliminated approximately 70% of the radiolabel in the urine and feces (high dose females excreted 38% of the dose). Radioactivity was found in the following tissues (in descending order) were liver, fat, kidney, blood, lung, spleen, bone, gonads, heart, muscle, and brain. Prodiamine appears to be metabolized rapidly by de-alkylation reactions to N,N-didespropyl metabolites. Cyclization also occurs 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 hydroxybenzimidazoles.

3. Developmental and Reproductive Effects

NOELs of 100 and 300 mg/kg/day for developmental and

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maternal toxicity, respectively, were established in a rat teratology study based on LOELs of 300 mg/kg/day for ocular anomalies in fetuses and 1000 mg/kg/day for maternal toxicity (Study No. Wil-15150). In a rabbit teratology study, the NOEL for maternal toxicity was 100 mg/kg/day based on depressed body weight gain at 300 mg/kg/day. There was no evidence of developmental toxicity at the highest dose tested (500 mg/kg/day) (Study No. Wil-15153).

A two generation reproduction study in rats found a NOEL of 200 ppm (approximately 10 mg/kg/day) based on reduced pup weight and increased relative liver weight at 2000 ppm (Study No. VCL 73/871075).

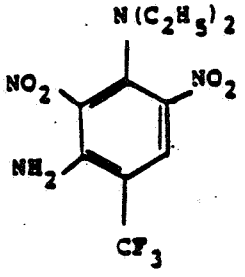
4. Structure-Activity Considerations

Diethamine and trifluralin were the only two pesticides found to resemble prodiamine. Acceptable toxicity studies (other than acute toxicity) were not available for diethamine. Trifluralin was previously reviewed by the Peer Review Committee (April 4, 1986, memo from R.B. Jaeger and R. Engler). It was recommended that it be classified as a Group C carcinogen on the basis of malignant or combined malignant and benign tumors of the renal pelvis in high dose male rats and benign tumors of the urinary bladder in high dose female rats. An increase in follicular cell adenoma and combined follicular cell adenomas/carcinomas of the thyroid also was noted in male rats receiving the highest dose of trifluralin. Submitted mutagenicity data for trifluralin is generally negative (Salmonella, mouse lymphoma, dominant lethal, sister chromatid exchange).

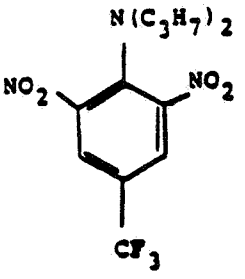
Prodiamine is metabolized to several benzimidazole-containing metabolites. 6-Nitrobenzimidazole was found to be positive for carcinogenicity in both sexes of mice (hepatic tumors) and negative in the rat in NTP testing.

Structures of Prodiamine, Diethamine and Trifluralin

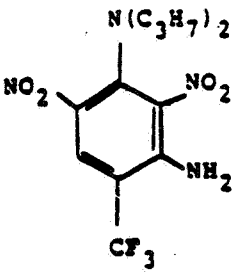
Diethamine



Trifluralin



Prodiamine



F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on prodiamine to be of importance in a weight-of-the-evidence determination of carcinogenic potential. Two studies, one in the rat and one in mouse, were considered to be adequate to assess the carcinogenic potential of prodiamine. In the rat study a compound-related increase in thyroid follicular cell neoplasia was observed at the high dose level in both sexes. The dose-trend was also found to be significant. In addition, a statistically significant positive trend in the incidence of pancreatic adenomas was observed although the incidence of carcinomas and adenomas (combined) was not significant. An increase in fibrosarcomas was found in the mouse carcinogenicity study. However, it was noted that dermal irritation resulting from fighting was also found in this group.

A close structural resemblance to trifluralin (classified as Group C for carcinogenicity) and biotransformation to benzimidazole metabolites, also of carcinogenic concern, was noted. The available genotoxicity testing for prodiamine was generally negative.

G. Classification of Carcinogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered. The Committee recommended that prodiamine should be classified as a Group C carcinogen based upon the evidence noted above. For the purpose of risk characterization, the Committee recommended that the Reference Dose approach (RfD) should be used for quantification of human risk. The recommendation to use the RFD approach was based upon several factors including the absence of genotoxicity, the nature of the response (benign thyroid follicular cell tumors), and the lack of a clear neoplastic response at sites other than the thyroid.

Concern was expressed regarding the metabolism of prodiamine to benzimidazole, the dose-trend for islet cell adenomas of the pancreas in females and the observation of fibrosarcomas in the mouse carcinogenicity study. Although the Committee noted the increase in tumors of the mammary gland in treated groups, the increase was not considered to be related to treatment based upon comparison with historical control data and the absence of a dose-response relationship.

H. PRC Recommendations

Acute and subchronic neurotoxicity testing is required to investigate potential behavioral effects suggested by the mouse carcinogenicity study.

Because perturbations of the hypothalamus/ pituitary/ thyroid axis often accompany thyroid follicular cell neoplasia[1], subchronic studies which measure alterations in TSH, T_3 and T_4 in prodiamine-treated rats would be useful to assess this effect.

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[1] See Paynter, O.E., Burin, G.J., Jaeger, R.B. and Gregorio, C.A. (1988). Goitrogens and thyroid follicular cell neoplasia: evidence for a threshold process. Regulatory Pharmacology and Toxicology 8:102-119

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1 APR 1991

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SUBSTANCES

Subject: Prodiamine , Qualitative Risk Assessment -
2-Year Sprague-Dawley Rat Dietary Study
Caswell no.727A

From: Bernice Fisher, Biostatistician Bernice Fisher 3/29/91
Science Support & Special Review Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

To: John H.S. Chen, Ph.D., Microbiologist
Review Section I
Herbicide/Fungicide/Antimicrobial Support Branch
Health Effects Division (H7509C)

Thru: for Esther Rinde, Ph.D., Acting Section Head Kyle M. Pettigrew 4/1/91
Science Support & Special Review Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

The qualitative risk assessment of prodiamine was based upon a two-year dietary study of Sprague-Dawley rats.

The attached tables present in tabular form, the results of the statistical analysis of data from the dietary study of Sprague-DAwley rats (MRID no. 409859-01-09, study no. HRC Report No. VCL 74/871495).

The sponsor of the study was Sandoz Crop Protection Corporation and the testing facility that they used was Huntingdon Research Centre, Ltd. The study was completed and issued in January, 1989.

Table 1. Prodiamine - Sprague-Dawley Rat Study, Male Mortality Rates[+] and Cox or Generalized K/W Test Results[++]

	Weeks				
Dose(ppm)	1-52	52[a]	53-78	79-107[b]	Total
0	0/70	20/70	9/50	22/41	31/50(62)*
50	1/70	20/69	3/49	18/46	22/50(44)
200	1/70	20/69	4/49	25/45	30/50(60)
800	2/70	19/68	8/49	22/41	32/51(63)
3200	1/70	20/69	13/49	19/36	33/50(66)

[+] Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[++] Thomas, D.G., Breslow, N. and Gart, J.J. - Trend and Homogeneity Analysis of Proportions and Life Table Data, version 2.0.

( ) percent

a Interim Sacrifice at week 52.
b Final Sacrifice at week 108.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
If * then $p<.05$ and if ** then $p<.01$.

Table 2. Prodiamine - Sprague-Dawley Rat Study, Female Mortality Rates[+] and Cox or Generalized K/W Test Results[++]

	Weeks					
Dose(ppm)	1-26	27-52	52[a]	53-78	79-107[b]	Total
0	0/70	1/70	20/69	8/49	22/41	31/50(62)
50	1/70	1/69	19/68	6/49	20/43	28/51(55)
200	1/70	1/69	18/68	4/50	24/46	30/52(58)
800	1/70	1/69	20/68	4/48	15/44	21/50(42)
3200	1/70	1/69	20/68	5/48	22/43	29/50(58)

[+] Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[++] Thomas, D.G., Breslow, N. and Gart, J.J. - Trend and Homogeneity Analysis of Proportions and Life Table Data, version 2.0.

( ) percent

a Interim Sacrifice at week 52.
b Final Sacrifice at week 108.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
If * then $p<.05$ and if ** then $p<.01$.

Table 3. Prodiamine - Sprague-Dawley Male Rats, Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
Tumors	0	50	200	800	3200
Carcinomas	1/50	0/49	1/49	3/49	2[a]/49
(%)	(2)	(0)	(2)	(6)	(2)
p=	0.165	0.505(n)	0.747	0.301	0.492
Adenomas	1/50	4/49	0/49	3/49	6[b]/49
(%)	(2)	(8)	(0)	(6)	(12)
p=	0.011*	0.175	0.505(n)	0.301	0.053
Both	2/50	4/49	1/49	6/49	8/49
(%)	(4)	(8)	(2)	(12)	(16)
p=	0.007**	0.329	0.508(n)	0.128	0.043*

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before 53 weeks.

(n) negative change from control

[a] First carcinoma observed at week 74, dose 3200 ppm.
[b] First adenoma observed at week 72, dose 3200 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
If * then $p<.05$ and if ** then $p<.01$.

Table 4. Prodiamine - Sprague-Dawley Female Rats, Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
Tumors	0	50	200	800	3200
Carcinomas (%)	0/49 (0)	0/47 (0)	0/50 (0)	2[a]/48 (4)	0/48 (0)
p=	0.456	1.000	1.000	0.242	1.000
Adenomas (%)	0/49 (0)	2/47 (4)	0/50 (0)	0/48 (0)	6[b]/48 (12)
p=	0.000**	0.237	1.000	1.000	0.012*
Both (%)	0/49 (0)	2/47 (4)	0/50 (0)	2/48 (4)	6/48 (12)
p=	0.000**	0.237	1.000	0.242	0.012*

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before 53 weeks.

[a] First carcinoma observed at week 89, dose 800 ppm.
[b] First adenoma observed at week 90, dose 3200 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
If * then $p<.05$ and if ** then $p<.01$.

Table 5. Prodiamine - Sprague-Dawley Female Rats, Mammary Gland Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
Tumor	0	50	200	800	3200
Adenocarcinoma	6/70	10/55	14/55	14[a]/56	14/68
(%)	(9)	(18)	(25)	(25)	(21)
p=	0.225	0.093	0.010*	0.012*	0.038*
Adenoma, Fibroadenoma & Fibroma (%)	19[b]/70 (27)	28/55 (51)	23/55 (42)	21/56 (38)	24/68 (35)
p=	0.326	0.006**	0.063	0.147	0.198
All Tumors	25/70	38/55	37/55	35/56	38/68
(%)	(36)	(69)	(67)	(62)	(56)
p=	0.423	0.000**	0.000**	0.002**	0.013*

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of first tumor.

[a] First Adenocarcinoma observed at week 52, dose 800 ppm.
[b] First benign tumor observed at week 48, dose 0 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Table 6. Prodiamine - Sprague-Dawley Female Rats, Pancreatic Islet Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
Tumors	0	50	200	800	3200
Carcinoma	3/49	3/48	3[a]/49	0/48	1/48
(%)	(6)	(6)	(6)	(0)	(2)
p=	0.114	0.651	0.661	0.125(n)	0.316(n)
Adenomoma	2/49	2[b]/48	1/49	2/48	7/48
(%)	(4)	(4)	(2)	(4)	(15)
p=	0.002**	0.684	0.500(n)	0.684	0.075
Both	5/49	5/48	4/49	2/48	8/48
(%)	(10)	(10)	(8)	(4)	(17)
p=	0.075	0.617	0.500(n)	0.226(n)	0.263

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before 53 weeks.

(n) negative change from control.

[a] First carcinoma observed at week 93, dose 200 ppm.
[b] First adenoma observed at week 79, dose 50 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ if ** then $p<.01$.

I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfDo)

Chemical -- Prodiamine (Rydex)
CASRN -- 29091-21-2
CASWELL -- 727A
On-line: 10/30/90

I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Increased liver weight and minor biochemical disturbances 2-Year Rat Feeding/ Oncogenicity Study Sandoz Crop Protection Corp., 1989	NOEL: 200 ppm (Male: 7.2 mg/kg/day; Female: 9.1 mg/kg/day) LEL: 800 ppm (Male: 29.4 mg/kg/day; Female: 37.0 mg/kg/day)	300	1	2E-2 mg/kg/day

*Conversion Factors: Actual dose tested

I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Powell, A.J.; Peters, H.; Gopinath, S; et al.
Prodiamine Potential Tumorigenic and Toxic Effects of Prolonged Dietary Administration to Rats
Huntingdon Research Centre, Ltd.
Sandoz Crop Protection Corporation
Study No. VCL 74/871495; January 29, 1989
MRID No. 40985901

Prodiamine was fed to male and female rats (50/sex/dose with a satellite group of 20/sex/dose) for 25 months at dietary levels of 0, 50, 200, 800, and 3200 ppm (Male: 0, 1.8, 7.2, 29.4, and 115.2 mg/kg/day; Female: 0, 2.3, 9.1, 37.0, and 151 mg/kg/day). 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 ($p<0.01$) 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.

Based on increased liver weights and minor biochemical disturbances, the LEL for systemic toxicity is 800 ppm (Male: 29.4 mg/kg/day; Female: 37.0 mg/kg/day). The NOEL for systemic toxicity is 200 ppm (Male: 7.2 mg/kg/day; Female: 9.1 mg/kg/day).

___I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF = 300. An uncertainty factor of 100 was used to account for the inter- and intraspecies differences. An additional UF of 3 was used to account for the lack of a chronic dog feeding study.

MF = 1.

___I.A.4. ADDITIONAL COMMENTS (ORAL RfD)

Data Considered for Establishing the RfD

1) 2-Year Feeding/Oncogenicity - rat: Principal study -- see previous discussion; core grade guideline (Sandoz Crop Protection Corp., 1989)

2) 2-Generation Reproduction - rat: Dietary levels tested: 0, 50, 200, and 2000 ppm (0, 2.5, 10, and 100 mg/kg/day); Groups of Crl:COBS CD(SD)BR rats (24/sex/dose) were administered prodiamine in the diet over two generations. At 2000 ppm a reduction in body weights and an increase in relative liver weights for males and females of all generations was observed. Since liver abnormalities were not observed in high-dose males and females of the F0 and F1b generations, the findings are suggestive of adaptive changes rather than overt toxicological effects. Therefore based on the above effects, the NOEL and LEL for parental toxicity are 200 and 2000 ppm (10 and 100 mg/kg/day), respectively. Prodiamine had no effect on mating or pregnancy rates. Litter size, sex ratios, and development of pups were also unaffected. 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. Therefore based on the above effects, the NOEL and LEL for reproductive toxicity are 200 and 2000 ppm (10 and 100 mg/kg/day), respectively.; core grade guideline (Sandoz Crop Protection Corp., 1988a)

3) Developmental toxicity - rat: Dose levels tested: 0, 100, 300, and 1000 mg/kg/day; Groups of pregnant CDL:CD (SB) BR rats (25/dose) were administered prodiamine orally by gavage, once daily for 10 consecutive days from gestation day 6 through 15. Control rats received corn oil on a comparable regimen. At the high-dose a decrease in body weight gain was observed. Although the depressed body weight was low (4%), this group's difference in gain reached the 0.01 level of significance and a depressed gain was consistently seen after treatment began. Therefore based on depressed body weight gain, the LEL for maternal toxicity is 1000 mg/kg/day. The NOEL for maternal toxicity is 300 mg/kg/day. Evidence of ocular anomalies was observed at all dose levels. However, the incidences of ocular malformations at the 100 mg/kg/day level were found to be with the range for the historical control data. Therefore based on the increased incidence of omphalocele, the LEL for developmental toxicity is 300 mg/kg/day. The NOEL for developmental toxicity is 100 mg/kg/day.; core grade guideline (Velsicol Chemical Corp., 1985a)

4) Developmental toxicity - rabbit: Dose levels tested: 0, 100, 300, and 500 mg/kg/day; Groups of pregnant New Zealand White rabbits (18/dose) were administered prodiamine by gastric intubation once daily for 13 consecutive days (6 through 18 of gestation). Maternal weight loss was statistically significant at both the 300 and 500 mg/kg/day levels during the dosing period, but not at the 100 mg/kg/day level. Based on decreased body weight gain, the LEL for maternal toxicity is 300 mg/kg/day. The NOEL for maternal toxicity is 100 mg/kg/day. No evidence of developmental toxicity was noted at any dose tested. Therefore, the NOEL for developmental toxicity is equal to or greater than 500 mg/kg/day, the highest dose tested.; core grade guideline (Vesicol Chemical Corp., 1985b)

Other Data Reviewed:

1) 2-Year Feeding/Oncogenicity - mouse: Dietary levels tested: 0, 50, 500, and 5000 ppm (Male: 0, 6.2, 59.4, and 606.6 mg/kg/day; Female: 0, 6.8, 64.6, and 646.2 mg/kg/day); Prodiamine was administered to CD-1 mice (52/sex/dose) in the diet for 99 weeks. 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 incidences 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 and 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. Based on mortality, reduced body weight gains, and increased liver weights the LEL for systemic toxicity is 5000 ppm (Male: 606.6 mg/kg/day; Female: 646.2 mg/kg/day). The NOEL for systemic toxicity is 500 ppm (Male: 59.4 mg/kg/day; Female: 64.6 mg/kg/day).; core grade guideline (Sandoz Crop Protection Corp., 1988b)

ata Gap(s): Chronic Dog Feeding Study

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study: High
Data Base: Medium
RfD: Medium

The critical study is of good quality and is given a high confidence rating. Additional studies are also of good quality, however, a chronic dog feeding study is lacking. Therefore the data base is given a medium confidence rating. Medium confidence in the RfD follows.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Registration Files

Agency RfD Work Group Review: ../../..

Verification Date: ../../..

___I.A.7. EPA CONTACTS (ORAL RfD)

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_VI. REFERENCES

Sandoz Crop Protection Corp., 1989. MRID No. 40985901
Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

Sandoz Crop Protection Corp., 1988a. MRID No. 40593421, 40593422, 41371901
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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

REVIEWED BY:

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Date: February 28, 1991

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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.

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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.

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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.

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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 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)
Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation: thrombotest (TT)

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		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†
	Creatine phosphokinase		Triglycerides
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

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
X	Glucose (qualitative)†		(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:

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†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.

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

APPROVED BY:

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

Signature: Roman J Prieto for

Date: 9-12-89

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

REVIEWED BY:

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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 ($p \leq 0.01$).

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

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**	330 ± 266.4**
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

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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.

HUNTINGDON RESEARCH CENTRE Ltd.,
HUNTINGDON, CAMBS, PE18 6ES, ENGLAND.
FACSIMILE NO: (0480) 890693. Group 3.

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ATTENTION OF: **Mildred Root** FROM: **John Colley/Lindsey Powell**

COMPANY: **Sandoz Crop Protection Corp.** DATE: **23 January 1991**

FAX NUMBER: **708 - 390 - 3944**

MESSAGE:

Re: Prodiamine: Toxicity/Carcinogenicity Study in Rats (Report No. VCL74/871495)

In response to your request of 9/10 January please find attached a table of historical control data for various tumour types (Table 1) and details of the studies used (Table 2).

We hope this data is sufficient for your requirements and please do not hesitate to contact us if you require any further information.

With kind regards

John Colley
Deputy Head
Department of Rodent Toxicology

Lindsey Powell
Study Director
Division of Toxicology

HRC FAX CONTROL
OUTGOING
23 JAN 1991

LOG	SHEETS	1	2
	3		X
		3	4
		X	X

FOR HRC USE ONLY

(15)

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TABLE 1

Background Tumour Incidence

Study Code	83A		83B		83C		84A		84B		84C		85A		85B		85C		85D		86A		86B		86C		86D		VCL 74	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Thyroids examined	50	50	50	50	54	55	100	100	50	50	49	50	48	50	50	50	54	55	53	55	60	60	55	55	55	55	100	99	50	50
Nos. of rats with :-																														
Follicular adenoma	4	1	4	2	7	1	4	2	4	3	6	0	0	0	4	1	3	0	0	3	1	0	2	1	4	0	8	2	1	0
Follicular adenocarcinoma	0	2	1	0	3	0	3	2	4	1	1	1	2	1	0	0	1	0	0	2	1	0	1	0	2	0	1	1	1	0
Pancreas examined	49	50	50	50	54	55	100	100	50	50	49	50	50	48	49	49	51	55	54	55	60	60	55	55	55	54	100	99	50	50
Nos. of rats with :-																														
Islet cell adenoma	7	3	7	4	7	11	12	4	5	2	14	6	9	2	4	4	11	4	15	2	6	3	4	2	4	0	24	4	4	2
Islet cell carcinoma	2	1	1	1	4	2	10	3	1	0	1	2	3	0	0	0	1	1	1	2	3	1	2	1	0	2	0	0	4	3
Nos. of rats with :-																														
Mammary adenoma	1	1	0	3	0	0	0	24	0	1	0	1	0	1	0	2	0	0	1	0	0	3	0	1	1	1	1	3	0	0
Mammary fibroadenoma	3	29	3	35	1	32	3	47	1	22	2	32	2	34	2	21	0	22	1	22	0	19	2	16	0	24	0	45	0	23
Mammary fibroma	1	0	1	1	0	0	1	7	0	0	0	3	0	2	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0
Mammary adenocarcinoma	0	7	0	8	0	9	0	26	0	6	0	14	0	14	0	1	0	12	0	11	0	20	1	9	1	8	0	17	1	6
Nos. of rats examined	50	50	50	50	55	55	100	100	50	50	50	50	50	50	50	50	55	55	55	55	60	60	55	55	55	55	100	100	50	50

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TABLE 2

Details of Background Studies

Study Code	Started	Duration	Diet		Diet/ Gavage
83A	21.07.83	104 Weeks	Labsure	Diet-2	Dietary
83B	29.11.83	"	"	"	"
83C	06.12.83	"	"	Diet-1	Oral Gavage
84A	20.02.84	"	"	Diet-2	Dietary
84B	05.07.84	"		Diet-2	"
84C	17.10.84	"		Diet-2	"
85A	03.12.85	"		Diet-2	Dietary
85B	05.02.85	"		Diet-2	Dietary
85C	30.09.85	112 Weeks	SDS	Diet-1	Oral Gavage
85D	"	"	"	"	"
86A	01.09.86	104 Weeks	SDS	Diet-1	Inhalation
86B	27.02.86	"	"	"	Oral Gavage
86C	"	"	"	"	"
86D	28.07.86	"	"	"	Inhalation
VCL/74	16.09.85	108 Weeks	Lab	Diet-2	Dietary

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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(40)a

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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.

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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.

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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 ($p < 0.01$).

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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 ($10^3/mm^3$) Study Week 52	78	99	Lymphocytes ($10^3/mm^3$) Study Week 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 ($p < 0.01$).

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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+
	X Seminal vesicle	Harderian glands
Respiratory	X Ovaries	
X Trachea+	X Uterus	
X Lung+		
		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.

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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 ($p \leq 0.01$).

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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 ($p < 0.01$).

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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.

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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 [redacted]thalmological 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 ($p < 0.03$).

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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.

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Reviewed by: John H.S. Chen, D.V.M. John H S Chen 12/5/89
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. [illegible] 12/6/89
Section I, Toxicology Branch II (H7509C)

Review of the Registrant's Response to the Previous Review Comments Concerning the Rat Teratology Study with Prodiamine (Toxicology Branch Memorandum of January 12, 1987, Winnie Teeters)

Registrant's Response: "EPA has concluded that a NOEL for developmental toxicity has not been established based on the incidence of ocular abnormalities observed at the lowest dose tested, 100 mg/kg. The Agency's conclusions were primarily (if not solely) based on historical control data provided by the testing laboratory. While we believe such data are useful, it should not preclude statistical or other evidence which does not support this conclusion." "The Agency also determined that microphthalmia and/or anophthalmia exceeded overall historical control incidence for these ocular abnormalities, thus demonstrating compound relationship. As with the finding of omphalocele, there was no dose-response relationship. In this case, however, these observations were noted at the low and high dose levels, but not the mid-dose. As we previously indicated, these malformations are reported to occur in this strain as congenital anomaly which is inherited as an autosomal recessive trait (Appendix 2 attached). Furthermore, the incidence of these malformations occurring spontaneously is quite variable and recent historical control data clearly show these lesions to be increasing in occurrence (Appendix 1 attached)."

"Finally, malformations reported in this study would readily be demonstrated in a proper reproductive effects study if conducted at adequately high levels. The in-life portion of a two-generation reproduction study in rats with prodiamine will be completed shortly. This study is being conducted at levels up to 2000 ppm prodiamine in the diet (roughly equivalent to 200 mg/kg). No ocular abnormalities attributable to prodiamine and no evidence of omphalocele, microphthalmia or anophthalmia have been observed in any of the treatment or control groups (Appendix 3 attached). This observation further leads us to believe the abnormalities are random and laboratory and/or population specific." "A NOEL for developmental toxicity has been demonstrated for this study because (a) No dose-response for any reported malformations was observed, (b) Microphthalmia and anophthalmia occur in this strain as congenital anomaly, (c) Recent historical control data show these malformations are variable and spontaneously increasing in occurrence and (d) No evidence of these malformations has been observed in a rat reproduction study conducted at level higher than presumed effect levels in the teratology study."

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Reviewer's Comments: The submitted addendum with the most recent historical control data for Charles River COBS CD rats (Appendix 1) and a copy of the manuscript by Kinney et al. concerning ocular defects in the Charles River CD rats (Appendix 2) provide adequate information for the spontaneous occurrences of microphthalmia and amophthalmia in the Charles River COBS CD rats. The incidences of ocular malformations at the 100 mg/kg level were found within the range for the historical control data recently submitted. The Registrant's explanations for the unusual incidences of such ocular abnormalities found at the 100 mg/kg dose group are considered to be reasonable. Since these incidences of omphalocele and ocular malformations cannot be confirmed in a rat reproduction study (Hungtington Research Center No. VCL 73/871075, February 22, 1988; Appendix 3 attached) at levels up to 2000 ppm prodiamine in the diet (equivalent to 100 mg/kg), we agree that the NOEL for developmental toxicity should be 100 mg/kg.

Recommendation: Registrant's response to the deficiencies 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 (based on increased incidences of omphalocele)

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005656

Reviewed by: Winnie Teeters
Section V , Tox. Branch (TS-769C)
Secondary reviewer: Laurence D. Chitlik, D.A.B.T. Chitlik for LDC 4/24/86
Section V , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

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

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

TEST MATERIAL: Prodiamine Technical

SYNONYMS: (Rydex)

STUDY NUMBER(S): WIL-15150

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: WIL Research Labs., Inc.

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

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

REPORT ISSUED: 11-11-85

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

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

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

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additionally 1 hour after each dose.

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

2. Body weight:

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

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

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

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

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

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

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

4. Fetal Morphological Data:

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

TABLE 4

PROJECT NO.:MIL-15150 — A TERATOLOGY STUDY IN RATS WITH PRODIAMINE TECHNICAL — PAGE 1

SPONSOR:VELSICOL CHEMICAL CORP. — MEAN BODY WEIGHT CHANGES (GRAMS) DURING GESTATION

DOSE GROUP:	0 MG/KG/DAY	100 MG/KG/DAY	300 MG/KG/DAY	1000 MG/KG/DAY
DAY 0- 6 MEAN	24.	24.	27.	25.
S.D./N	9./20	9./21	7./20	8./21
DAY 6- 9 MEAN	9.	7.	3.0	3.0
S.D./N	11./20	7./21	5./20	7./21
DAY 9- 12 MEAN	18.	16.	18.	17.
S.D./N	8./20	9./21	7./20	8./21
DAY 12- 16 MEAN	31.	27.	29.	26.
S.D./N	8./20	7./21	7./20	8./21
DAY 16- 20 MEAN	57.	58.	61.	55.
S.D./N	15./20	14./21	11./20	13./21
DAY 6- 16 MEAN	59.	58.	58.0	46.00
S.D./N	14./20	11./21	11./20	12./21
DAY 6- 20 MEAN	116.	109.	111.	102.
S.D./N	25./20	23./21	18./20	23./21
DAY 0- 20 MEAN	140.	133.	138.	127.
S.D./N	24./20	24./21	21./20	23./21

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST

** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .01 LEVEL USING DUNNETT'S TEST

MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

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

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

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

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

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

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

5. Discussion and Conclusions:

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

4B

PROJECT NO.:WIL-15150
SPONSOR:VELSICOL CHEMICAL CORP.

TABLE 6
A TERATOLOGY STUDY IN RATS WITH PRODIAMINE TECHNICAL
NUMBER OF FETUSES AND LITTERS WITH MALFORMATIONS

PAGE 1

DAY 20

DOSE GROUP:	FETUSES 1	FETUSES 2	FETUSES 3	FETUSES 4	LITTERS 1	LITTERS 2	LITTERS 3	LITTERS 4
NUMBER EXAMINED EXTERNALLY	257	269	271	247	20	21	20	21
OMPHALOCELE	1	0	4	1	1	0	2	1
CLEFT PALATE	0	0	0	1	0	0	0	1
MICROPHTHALMIA AND/OR ANOPHTHALMIA	0	2	0	4	0	1	0	2
NUMBER EXAMINED VISCERALLY	127	139	129	134	20	21	19	21
MALPOSITIONED TESTICLE(S)	0	0	0	1	0	0	0	1
NUMBER EXAMINED SKELETALLY	130	130	142	133	20	20	20	21
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	1	1	0	1	1	1	0	1
STERNOSCHISIS	0	0	0	1	0	0	0	1
TOTAL MALFORMATIONS								
NUMBER WITH EXTERNAL MALFORMATIONS:	1	2	4	6	1	1	2	4
NUMBER WITH SOFT TISSUE MALFORMATIONS:	0	0	0	1	0	0	0	1
NUMBER WITH SKELETAL MALFORMATIONS:	1	1	0	1	1	1	0	1
TOTAL NUMBER WITH MALFORMATIONS:	2	3	4	7	2	2	2	4

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL AT 0.05 LEVEL USING FISHER'S EXACT TEST

TABLE 9

PROJECT NO.:HIL-15150
SPONSOR:HELSICOL CHEMICAL CORP.

A TERATOLOGY STUDY IN RATS WITH PRODIAMINE TECHNICAL
PERCENT OF FETUSES AND LITTERS WITH VARIATIONS

DAY 20

DOSE GROUP:	FETUSES 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED EXTERNALLY NO FINDINGS	257	269	271	267	20	21	20	21
NUMBER EXAMINED VISCERALLY	127	139	129	134	20	21	19	21
RENAL PAPILLA(E) NOT DEVELOPED AND/OR DISTENDED URETER(S)	4.7	4.3	1.6	3.7	20.0	23.8	10.5	14.3
NUMBER EXAMINED SKELETALLY	130	130	142	133	20	20	20	21
14TH RUDIMENTARY RIB(S)	3.1	0.0	7.0	3.0	15.0	0.0	30.0	19.0
STERNEBRA(E) 05 AND/OR 06 UNOSSIFIED	6.2	14.6	3.5	7.5	30.0	45.0	25.0	28.6
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	3.1	4.6	2.8	4.5	10.0	30.0	15.0	23.8
BENT RIB(S)	0.0	2.3	0.0	0.0	0.0	5.0	0.0	0.0
REDUCED OSSIFICATION OF THE SKULL	0.0	0.8	0.0	0.8	0.0	5.0	0.0	4.8
7TH CERVICAL RIB(S)	0.0	1.5	0.7	1.5	0.0	10.0	5.0	4.8
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	1.5	0.0	0.7	0.8	10.0	0.0	5.0	4.8
REDUCED OSSIFICATION OF THE 13TH RIB(S)	0.8	2.3	0.7	0.0	5.0	10.0	5.0	0.0
25 PRESACRAL VERTEBRAE	0.8	0.0	0.0	0.0	5.0	0.0	0.0	0.0
HYOID UNOSSIFIED	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

WIL HISTORICAL CONTROL DATA
NEW ZEALAND WHITE RABBITS

APPENDIX A
SUMMARY INCIDENCE OF MALFORMATIONS

TOTAL NUMBER OF LITTERS EXAMINED	355
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	2409
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	2037
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	2247

	NUMBER FETUSES	NUMBER LITTERS	PERCENT (RANGE) FETUSES	PERCENT (RANGE) LITTERS
EXTERNAL MALFORMATIONS				
OMPHALOCELE	4	4	(0.0- 1.0)	(0.0- 6.2)
MALROTATED HINDLEGS	1	1	(0.0- 0.7)	(0.0- 6.7)
HERMAPHRODITE	1	1	(0.0- 0.7)	(0.0- 6.7)
SPINA BIFIDA	1	1	(0.0- 0.6)	(0.0- 5.0)
EXENCEPHALY WITH OR WITHOUT OPEN EYE LID	2	2	(0.0- 1.6)	(0.0- 14.3)
TAIL ANOMALY	2	2	(0.0- 1.2)	(0.0- 8.3)
MICROPHTHALMIA AND/OR ANOPHTHALMIA	1	1	(0.0- 1.2)	(0.0- 7.7)
MICROGNATHIA (MAXILLAE)	1	1	(0.0- 1.2)	(0.0- 7.7)
VERTEBRAL AGENESIS	1	1	(0.0- 1.0)	(0.0- 6.2)
CARPAL AND/OR TARSAL FLEXURE	2	2	(0.0- 1.2)	(0.0- 7.1)
DOME-SHAPED HEAD WITH HYDROCEPHALY	4	1	(0.0- 3.0)	(0.0- 5.6)
TOTAL NUMBER OF FETUSES WITH EXTERNAL MALFORMATIONS	19	16		

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APPENDIX A

SUMMARY INCIDENCE OF MALFORMATIONS

UK HISTORICAL CONTROL DATA
NEW ZEALAND WHITE RABBITS

TOTAL NUMBER OF LITTERS EXAMINED	355			
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	2409			
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	2037			
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	2247			
		NUMBER	PERCENT (RANGE)	
		FETUSES LITTERS	FETUSES	LITTERS
SKELETAL MALFORMATIONS				
SKULL ANOMALY	3	3	(0.0- 2.0)	(0.0- 11.8)
STERNEBRAE FUSED	15	13	(0.0- 3.4)	(0.0- 15.4)
6 CERVICAL VERTEBRAE	2	2	(0.0- 1.1)	(0.0- 7.1)
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	31	27	(0.0- 4.9)	(0.0- 33.3)
RIB ANOMALY	3	3	(0.0- 1.7)	(0.0- 14.3)
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRAE 11	2	1	(0.0- 2.0)	(0.0- 7.1)
STERNEBRAE(E) MALALIGNED (SEVERE)	5	5	(0.0- 1.9)	(0.0- 7.7)
CAUDAL VERTEBRAE ANOMALY	1	1	(0.0- 1.2)	(0.0- 7.7)
RIB(S) WITH SPHERICAL ENLARGEMENT	1	1	(0.0- 1.2)	(0.0- 7.1)
EXTRA SITE OF OSSIFICATION IN THE CERVICAL REGION	1	1	(0.0- 1.0)	(0.0- 4.2)
FUSED NASAL BONES	2	2	(0.0- 1.7)	(0.0- 8.3)
TOTAL NUMBER OF FETUSES WITH SKELETAL MALFORMATIONS	42	53		
TOTAL NUMBER OF FETUSES WITH MALFORMATIONS	92	76	3.7	21.4

APPENDIX A

SUMMARY INCIDENCE OF FETAL VARIATIONS

NTL HISTORICAL CONTROL DATA
NEW ZEALAND WHITE RABBITS

TOTAL NUMBER OF LITTERS EXAMINED	355
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	2409
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	2037
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	2247

	NUMBER		PERCENT (RANGE)	
	FETUSES	LITTERS	FETUSES	LITTERS
SKELETAL VARIATIONS				
STERNEBRA(E) #5 ABOVE #6 UNOSSIFIED	491	201	(5.4- 50.5)	(23.5- 91.7)
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	11	11	(0.4- 2.4)	(0.4- 15.0)
13TH RUDIMENTARY RIB(S)	244	157	(0.4- 23.2)	(0.4- 82.4)
13TH FULL RIB(S)	440	231	(13.0- 56.0)	(30.8- 92.9)
REDUCED OSSIFICATION OF THE SKULL	14	13	(0.4- 5.3)	(0.4- 25.0)
ACCESSORY SKULL BONE(S)	15	14	(0.4- 3.3)	(0.4- 15.4)
7TH CERVICAL RIB(S)	87	21	(0.4- 7.4)	(0.4- 33.3)
STERNEBRA(E) MALALIGNED,SLIGHT OR MODERATE)	63	34	(0.4- 22.3)	(0.4- 52.9)
HYOID ARCH(ES) KINK	79	55	(0.4- 13.2)	(0.4- 57.1)
HYOID BODY AND/OR ARCH(ES) UNOSSIFIED	18	13	(0.4- 7.0)	(0.4- 30.0)
PUBIS UNOSSIFIED	2	2	(0.4- 1.0)	(0.4- 10.0)
27 PRESACRAL VERTEBRAE	248	125	(0.4- 29.5)	(0.4-100.0)
25 PRESACRAL VERTEBRAE	5	5	(0.4- 1.9)	(0.4- 9.1)
ADDITIONAL OSSIFICATION CENTER	-	-	(0.4- 0.9)	(0.4- 2.1)
MALALIGNED PHALANGE(S)	-	-	(0.4- 0.9)	(0.4- 2.1)
7TH STERNEBRA	-	-	(0.4- 0.9)	(0.4- 2.1)
11 FULL PAIRS OF RIBS	2	2	(0.4- 1.2)	(0.4- 4.7)
RIB ENLARGEMENT	-	-	(0.4- 1.2)	(0.4- 7.7)
INTERRUPTED OSSIFICATION OF THE 13TH RIB(S)	3	3	(0.4- 1.2)	(0.4- 7.1)
CENTRUM MISSHAPEN AND MALIGNED	-	-	(0.4- 2.4)	(0.4- 14.3)
SACRA UNOSSIFIED	1	1	(0.4- 0.9)	(0.4- 5.9)
	-	-	(0.4- 0.9)	(0.4- 5.9)

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APPENDIX A

VIL HISTORICAL CONTROL DATA — SUMMARY OF MATERNAL AND FETAL OBSERVATIONS AT CESAREAN SECTION — PAGE 1

CHARLES RIVER CD® RATS

NO. OF ANIMALS IN THE HISTORICAL CONTROL:	803
NO. OF ANIMALS THAT DIED:	0
NO. OF ANIMALS THAT DELIVERED:	0
NO. OF ANIMALS EXAMINED AT CESAREAN SECTION:	803
NO. NONGRAVID:	85
NO. GRAVID:	798
NO.OF DAMS WITH ONLY RESORPTIONS:	2
NO.OF DAMS WITH LIVE FETUSES:	796
NO. OF LIVE FETUSES/DAM:	14.0 (11.9 - 15.4)
NO. OF POSTIMPLANTATION LOSSES/DAM:	0.6 (0.1 - 1.2)
NO. OF IMPLANTATION/DAM:	14.6 (12.8 - 16.0)
NO. OF CORPORA LUTEA/DAM:	16.2 (14.4 - 18.1)
FETAL SEX RATIO: MALE:FEMALE	5511:5640
MEAN FETAL BODY WEIGHT (G):	3.51 (3.26 - 3.85)

( ) - RANGE OF MEANS

CONFIDENTIAL

(471)

TOTAL NUMBER OF LITTERS EXAMINED	796
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	11151
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	6635
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	7234

	NUMBER FETUSES	NUMBER LITTERS	PERCENT (RANGE) FETUSES	PERCENT (RANGE) LITTERS
SOFT-TISSUE MALFORMATIONS				
ENCEPHALOMENINGOCELE	3	2	(0.0- 1.3)	(0.0- 5.0)
KIDNEY AND/OR URETER ABSENT	1	1	(0.0- 0.3)	(0.0- 4.5)
HEART AND/OR GREAT VESSEL ANOMALY	6	2	(0.0- 1.8)	(0.0- 5.0)
HYDROCEPHALY	2	2	(0.0- 0.4)	(0.0- 3.6)
TESTICULAR HYPOPLASIA	1	1	(0.0- 0.3)	(0.0- 5.0)
TOTAL NUMBER OF FETUSES WITH SOFT TISSUES MALFORMATIONS	13	8		

CONFIDENTIAL

(841)

APPENDIX A

UNL HISTORICAL CONTROL DATA — SUMMARY INCIDENCE OF FETAL VARIATIONS — PAGE 5

CHARLES RIVER CD® CD RATS

TOTAL NUMBER OF LITTERS EXAMINED	796
TOTAL NUMBER OF FETUSES [redacted] EXTERNALLY	11151
TOTAL NUMBER OF FETUSES [redacted] VISCERALLY	6635
TOTAL NUMBER OF FETUSES [redacted] SKELETALLY	7236

	NUMBER		PERCENT (RANGE)	
	FETUSES	LITTERS	FETUSES	LITTERS
EXTERNAL VARIATIONS				
FOCAL SUBCUTANEOUS HEMORRHAGE(S)	11	10	(0.0- 1.0)	(0.0- 13.6)

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TOTAL NUMBER OF LITTERS EXAMINED	796
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	11151
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	6435
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	7236

	NUMBER FETUSES	NUMBER LITTERS	PERCENT (RANGE) FETUSES	PERCENT (RANGE) LITTERS
SKELETAL VARIATIONS				
STERNEBRA(E) 05 AND/OR 06 UNOSSIFIED	1598	517	(4.6- 37.1)	(25.0-100.0)
HYOID UNOSSIFIED	210	125	(0.0- 15.7)	(0.0- 50.0)
14TH RUDIMENTARY RIB(S)	345	192	(0.0- 20.4)	(0.0- 64.0)
STERNEBRA(E) 01,02,03 AND/OR 04 UNOSSIFIED	47	42	(0.0- 2.5)	(0.0- 22.7)
REDUCED OSSIFICATION OF THE 13TH RIB(S)	74	56	(0.0- 6.4)	(0.0- 22.7)
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	80	68	(0.0- 6.2)	(0.0- 40.9)
REDUCED OSSIFICATION OF THE SKULL	48	30	(0.0- 7.7)	(0.0- 19.0)
14TH FULL RIB(S)	10	7	(0.0- 1.4)	(0.0- 7.1)
25 PRESACRAL VERTEBRAE	3	3	(0.0- 0.7)	(0.0- 5.0)
ENTIRE STERNUM UNOSSIFIED	3	3	(0.0- 0.7)	(0.0- 5.0)
METACARPAL(S) AND/OR METATARSAL(S) UNOSSIFIED	5	5	(0.0- 0.9)	(0.0- 9.1)
27 PRESACRAL VERTEBRAE	11	8	(0.0- 2.1)	(0.0- 9.1)
7TH CERVICAL RIB(S)	10	10	(0.0- 1.5)	(0.0- 9.1)
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	10	9	(0.0- 1.9)	(0.0- 9.1)
PUBIS UNOSSIFIED	7	7	(0.0- 1.2)	(0.0- 8.3)
BENT RIB(S)	33	21	(0.0- 3.1)	(0.0- 9.5)
GENERAL REDUCED OSSIFICATION OF THE SKELETON	3	3	(0.0- 1.2)	(0.0- 8.0)
REDUCED OSSIFICATION OF THE ISCHIUM	1	1	(0.0- 0.3)	(0.0- 4.5)
REDUCED OSSIFICATION OF THE PELVIC GIRDLE	6	6	(0.0- 1.3)	(0.0- 14.3)
INTERRUPTED OSSIFICATION OF THE 13TH RIB(S)	1	1	(0.0- 0.4)	(0.0- 4.3)

005656

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

DATA EVALUATION REPORT

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

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

TEST MATERIAL: Prodiamine Technical

SYNONYMS: (Rydex)

STUDY NUMBER(S): WIL-15153

SPONSOR: Vesicol Chem. Corp.

TESTING FACILITY: WIL Research Labs., Inc.

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

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

REPORT ISSUED: 11-7-85

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

Classification: core-minimum

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

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

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

Hair loss appeared to be evenly distributed among the groups.

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

2. Body Weights:

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

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

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

3. Food consumption:

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

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

4. Gestation Day 29 Cesarean Section Data:

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

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

5. Necropsy Examination:

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

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

The most prevalent findings at scheduled necropsy were ascities,

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

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

6. Fetal Morphological Data:

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

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

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

TABLE 11

PROJECT NO.:BIL-15153 A TERATOLOGY STUDY IN RABBITS WITH FRODIAMINE TECHNICAL PAGE 1

SPONSOR:VELSICOL CHEMICAL CORP. PERCENT OF FETUSES AND LITTERS WITH VARIATIONS

MAY 29

DOSE GROUP:	FETUSES 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED EXTERNALLY AND FINDINGS	81	94	79	47	14	16	15	9
NUMBER EXAMINED VISCERALLY	81	94	79	47	14	16	15	9
MAJOR BLOOD VESSEL VARIATION	22.2	13.8	21.5	2.1	57.1	43.7	66.7	11.1
GALLBLADDER ABSENT OR SMALL	1.2	3.2	6.3	4.3	7.1	12.5	33.3	22.2
HEMORRHAGIC RING AROUND THE IRIS	1.2	0.0	0.0	0.0	7.1	0.0	0.0	0.0
NUMBER EXAMINED SKELETALLY	81	94	79	47	14	16	15	9
13TH RUDIMENTARY RIB(S)	11.1	22.3	16.5	21.3	35.7	68.7	66.7	55.6
13TH FULL RIB(S)	33.3	37.4	45.6	51.1	78.6	93.7	73.3	88.9
HYOID ARCH(ES) BENT	7.4	3.2	1.3	0.0	28.6	12.5	6.7	0.0
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	1.2	2.1	2.5	4.3	7.1	12.5	13.3	22.2
27 PRESACRAL VERTEBRAE	7.4	15.0	20.3	8.5	28.6	62.5	53.3	33.3
STERNEBRA(E) 05 AND/OR 06 UNOSSIFIED	2.5	11.7	25.3	14.9	7.1	50.0	33.3	44.4
HYOID BODY AND/OR ARCH(ES) UNOSSIFIED	1.2	0.0	0.0	2.1	7.1	0.0	0.0	11.1
7TH CERVICAL RIB(S)	4.9	0.0	1.3	2.1	21.4	0.0	6.7	11.1
ACCESSORY SKULL BONE(S)	2.5	2.1	2.5	4.3	14.3	12.5	6.7	11.1
25 PRESACRAL VERTEBRAE	0.0	0.6	3.8	0.0	0.0	0.0	13.3	0.0
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	1.2	0.0	0.0	0.0	7.1	0.0	0.0	0.0
7TH STERNEBRA	1.2	0.0	0.0	0.0	7.1	0.0	0.0	0.0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 500 MG/KG/DAY

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

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

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

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

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 12/6/89
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I. 12/6/89
Section I, Toxicology Branch II (H7509C)

Review of the Registrant's Response to the Previous Review Comments Concerning the Rabbit Teratology Study with Prodiamine (Toxicology Branch Memorandum of January 12, 1987, Winnie Teeters)

Registrant's Response: "On page 1 of the EPA Data Evaluation Report of this study, the Agency concluded that a NOEL for maternal toxicity was not established and the lowest effect level (LEL) was 100 mg/kg, the lowest dose tested (LDT). On page 7 of this same report, the Agency concluded that the LEL was 300 mg/kg (a contradiction to their earlier statement). "In the range-finding study with rabbits to establish levels for the teratology study, a clear demonstration of maternal weight loss was observed at both the 500 and 1000 mg/kg levels. Weight losses at these levels were pronounced, whereas at lower levels (50, 125, and 250 mg/kg) overall weight gains were similar to control. There is mutual agreement between the Agency and ourselves on this evaluation."

"Maternal weight loss was also reported in the teratology study and to a large extent demonstrated a similar and predictable pattern in that this observation was statistically significant at both 300 and 500 mg/kg levels during the dosing period, but not at the 100 mg/kg level. We agree with the Agency's analysis that small body weight changes are common in this species thus resulting in less confidence accepting a non-significant decrease as a true compound effect. We do not agree however with the Agency's conclusion that the weight variations between controls and the 100 mg/kg level were compound related. It is important to note that prior to dosing (Days 0-6), control animals esatblished a weight gain pattern which was approximately 1.5X that seen in the other three groups. A similar pattern can and should be expected during the dosing period and in our opinion did occur."

"The mean maternal body weight change for 25 studies conducted at the testing laboratory demonstrates the variability of this parameter in control animals and tends to give confidence only to those findings which are statistically significant (Appendix 1 attached). A NOEL for maternal toxicity has been demonstrated for this study becuase (a) No statistical significance was attributed to maternal weight loss at 100 mg/kg, (b) A range-finding study clearly demonstrated a NOEL for maternal weight loss at 50, 125, and 250 mg/kg, and (c) Non-statistical lower weight gains at the 100 mg/kg level in the teratology study can be attributed to dissimilar predosing weight gain patterns."

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Reviewers' Comments: The submitted addendum with table (Appendix 1) provides adequate information concerning the spontaneous maternal body weight changes in the New Zealand white rabbits established by the WIL testing laboratory (Historical control data for 25 studies). The small body weight changes at the 100 mg/kg level were found within the range for the historical control data recently submitted. the Registrant's explanations for the establishment of NOEL for maternal toxicity in this study are considered to be reasonable. We agree that the NOEL for maternal toxicity should be 100 mg/kg.

Recommendation: 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 (based on an adverse effect on body weight gain)

WIL HISTORICAL CONTROL DATA (NEW ZEALAND WHITE RABBITS)
MEAN MATERNAL BODY WEIGHTS (GRAMS)

STUDY	GESTATION DAY 0	6	9	12	15	18	24	29
1	3633.	3733.	N/A	N/A	N/A	3851.	3903.	3869.
2	3617.	3729.	N/A	N/A	N/A	N/A	3917.	3975.
3	3820.	3940.	N/A	N/A	N/A	4070.	4160.	4260.
4	4240.	4300.	N/A	N/A	N/A	4288.	4268.	4213.
5	3932.	4047.	N/A	N/A	N/A	4144.	4224.	4197.
6	3936.	4052.	4086.	4090.	4108	4181.	4257.	4351.
7	4154.	4184.	4165.	4183.	4246	N/A	4278.	4274.
8	3713.	3892.	N/A	3931.	N/A	4046.	4071.	4126.
9	3824.	3940.	N/A	4007.	N/A	4087.	4130.	4164.
10	4010.	4064.	4081.	4105.	4193.	4222.	4272.	4333.
11	3639.	3795.	N/A	3903.	N/A	4015.	4042.	4093.
12	3734.	3852.	N/A	3928.	N/A	4036.	4150.	4136.
13	3872.	3991.	N/A	4114.	N/A	4172.	4162.	4149.
14	3924.	4039.	N/A	4140.	4224.	4305.	4352.	4332.
15	3851.	3994.	N/A	4053.	N/A	4098.	4127.	4133.
16	4017.	4129.	4163.	4219.	4282.	4290.	4283.	4433.
17	3932.	4014.	N/A	4094.	N/A	4180.	4196.	4268.
18	3897.	3984.	4000.	4039.	4078.	4107.	4210.	4269.
19	3818.	3987.	N/A	4083.	4124.	4149.	4227.	4269.
20	3932.	4034.	N/A	4142.	N/A	4182.	4260.	4264.
21	3873.	3957.	4039.	4073.	4153.	4168.	4243.	4240.
22	3762.	3875.	N/A	3925.	4067.	4076.	4098.	4131.
23	4139.	4207.	N/A	4273.	N/A	4284.	4277.	4192.
24	3630.	N/A	N/A	N/A	N/A	N/A	4045.	4052.
25	3635.	3655.	N/A	3765.	N/A	3851.	3893.	3972.
MEAN	3861.4	3974.3	4089.0	4056.2	4163.9	4127.4	4161.8	4187.8
S.D.	170.50	153.66	65.95	123.28	76.45	123.35	125.80	130.05
N.	25	24	6	19	9	22	25	25

N/A = NOT MEASURED IN THIS STUDY

WIL HISTORICAL CONTROL DATA (NEW ZEALAND WHITE RABBITS)
MEAN MATERNAL BODY WEIGHT CHANGES (GRAMS)

STUDY	GESTATION DAY 0-6	6-12	6-18	18-29	0-29
1	101.	N/A	118.	41.	282.
2	113.	N/A	N/A	N/A	358.
3	120.	N/A	130.	190.	440.
4	60.	N/A	-12.	-75.	-28.
5	115.	N/A	97.	53.	265.
6	105.	49.	140.	170.	414.
7	30.	-1.	N/A	N/A	120.
8	178.	39.	155.	89.	434.
9	116.	67.	147.	81.	319.
10	53.	41.	158.	111.	322.
11	156.	67.	179.	78.	413.
12	118.	76.	184.	100.	402.
13	120.	122.	181.	-13.	291.
14	115.	101.	266.	28.	408.
15	143.	59.	104.	35.	282.
16	113.	90.	161.	57.	346.
17	83.	80.	166.	88.	336.
18	87.	55.	123.	153.	353.
19	169.	96.	162.	113.	453.
20	102.	108.	148.	82.	407.
21	83.	117.	211.	73.	367.
22	113.	50.	187.	55.	360.
23	68.	66.	77.	-91.	54.
24	N/A	N/A	N/A	N/A	419.
25	20.	110.	196.	122.	337.
MEAN	103.3	73.3	149.0	70.0	326.2
S.D.	38.92	31.62	54.76	68.24	118.96
N.	24	19	22	22	25

N/A = NOT MEASURED IN THIS STUDY

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] 12/11/89
Section I, Toxicology Branch - HFAS (H7509C)

007660

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:CDBS 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				Females			
	0	50	200	2000	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
F1a Males:	0	111	206	200	---
	50	119	202	196	---
	200	113	206	196	---
	2000	107	199	192	---
F1b 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	---	---
F1a Females:	0	98	145	136	---
	50	97	145	137	---
	200	95	140	131	---
	2000	93	137	130	---
F1b 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 [1]

Dose Level (ppm)	Bod	Absolute Weights 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.

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				F1b 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 F1b 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	7.3	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."

REVIEWER

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

007973

JUN 11 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Subject: Prodiamine: ID Number 55947-WR
Tox Chem No. 727A
HED Project No. 0-0687

From: John H.S. Chen, D.V.M. 6/8/90
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 6/8/90
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief 6/11/90
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Sandoz Crop Protection Co.
Des Plaines, Illinois 60018

Action Requested: Review of the Registrant's Response to the Previous Toxicology Branch II Comments concerning the Acute Rat Inhalation Study with Prodiamine 65 WDG, the L5178Y Mouse Lymphoma Mutation Assay with Technical Prodiamine, the Rat Production Study with Technical Prodiamine, and the Acute Toxicity Study with Technical Prodiamine.

Reviewer's Comments:

1. Acute Rat Inhalation Toxicity Study with Prodiamine 65 WDG. Huntingdon Research Center Study No. VCL 111/86831, November 7, 1986 (81-3).

Registrant's Response: "This study was done according to the guidelines in place during 1986 and technically is a sound study. It was submitted nearly two years before the SEP's latest amendment in April, 1989 and was put into

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review February 1989, two months before the amendment. The latest previous SEP, August, 1988, discusses the two rejection criteria for this study (particle size and maximum concentration) but no difinitive changes are made, unlike the April, 1989 amendments. Under the 1988 guidelines, we are confident the Agency will find this study acceptable. In retrospect, the need for this study is in question. Prodiamine 65 WG is an end-use product formulation. Acute inhalation toxicity on the technical product (Accession No. 257489), and the 75 WP formulation (Accession No. 257490) do not indicate any toxicological concerns based on inhalation. Both were classified as core guideline data and both have a toxicity III, caution rating. At a minimum, 95% granules are in the 420 to 2000 micron range."

Reviewer's Comments: The Registrant's explanations for the deficiencies of this study are considered to be reasonable. Because both the acute inhalation toxicity studies in rats previously accepted by the Agency (Acute inhalation toxicity with the technical prodiamine in rats, Huntingdon Res. Center #VCL 49/84839, LC50 > 0.256g/m3, Toxicity Category III, Core Guideline 005267; Acute inhalation toxicity with the Prodiamine 75 WP formulation in rats Huntingdon Res. Center #VCL 54/8385, LC50 > 3.8 g/m3, Toxicity Category III, Core Guideline 005267) do not indicate any toxicological concerns based on inhalation, it is unlikely that the results of this study with the Prodiamine 65 WDG formulation will fall outside the range of the Toxicity Category III if repeated.

Recommendation: Since the clarification of the particle size from 5.5 um to less than 1 um and the application of maximum attainable concentration higher than 1.81 mg/L have no determinable effects to altering the outcome of the study results, The study is upgraded to Core Minimum.

LC50 > 1.81 mg/L (both sexes)

Toxicity Category: III

2. Mouse Lymphoma Assay with Technical Prodiamine. Microbiological Associates Study No. T2840.701 (84-2)

Registrant's Response: "The mouse lymphoma assay was conducted as part of a battery of five mutagenicity tests.

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Four of these tests, covering the three mutagenicity categories, have been accepted by the Agency and all tested negative. As the mouse lymphoma assay is known to cause false positives and an acceptable study is on file for the guideline, we propose to conduct a CHO/HGPRT Forward Mutation Assay rather than to repeat the mouse lymphoma assay. The CHO/HGPRT Forward Mutation Assay is less likely to cause a false positive while measuring the same endpoint. We request the Agency's review and approval of this replacement study as outlined in the attached protocol."

Reviewer's Comments: The Registrant's request for conducting a CHO/HGPRT Forward Mutation Assay to replace the mouse lymphoma assay is considered reasonable. The test protocol for performing the CHO/HGPRT Forward Mutation Assay should be based on the method described by Hsie et al. (Hsie et al., A Report of U.S. EPA's Gene-Tox Program for CHO/HGPRT Assay, Mutation Res. 86: 193-214, 1981).

Recommendation: Toxicology Branch II has no objection to the Registrant's request. However, the mouse lymphoma assay with technical prodiamine remains unacceptable.

3. Effect of Prodiamine on Reproductive Function of Two Generations in the Rat. Huntingdon Research Center Study No. VCL 73/871075 (83-4)

Registrant's Response: "The purity of the test material was 94.3% Prodiamine."

Reviewer's Recommendation: The study is upgraded to Core Guideline. Parental Toxicity NOEL = 200 ppm; Reroductive Toxicity NOEL = 200 ppm.

4. Acute Dermal Toxicity Study with Technical Prodiamine (81-2)

Registarnt's Response: "An acute dermal study was previously submitted on technical prodiamine January 22, 1985 to EUP File 876-EUP-44 (Endurance 65 WDG Herbicide) and assigned Accession #256459. Results indicate an LD50 of >2000 mg/kg. We were under the impression that this study was accepted. Please advise."

Reviewer's Comments: The acute rat dermal study with an LD50 of >2000 mg/kg was tested on the 65 WDG formulation (Core Minimum 005656; Accession No. 263738). There is no acute dermal study with technical prodiamine in our toxicology data file.

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

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity

TOX. CHEM. NO.: 727-A

ACCESSION NUMBER: 260680

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

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

SYNONYMS: (Rydex)

STUDY NUMBER(S): T2840.380

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

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

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

REPORT ISSUED: 4-26-85

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

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

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

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

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

The report contained signed statements regarding quality assurance procedures.

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

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

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

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

Classification: Acceptable

Study No. T2840.380

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TABLE 2

PARALLEL TOXICITY TEST

UNSCHEDULED DNA SYNTHESIS

TREATMENT	DISHES COUNTED	% VIABLE CELLS	VIABLE CELLS/DISH (X10^5)	SURVIVAL INDEX	RELATIVE SURVIVAL	RELATIVE TOXICITY
PRODIAMINE TECHNICAL (96.3%)						
100 ug/ml	3	0.0%	0.000	0.0%	0.0%	100.0%
30 ug/ml	3	13.8%	0.270	5.4%	12.1%	87.9%
20 ug/ml	3	64.6%	1.460	29.2%	65.5%	34.5%
10 ug/ml	3	70.5%	1.810	36.2%	81.2%	18.8%
3.3 ug/ml	3	72.5%	1.960	39.2%	87.9%	12.1%
1.0 ug/ml	3	77.8%	2.120	42.4%	95.1%	4.9%
0.3 ug/ml	3	85.2%	2.090	41.8%	93.7%	6.3%
DMBA						
10 ug/ml	3	55.7%	0.860	17.2%	38.6%	61.4%
3 ug/ml	3	60.7%	0.860	17.2%	38.6%	61.4%
DMSO						
10 ul/ml	3	78.5%	2.230	44.6%	100.0%	0.0%
UNE	3	77.7%	2.270	45.4%	101.8%	-1.8%

Cells plated per dish: 500,000

Survival Index = $\frac{\text{Average Viable Cells per Dish} \times 100}{\text{Cells Plated per Dish}}$

Relative Survival = $\frac{\text{Survival Index} \times 100}{\text{Survival Index of Control}}$

Relative Toxicity = 100 - Relative Survival

UNE = Untreated Control

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TABLE 3
UNSCHEDULED DNA SYNTHESIS SUMMARY RESULTS

Treatment	Relative Survival	Slide Designation	No. of Nuclei Counted	Average Net Grains per Nucleus (±S.E.)	Grand Mean (± S.E.)	Percent cells with ≥ 5 Net Nuclear Grains
Prodiamine Technical (96.3)						
100 ug/ml	0.0%	A	25	Not counted due to toxicity		
		B	25			
		C	25			
30 ug/ml	12.1%	A	25	0.8 ± 0.9		
		C	25	0.4 ± 0.7	0.4 ± 0.8	0.0%
		C	25	-0.1 ± 0.6		
20 ug/ml	65.5%	A	25	0.0 ± 0.8		
		B	25	-0.2 ± 1.3	0.0 ± 1.0	0.0%
		C	25	0.2 ± 0.8		
10 ug/ml	81.2%	A	25	-0.3 ± 1.4		
		B	25	-0.1 ± 1.0	-0.1 ± 1.1	0.0%
		C	25	0.0 ± 0.8		
3.3 ug/ml	87.9%	A	25	0.0 ± 0.9		
		B	25	0.3 ± 1.0	0.2 ± 1.6	1.3%
		C	25	0.3 ± 2.4		
1.0 ug/ml	95.1%	A	25	-0.3 ± 1.5		
		B	25	-0.1 ± 1.2	-0.1 ± 1.5	0.0%
		C	25	0.1 ± 1.7		
0.3 ug/ml	93.7%	A	25	-0.1 ± 0.7		
		B	25	0.1 ± 1.0	-0.1 ± 0.9	0.0%
		C	25	-0.2 ± 1.0		
DMBA						
10 ug/ml	38.6%	A	25	24.1 ± 4.5		
		B	25	25.8 ± 5.1	23.7 ± 4.9	100%
		C	25	21.2 ± 4.0		
DMBA						
3.0 ug/ml	38.6%	A	25	25.5 ± 5.8		
		B	25	11.7 ± 2.1	18.8 ± 7.1	100%
		C	25	19.1 ± 4.1		
DMSO						
10 ul/ml	100.0%	A	25	0.4 ± 1.1		
		B	25	-0.1 ± 0.9	0.0 ± 1.0	0.0%
		C	25	-0.2 ± 0.8		
NNE[1]	101.8%	A	25	0.5 ± 1.0		
		B	25	0.4 ± 1.0	0.1 ± 1.0	0.0%
		C	25	-0.5 ± 0.8		

[1] Untreated Control.

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005656

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

10-28-86

12/3/86

DATA EVALUATION REPORT

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

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

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

SYNONYMS: (Rydex)

STUDY NUMBER(S): T4022.501

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

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

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

REPORT ISSUED: 6-27-85

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

RESULTS: The toxicity tests, including concentrations which caused

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

Averaged Revertants/Plate

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

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

Classification: Acceptable

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 12/14/90
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. 12/18/90
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

CHEMICAL: Prodiamine

Tox. Chem. No.: 727A

EPA File Symbol: 55947-UR

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

ACCESSION NUMBER:
MRID Number: 415581-01
SYNONYMS/CAS No.:

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

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

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

AUTHOR(S): Robert R. Young

STUDY NUMBER(S): 12200-0-431

REPORT ISSUED: June 27, 1990

CONCLUSION(S) - Executive Summary:

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

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

Study: Acceptable

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

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

2. **Control Materials**:
   Negative: DMSO
   Solvent/final concentration: DMSO
   Positive: Non-activation (concentrations, solvent):
   Ethylmethane sulfonate (EMS; 0.25 & 0.4 ul/ml)

   Activation (concentrations, solvent):
   3-methylcholanthrene (MCA; 2.5 & 4.0 ug/ml)

3. **Activation**: S9 derived from
   ___X Aroclor 1254 __X induced Male rat X liver
   ___ phenobarbital ___ non-induced ___ mouse ___ lung
   ___ none ___ hamster ___ other
   ___ other ___ other
   If other, describe below
   Describe S9 mix composition (if purchased, give details):

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

4. **Test Cells**: mammalian cells in culture
   __X mouse lymphoma L5178Y cells
   ___ Chinese hamster ovary (CHO) cells
   ___ V79 cells (Chinese hamster lung fibroblasts)
   ___ other (list):

   Properly maintained? (Y) / N (circle one)
   Periodically checked for Mycoplasma contamination?
   (Y) / N (circle one)
   Periodically checked for karyotype stability?
   (Y) / N (circle one)
   Periodically "cleansed" against high spontaneous background?
   (Y) / N (circle one)

5. Locus Examined:

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

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

___ Na^+/K^+ ATPase
Selection agent:
(give concentration)
_____ ouabain

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

6. Test compound concentrations used:

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

Activated conditions:

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

B. TEST PERFORMANCE

1. Cell treatment:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

placed in a humidified incubator at approximately 37°C with approximately five percent CO_2:95 percent air for colony development. After ten to fourteen days in the incubator, the colonies were counted on an Artek Model 880 colony counter fitted with a ten turn potentiometer for discrimination of colony size. The smallest detectable colony was between 0.2 and 0.3 mm in diameter, depending on its position in the agar matrix.

The mutant frequency was calculated as the ratio of the total number of mutant colonies found in each set of three mutant selection dishes to the total number of cells seeded, adjusted by the absolute selection cloning efficiency. If one dish in either set was lost due to contamination or other cause, the colony count of the missing dish was determined by a proportion equation based upon the weights of the three dishes of the set and the colony counts in the two acceptable dishes. If a lost plate was not available for weighing, the colony count of the lost plate was determined from the average of the two remaining acceptable plates. Any mutant frequency calculated by either method was identified by footnote in the data tables as a reminder of the reduced sample size in the event of a spurious variation.

The measurement of the toxicity of each treatment was the relative suspension growth of the cells over the two-day expression period multiplied by the relative cloning efficiency at the time of selection. Although not strictly a measure of cell survival, this parameter (called percent relative growth) provides a measure of the effectiveness of treatment and is used as the basis for selecting doses for any necessary repeat trials.

2. Activation Assay

The activation assay is often run concurrently with the nonactivation assay; however, it is an independent assay performed with its own set of vehicle and positive controls. The two assays were identical except for the addition of the S9 fraction of rat liver homogenate and necessary cofactors (CORE) during the four-hour treatment period. The 10 ml volume during treatment included this S9 activation mix which was prepared just prior to use and kept on ice. CORE consisted of nicotinamide adenine dinucleotide phosphate (NADP, sodium salt) and isocitrate (isocitric acid). The S9 homogenate was commercially prepared (Molecular Toxicology, Inc., lot number 0282) and consisted of the 9000 x g supernatant from the homogenized livers of Aroclor 1254-induced adult male Sprague Dawley rats.

C. Mutagenicity Testing

1. Nonactivation Assay

The assay procedure used was based on that reported by Clive and Spector (1975) and Clive, et al. (1979). The cells for the experiment were obtained from logarithmically growing laboratory stock cultures and were seeded into a series of tubes at 6 x 10^5 cells per tube. The cells were pelleted by centrifugation, the culture medium removed, and the cells resuspended in a final volume of 10.0 ml of treatment medium that contained five percent heat inactivated horse serum. The dosed tubes were closed, vortexed and placed in an orbital shaker incubator at approximately 37°C at 80 ± 10 orbits per minute for an exposure period of about four hours. Afterwards, the cells were washed twice, resuspended in 20.0 ml of growth medium and returned to the orbital shaker incubator as closed-tube cultures.

The appearance of the treated cultures (precipitate formation, oil separation, pH change) was recorded both at the time of treatment and after the four hour treatment period.

A standard expression period of two days was used to allow recovery, growth and expression of the TK-/- phenotype. Cell densities were determined on day one (about twenty-four hours after treatment) and each culture was adjusted to 3 x 10^5 cells/ml in 20.0 ml of growth medium to maintain optimal growth rates. If the cells in a culture failed to multiply to a density of 4 x 10^5/ml on the first day after treatment, the culture was returned to the incubator without being subcultured. On day two, cell counts were again determined, and appropriate cultures were selected for cloning and mutant selection.

At least five doses were selected for mutant analysis. If possible, doses were selected to include nontoxic to highly toxic (approximately ten to twenty percent relative growth) treatment conditions. Cultures with cell densities less than approximately 3 x 10^5 cells/ml on day 2 were not considered for analysis.

Each culture selected for analysis was sampled to obtain cells for exposure to the selection agent and to determine the cloning efficiency of the population. A total sample size of 3 x 10^6 cells was suspended in selection medium to selectively recover only TK-/- mutants. This sample was distributed into three 100 mm dishes so that each dish contained approximately 1 x 10^6 cells. The cloning efficiency was determined by serially diluting the cells and seeding each of three dishes with approximately 200 cells in cloning medium. All of the dishes were

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TABLE 1

CYTOTOXICITY ASSAY WITH PRODIAMINE TECHNICAL - TRIAL I

SAMPLE IDENTITY: PRODIAMINE TECHNICAL, LOT NUMBER C-85177, 94.3%
ASSAY NUMBER: 12200
TEST DATE: MAY 1, 1990
SOLVENT: DIMETHYL SULFOXIDE
COMMENTS ON TREATMENT: FOUR HOUR EXPOSURE PERIOD.

APPLIED CONCENTRATION MG/ML	WITHOUT S9 ACTIVATION		WITH S9 ACTIVATION	
	CELL DENSITY/ML ($X10^5$)[a]	% VEHICLE CONTROL[b]	CELL DENSITY/ML ($X10^5$)[a]	% VEHICLE CONTROL[b]
NEGATIVE CONTROL[c]	21.7	109.6	17.6	119.7
VEHICLE CONTROL[d]	19.8	100.0	14.7	100.0
0.00195	20.1	101.5	8.5	57.8
0.00391	19.5	98.5	7.4	50.3
0.00781	18.1	91.4	3.6	24.5
0.0156	17.1	86.4	1.2	8.2
0.0313	17.8	89.9	0.5	3.4
0.0625	12.9	65.2	0.3	2.0
0.125	2.1	10.6	0.0	0.0
0.25	0.7	3.5	0.0	0.0
0.5	1.4	7.1	0.0	0.0
1.0	3.8	19.2	0.0	0.0

[a]Cell density determined by hemocytometer approximately 24 hours after treatment initiation.
[b]Relative to vehicle control cell density for all treatments.
[c]Negative Control = Negative (culture media) control.
[d]Vehicle Control = 1% dimethyl sulfoxide in culture media

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TABLE 2

CYTOTOXICITY ASSAY WITH PRODIAMINE TECHNICAL - TRIAL II WITH ACTIVATION

SAMPLE IDENTITY: PRODIAMINE TECHNICAL, LOT NUMBER C-85177, 94.3%
ASSAY NUMBER: 12200
TEST DATE: MAY 7, 1990
SOLVENT: DIMETHYL SULFOXIDE
COMMENTS ON TREATMENT: FOUR HOUR EXPOSURE PERIOD.

APPLIED CONCENTRATION MG/ML	WITHOUT S9 ACTIVATION		WITH S9 ACTIVATION	
	CELL DENSITY/ML ($X10^5$)[a]	% VEHICLE CONTROL[b]	CELL DENSITY/ML ($X10^5$)[a]	% VEHICLE CONTROL[b]
NEGATIVE CONTROL[c]	-	-	20.3	125.3
VEHICLE CONTROL[d]	-	-	16.2	100.0
0.000195	-	-	14.9	92.0
0.000391	-	-	17.8	109.9
0.000781	-	-	16.0	98.8
0.00156	-	-	12.1	74.7
0.00313	-	-	12.0	74.1
0.00625	-	-	7.6	46.9
0.0125	-	-	3.0	18.5
0.025	-	-	0.9	5.6
0.05	-	-	0.2	1.2
0.1	-	-	0.0	0.0

[a]Cell density determined by hemocytometer approximately 24 hours after treatment initiation.
[b]Relative to vehicle control cell density for all treatments.
[c]Negative Control = Negative (culture media) control.
[d]Vehicle Control = 1% dimethyl sulfoxide in culture media

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TABLE 3

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL I

NAME OR CODE DESIGNATION OF THE TEST COMPOUND: PRODIAMINE TECHNICAL, LOT C-85177, 94.3%
GENETICS ASSAY NO: 12200
SOLVENT: DIMETHYL SULFOXIDE
SELECTIVE AGENT: 3.0 UG/ML TFT
TEST DATE: 05/09/90

CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
ACTIVATION CONTROLS[e]				AVG SOLV CONTROL				AVG SOLV CONTROL		
T CONTROL	18.8	12.1	25.3		146.0	503.0	83.8		100.0	58.1
T CONTROL	17.4	13.6	26.3		123.0	494.0	82.3		100.0	49.8
T CONTROL	17.2	12.7	24.3	25.3	120.0	481.0	80.2	82.1	100.0	49.9
25 UL/ML	15.5	13.7	23.6		881.0	407.0	67.8		77.1	432.9[f]
50 UL/ML	16.3	10.8	19.6		1377.0	401.0	66.8		62.9	686.8[f]
COMPOUND			RELATIVE TO SOLV CONTROL (%)				RELATIVE TO SOLV CONTROL (%)			
UG/ML	16.2	12.4	88.2		167.0	543.0	110.2		97.2	61.5
UG/ML	13.3	12.8	74.8		NS[g]	---	---		-----	------
UG/ML	15.1	10.9	72.3		120.0	493.0	100.1		72.4	48.7
UG/ML	11.8	12.2	63.2		129.0	549.0	111.4		70.4	47.0
UG/ML	4.1	9.5	17.1		160.0	353.0	71.6		12.2	90.7
UG/ML	3.0[h]	6.2	8.2		154.0	281.0	57.0		4.7	109.6[f,i]
UG/ML	2.7[h]	6.9	9.1		141.0	327.0	66.4		6.0	86.2
UG/ML I[j]	2.7[h]	3.5	4.6		212.0	212.0	43.0		2.0	200.0[f,i]
UG/ML I	1.0[h]	1.3	1.7		T[k]	---	---		-----	------
UG/ML I	1.8[h]	3.5	4.6		589.0	188.0	38.1		1.8	626.6[f,i]

[a] SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b] CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED

[c] RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d] MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e] SOLVENT CONTROL 1% DMSO, EMS = ETHYLMETHANE SULFONATE POSITIVE CONTROL

[f] MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 105.2 X 10^{-6}

[g] NOT PLATED FOR SELECTION, SUFFICIENT SURVIVING NONTOXIC DOSE LEVELS

[h] SPLIT BACK DUE TO TOXICITY

[i] LESS THAN 5% RELATIVE GROWTH, DATA REPORTED BUT NOT USED IN EVALUATION

[j] INSOLUBLE MATERIAL VISIBLE, ACCURATE OBSERVATION DIFFICULT DUE TO PRESENCE OF CELLS

[k] CULTURE TERMINATED DUE TO EXCESSIVE TOXICITY

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TABLE 4

MUTATION ASSAY ACTIVATION - TRIAL I

NAME OR CODE DESIGNATION OF THE TEST COMPOUND: PRODIAMINE TECHNICAL, LOT C-85177, 94.3%
GENETICS ASSAY NO: 12200
SOLVENT: DIMETHYL SULFOXIDE
SELECTIVE AGENT: 3.0 UG/ML TFT
TEST DATE: 05/09/90

CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)
TIVATION CONTROLS[e]		BATCH NO: 0282		AVG SOLV CONTROL				AVG SOLV CONTROL		
NT CONTROL	16.7	14.8	27.5		168.0	626.0	104.3		100.0	53.7
NT CONTROL	12.4	16.8	23.1		143.0	561.0	93.5		100.0	51.0
NT CONTROL	18.4	13.9	28.4	26.3	107.0	527.0	87.8	95.2	100.0	40.6
.50 UG/ML	7.3	15.6	12.7		717.0	459.0	76.5		38.7	312.4[f]
.00 UG/ML	5.6	13.4	8.3		725.0	268.0	44.7		14.9	541.0[f]
COMPOUND			RELATIVE TO SOLV CONTROL (%)				RELATIVE TO SOLV CONTROL (%)			
5 UG/ML	11.1	13.5	63.3		178.0	585.0	102.4		64.8	60.9
.0 UG/ML	10.5	15.0	66.5		NS[g]	---	---		-----	------
0 UG/ML	8.5	13.4	48.1		183.0	557.0	97.5		46.9	65.7
.0 UG/ML	6.0	15.9	40.3		171.0	570.0	99.8		40.2	60.0
.0 UG/ML	6.6	12.6	35.1		146.0	518.0	90.7		31.8	56.4
.0 UG/ML	3.5[h]	17.0	21.5		166.0	496.0	86.9		18.7	66.9
.0 UG/ML	3.7[h]	12.2	15.5		200.0	508.0	89.0		13.8	78.7
.0 UG/ML	2.0[h]	8.5	10.8		214.0	548.0	95.9		10.4	78.1
.0 UG/ML	1.4[h]	3.7	4.7		205.0	472.0	82.7		3.9	86.9[i]
.0 UG/ML	0.3[h]	0.8	1.0		TJ	---	---		-----	------

SPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

.ONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED

ELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

ITANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE REQUENCY IN UNITS OF 10E-6

OLVENT CONTROL 1% DMSO, MCA = 3-METHYLCHOLANTHRENE POSITIVE CONTROL

UTAGENIC, EXCEEDS MINIMUM CRITERION OF 96.9 X 10^{-6}

S = NOT PLATED FOR SELECTION, SUFFICIENT SURVIVING NONTOXIC DOSE LEVELS.

OT SPLIT BACK DUE TO TOXICITY

ESS THAN 5% RELATIVE GROWTH, DATA REPORTED BUT NOT USED IN EVALUATION

= CULTURE TERMINATED DUE TO EXCESSIVE TOXICITY

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APPENDIX A HISTORICAL MOUSE LYMPHOMA ASSAY CONTROL MUTANT FREQUENCY DATA

A. Nonactivation Studies

1. Pooled negative and solvent controls

Mean (± SD)	$30.2 \pm 10.9 \times 10^{-6}$
Range	9.4 to 89.8×10^{-6}
Number of experiments	50
Number of controls	155

2. Positive controls (0.25 µl/ml ethylmethane sulfonate)

Mean (± SD)	$394.9 \pm 84.6 \times 10^{-6}$
Range	167.4 to 616.4×10^{-6}
Number of experiments	50
Number of controls	50

3. Positive controls (0.4 µl/ml ethylmethane sulfonate)

Mean (± SD)	$645.8 \pm 119.7 \times 10^{-6}$
Range	451.4 to 1048.6×10^{-6}
Number of experiments	50
Number of controls	50

B. Activation Studies

1. Pooled negative and solvent controls

Mean (± SD)	$37.8 \pm 14.1 \times 10^{-6}$
Range	11.9 to 90.4×10^{-6}
Number of experiments	50
Number of controls	156

2. Positive controls (2.5 µg/ml 3-methylcholanthrene)

Mean (± SD)	$276.3 \pm 92.3 \times 10^{-6}$
Range	58.6 to 509.5×10^{-6}
Number of experiments	50
Number of controls	50

3. Positive controls (4.0 µg/ml 3-methylcholanthrene)

Mean (± SD)	$412.7 \pm 128.5 \times 10^{-6}$
Range	175.6 to 793.9×10^{-6}
Number of experiments	50
Number of controls	50

The historical control data was compiled from the most recent fifty experiments when possible. The mean (± one standard deviation) and the range of the mutant frequencies were reported for each control condition. Because some experiments contained multiple controls, the number of independent control cultures exceeded the number of experiments.

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

11-02-86

12/3/86

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity

TOX. CHEM. NO.: 727 A

ACCESSION NUMBER: 260680

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

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

SYNONYMS: (Rydex)

STUDY NUMBER(S): T2840.337

SPONSOR: Vesicol Chemical Corp.

TESTING FACILITY: Microbiological Associates, Inc.

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

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

REPORT ISSUED: 5-21-85

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

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

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

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

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

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

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

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

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

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

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

Classification: Acceptable.

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TABLE 5

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH T2840 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Cells Scored	Number and Types of Aberrations Scored[2]	Structural Aberrations Per Cell[3,4]	Cells with Structural Aberrations (%)[4,5]	Cells with Numerical Aberrations (%)[4]
Untreated Cells	100	5 tg	0	0	0
DMSO	100	7 tg, 1 sg	0	0	0
T2840 30 ug/ml	100[7]	2 tg, 2 tb, 1 af	0.03	2	0
15 ug/ml	100	3 tg, 1 tf	0.01	1	0
8 ug/ml	100	2 tg, 1 sg	0	0	0
4 ug/ml	100	5 tg, 1 sb	0.01	1	0
TEM 1 ug/ml	100	14 tg, 1 sg, 32 tb, 4 sb, 3 tf 1 r, 27 tr, 9 qr, 2 cr	0.78	50**	0

[1]CHO cells were treated for 16 hours at 37±1°C in the absence of an exogenous source of metabolic activation.
[2]Chromatid gap, tg; chromatid break, tb; chromosome gap, sg,; chromosome break, sb; chromatid fragment, tf; acentric fragments, af; dicentric, d; ring, r; triradial, tr; quadriradial, qr; complex rearrangements, cr; pulverized chromosome, pu; more than one pulverized chromosome, pu+; pulverized cell, pc; endoreduplication, e; greater than ten aberrations/cell, >10.
[3]>10 and pulverization were counted as 10 aberrations.
[4]Excluding gaps
[5]*, $p \leq 0.05$; **, $p \leq 0.01$ (Chi-square Analysis).
[6]Includes endoreduplications
[7]100 cells scored from single treatment flask due to absence of metaphase spreads in second flask due to apparent toxicity of treatment.

TABLE 6

CYTOGENETIC ANALYSIS OF CHO CELLS TREATED WITH T2840 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment[1]	Cells Scored	Number and Types of Aberrations Scored[2]	Structural Aberrations Per Cell[3,4]	Cells with Structural Aberrations (%)[4,5]	Cells with Numerical Aberrations (%)[6]
Untreated Cells	100	6 tg	0	0	0
DMSO	100	3 tg, 1 tb	0.01	1	0
T2840					
30 ug/ml	100	3 tg, 1 tb	0.01	1	0
15 ug/ml	100	3 tg	0	0	0
8 ug/ml	100	7 tg	0	0	0
4 ug/ml	100	3 tg, 1 d	0.01	1	0
CP 50 ug/ml	100	5 tg, 1 sg, 25 tb, 1 sb, 10 tf 1 r, 37 tr, 6 qr, 3 cr, 1 pc	0.93	46**	0

[1] CHO cells were treated for 2 hours at 37±1°C in the presence of an exogenous source of metabolic activation.

[2] Chromatid gap, tg; chromatid break, tb; chromosome gap, sg,; chromosome break, sb; chromatid fragment, tf; acentric fragments, af; dicentric, d; ring, r; triradial, tr; quadriradial, qr; complex rearrangements, cr; pulverized chromosome, pu; more than one pulverized chromosome, pu+; pulverized cell, pc; endoreduplication, e; greater than ten aberrations/cell, >10.

[3] >10 and pulverization were counted as 10 aberrations.

[4] Excluding gaps

[5] *, $p \leq 0.05$; **, $p \leq 0.01$ (Chi-square Analysis).

[6] Includes endoreduplications

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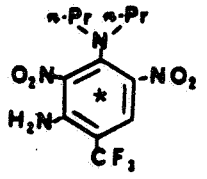
1. CHEMICAL: N^3,N^3-Dipropyl-2,4-dinitro-6-trifluoromethyl-1,3-benzenediamine; prodiamine.

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.

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(85)
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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.

Table I. Silica gel TLC R_f values for prodiamine, model metabolites and rat metabolites.

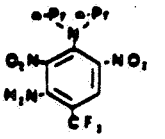
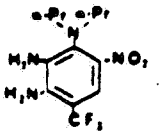
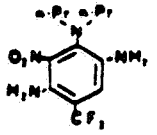
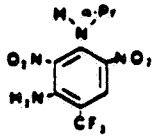
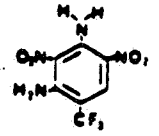
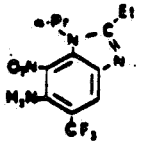
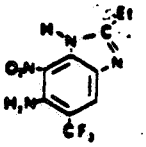
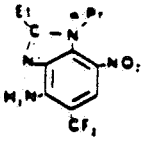
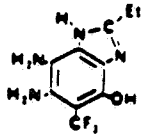
Designation and Trivial name	Structure	R_f in solvent system [a/] A	B	C
Prodiamine		0.62	0.90	-
Reduced A		0.43	0.89	-
Reduced B		0.60	0.91	-
N-despropyl		0.53	0.91	-
N,N-didespropyl		0.42	0.89	-
N-propyl benzimidazole B		0.27	0.69	0.46
6-Amino benzimidazole		0.13	0.68	-
Fecal metabolites:				
F1		0.71	0.93	-
F2 Prodiamine		0.63	0.90	-
F3		0.47	0.87	-

Table 1 cont'd

F4 N-propyl benzimidazole B	0.29	0.83	0.46
F5 N-propyl benzimidazole A 	0.24	0.84	0.44
TLC origin	0.00	0.90, 0.82 0.23, 0.00	-
Urinary metabolites:			
U1-A Prodiamine	0.63	0.90	-
U1-B N,N-didespropyl	0.43	0.89	-
U1-C	0.30	0.88	-
U2	0.07	0.80	-
U3	0.03	0.75	-
U4	0.03	0.75	-
U5	-	0.65	-
U6 Hydroxy benzimidazole 	0.00	0.45	-
U7	0.00	0.11	-
U8-A	0.00	0.21	-
U8-B	0.00	0.00	-

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a/ A = hexane/ethyl acetate (70:30); B = ethyl acetate/toluene/acetone /acetic acid/water (85:3:2:5:5); C = toluene/acetone/acetic acid (75:20:5).

Source: CBI Table I, CBI pp. 17-18.

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.

Table III. Percent of radiocarbon excreted in urine and feces of rats orally administered ^{14}C-prodiamine.[a/,b/]

		% of dose			
		Male		Female	
Group	Time (hr)	Urine	Feces	Urine	Feces
A	0-7	10.24 ± 3.21	5.27 ± 8.44	8.70 ± 1.16	0.00 ± 0.01
	7-24	18.86 ± 3.55	45.29 ± 10.51	14.70 ± 2.82	39.01 ± 16.23
	24-48	1.99 ± 0.65	12.81 ± 6.02	2.63 ± 1.91	24.67 ± 8.53
	48-72	0.45 ± 0.17	3.72 ± 5.85	0.54 ± 0.19	3.57 ± 2.34
	72-96	0.23 ± 0.08	0.32 ± 0.15	0.28 ± 0.06	1.24 ± 1.36
B	0-7	0.63 ± 0.16	0.17 ± 0.38	0.70 ± 0.41	0.00 ± 0.00
	7-24	5.47 ± 0.53	63.93 ± 16.96	4.78 ± 0.31	32.11 ± 18.56
	24-48	0.87 ± 0.22	21.90 ± 15.35	2.21 ± 1.09	50.67 ± 18.73
	48-72	0.18 ± 0.04	1.57 ± 0.75	0.31 ± 0.08	5.21 ± 5.10
	72-96	0.11 ± 0.04	0.19 ± 0.08	0.12 ± 0.03	0.55 ± 0.39
C	0-7	10.36 ± 3.75	0.00 ± 0.00	8.71 ± 2.19	0.37 ± 0.77
	7-24	18.30 ± 3.38	41.88 ± 15.47	17.64 ± 3.60	45.53 ± 21.62
	24-48	1.05 ± 0.27	18.97 ± 11.52	2.42 ± 1.56	18.46 ± 13.52
	48-72	0.32 ± 0.06	2.46 ± 1.94	0.72 ± 0.43	4.46 ± 5.68
	72-96	0.18 ± 0.03	0.44 ± 0.20	0.48 ± 0.36	0.70 ± 0.57

a/ Expressed as mean ± S.D. (n=5).

b/ A = 10 mg/kg; B = 400 mg/kg; C = continuous 10 mg/kg/day for 14 days.

Source: CBI Table III, CBI p. 20.

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Table IV. Percentage of radiocarbon in tissues of rats orally dosed ^{14}C-prodiamine. a/,b/

	% of dose					
	Group A		Group B		Group C	
Tissue	Male	Female	Male	Female	Male	Female
Heart	0.002	0.003	0.000	0.001	0.002	0.002
Spleen	0.001	0.002	0.000	0.001	0.001	0.002
Gonad	0.004	0.003	0.001	0.001	0.004	0.003
Lung	0.005	0.007	0.002	0.003	0.004	0.006
Brain	0.000	0.002	0.000	0.001	0.001	0.002
Liver	0.372	0.316	0.104	0.103	0.342	0.294
Kidney	0.028	0.035	0.009	0.013	0.028	0.034
Carcass	0.740	0.998	0.288	0.430	0.790	1.028
Total	1.152	1.367	0.403	0.553	1.172	1.370

a/ Average of 5 animals. Group A = 10 mg/kg; B = 400 mg/kg; C = continuous 10 mg/kg/day for 14 days.

b/ Animals killed at 96 hr.

Source: CBI Table V, CBI p. 22.

Table V. Radiocarbon concentration (ppm equivalent) in tissues of rats orally dosed ^{14}C-prodiamine. a/, b/

	ppm equivalent					
	Group A		Group B		Group C	
Tissues	Male	Female	Male	Female	Male	Female
Heart	0.046	0.103	0.612	1.608	0.051	0.068
Muscle	0.029	0.047	0.436	0.686	0.026	0.038
Spleen	0.056	0.110	0.711	1.798	0.058	0.124
Gonad	0.036	0.103	0.499	1.891	0.036	0.094
Lung	0.114	0.155	1.535	2.244	0.093	0.113
Bone	0.058	0.106	0.858	1.478	0.053	0.075
Brain	0.016	0.022	0.222	0.399	0.015	0.019
Fat	0.364	0.907	3.988	9.087	0.223	0.503
Liver	0.795	0.849	8.268	10.941	0.686	0.683
Kidney	0.335	0.490	3.979	6.645	0.313	0.405
Blood	0.155	0.197	2.106	3.527	0.135	0.173

a/ Average of 5 animals. Group A = 10 mg/kg; B = 400 mg/kg; C = continuous 10 mg/kg/day for 14 days.

b/ Animals killed at 96 hr.

Source: CBI Table VI, CBI p. 23.

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

Table VI. Radiocarbon characteristics and identity in the feces of rats orally administered ^{14}C-prodiamine. [a/,b/]

	% of dose					
	Group A		Group B		Group C	
Fraction and Identity	Male	Female	Male	Female	Male	Female
I. Acetone[c/]						
F1	0.26	0.16	1.68	1.14	0.24	0.25
F2 Prodiamine	0.71	0.39	51.75	50.11	0.54	0.54
F3	0.08	0.05	0.39	0.31	0.08	0.08
F4 N-propyl benzimidazole B	0.11	0.07	0.32	0.30	0.08	0.10
F5 N-propyl benzimidazole A	0.12	0.10	1.21	1.12	0.13	0.10
TLC origin[d/]	19.61	17.48	11.45	12.99	18.25	20.07
Remainder	1.36	0.91	3.16	3.89	1.43	1.05
Subtotal	22.25	19.16	69.95	69.86	20.75	22.20
II. Acetone precipitate	17.81	20.67	8.56	7.87	16.00	18.01
III. Aqueous	4.35	3.01	0.33	1.20	6.56	6.41
IV. Solids	24.19	27.60	9.17	9.41	21.04	21.97
V. Total recovery	68.60	70.45	88.01	88.33	64.34	68.58

a/ Average of 3 animals.

b/ Group A = 10 mg/kg; B = 400 mg/kg; C = continuous 10 mg/kg/day for 14 days.

c/ TLC analysis using solvent system A.

d/ After acid hydrolysis and re-TLC in solvent system B, it resolved into prodiamine, N-propyl benzimidazole B and unknown metabolites (Table I).

Source: CBI Table VIII, CBI p. 25.

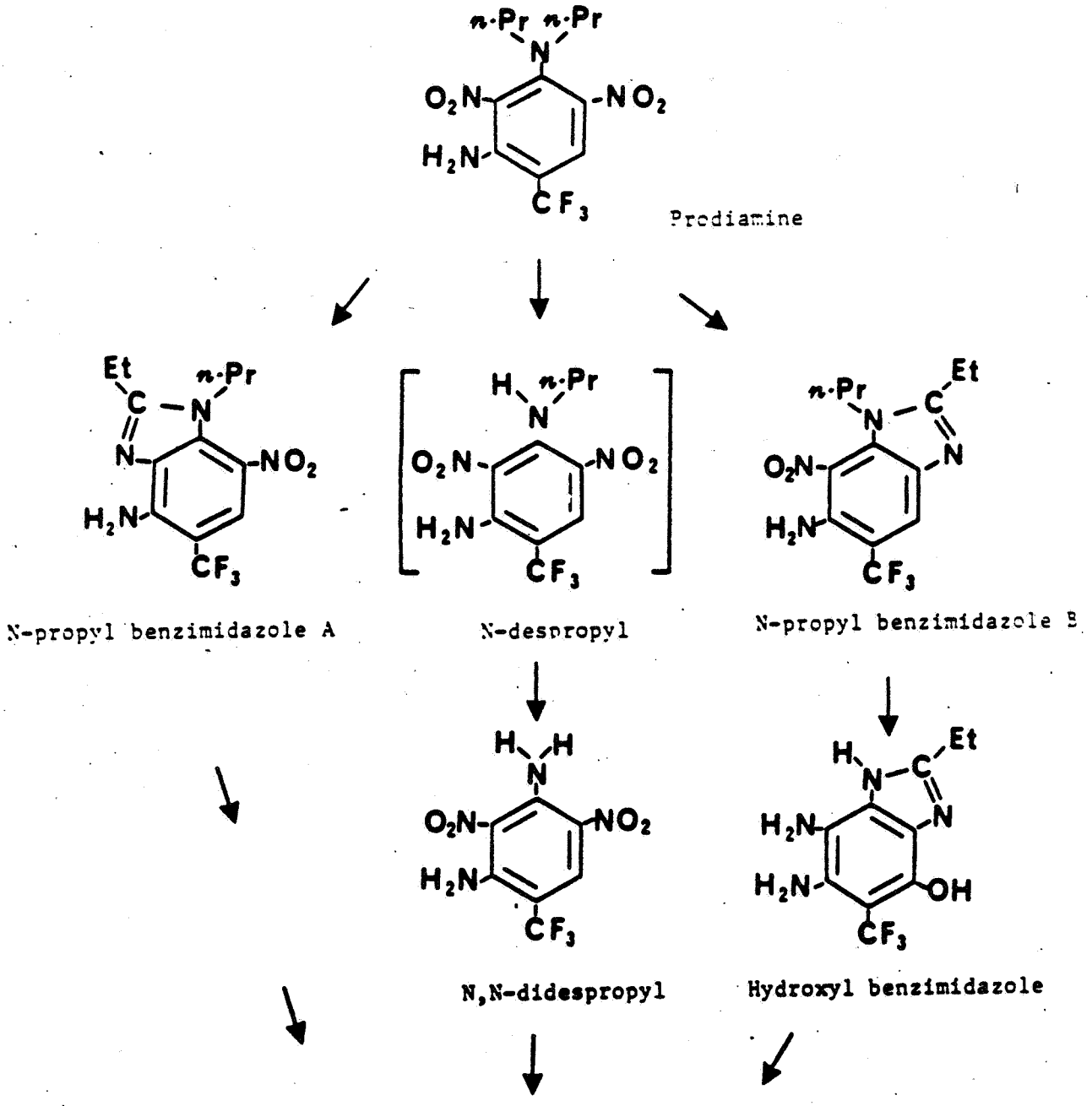

Figure 1. Proposed metabolic pathways for prodiamine in the rat.

Source: CBI Figure 17, CBI p. 45.

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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**

Table VII. Radiocarbon characteristics and identity in the urine of rats orally administered ^{14}C-prodiamine. [a/,b/]

Fraction and identity	% of dose					
	Group A		Group B		Group C	
	Male	Female	Male	Female	Male	Female
I. Methanol[c/]						
U1 Prodiamine + N,N-didespropyl	0.34	0.31	0.07	0.09	0.42	0.53
U2	0.63	0.43	0.07	0.17	0.40	0.44
U3	0.61	0.51	0.07	0.16	0.36	0.46
U4	0.84	0.65	0.06	0.25	0.36	0.30
U5	0.58	0.61	0.07	0.25	0.20	0.33
U6 Hydroxy benzimidazole	1.56	2.13	0.26	0.88	1.04	1.27
U7	1.37	1.31	0.55	0.49	0.76	1.19
U8 Prodiamine conjugate	11.12	8.35	2.24	1.57	6.94	5.64
TLC origin[d/]	9.13	6.78	2.51	2.47	12.03	10.37
Subtotal	26.20	21.10	5.89	6.34	22.50	20.54
II. Acetone	0.70	1.26	0.35	0.25	1.18	5.23
III. Water soluble	4.60	3.05	1.05	1.29	5.80	3.61
IV. Total recovery	31.50	25.41	7.29	7.88	29.48	29.38

a/ Average of 3 animals.

b/ Group A = 10 mg/kg; B = 400 mg/kg; C = continuous 10 mg/kg/day for 14 days.

c/ TLC analysis using solvent system B.

d/ After acid hydrolysis and re-TLC using solvent system B, it resolved into U1 to U8.

Source: CBI Table X, CBI p. 27.

Table VIII. Extraction characteristics of radiocarbon in liver of rats orally administered ^{14}C-prodiamine. a/,b/

Group	Sex	% of radiocarbon in liver					
		Free	Acid-rel	Base-rel	Aqueous	Solids	Total
A	M	7.58	5.95	3.03	57.49	17.51	91.56
	F	10.07	5.34	3.08	57.46	17.95	93.89
B	M	9.47	8.62	6.28	58.07	11.87	94.30
	F	13.63	8.99	5.23	51.55	9.73	89.11
C	M	6.99	6.91	4.51	56.98	17.70	93.08
	F	9.44	5.22	3.42	57.82	17.26	93.14

a/ Average of 2 animals. Group A = 10 mg/kg; B = 400 mg/kg; C = continuous 10 mg/kg/day for 14 days.

b/ Animals killed at 96 hr.

Source: CBI Table XI, CBI p. 28.

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

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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

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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)

Sandoz Crop Protection Corp.
341 East Ohio Street
Chicago, IL 60611

August 6, 1986

Protocol

METABOLISM OF PRODIAMINE IN RATS

OBJECTIVE

To conduct a metabolism study of prodiamine in rats according to EPA guidelines (Pesticide Assessment Guideline, Subdivision F, November 1982).

INTRODUCTION

Prodiamine absorption, distribution and metabolite characterization in rats were previously carried out (Dressler 1975 a,b). About 58% of the dose was recovered in feces (90% of them excreted within 48 hr). Urinary ^{14}C accounted for 29% of the dose and expired $^{14}CO_2$ accounted for 0.14%.

A comparative metabolism study of prodiamine in rats and goats was conducted by Nietschmann (1985). Most of the oral dose was absorbed by rats and then eliminated via bile to feces. Prodiamine was extensively metabolized to polar metabolites. Only trace amount of ^{14}C in feces and bile was identified as unchanged prodiamine.

In these studies, a conclusion was reached that prodiamine was rapidly absorbed and eliminated by rats. Very low residue level was found in animal tissues. However, a majority of the metabolites was characterized as polar metabolites. These metabolism studies were not carried out sufficiently to meet the current EPA guideline. Therefore, a new metabolism study will be conducted to meet this guideline.

TIME SCHEDULE

This study should be completed by March 1987.

MATERIALS AND METHODS

This study is to be conducted under GLP and pertinent standard operating procedures will be followed.

1. Labeled compound: ^{14}C-prodiamine (2,4-dinitro-N^3,N^3-dipropyl-6-trifluoromethyl-1,3-benzenediamine, UL-^{14}C), with a specific activity of 25.0 mCi/mmole and radiochemical purity of greater than 98% was synthesized by Wizard Laboratories, Davis, CA. Unlabeled analytical grade prodiamine (greater than 99% pure) and model metabolites will be provided by Sandoz Crop Protection Corporation.

2. Animals: Seven to nine weeks old Charles River Sprague-Dawley (CD) rats will be used.

   a. Sex: Males and females.

   b. Number: Five male and five female rats at each dose level.

   c. Quarantine: At least 7 days.

   d. Randomization: Animals from each sex will be assigned at random to each group. The weight variation of the animals of each sex should not exceed 10% of the mean weight.

3. Dose groups: EPA guidelines describes that oral (low, high and consecutive doses) and intravenous doses of test compound should be conducted. However, the intravenous administration will not be conducted because of poor solubility of prodiamine in physiological solutions or water (0.013 ppm).

a. Low dose level: A single oral dose of ^{14}C-prodiamine in Emulphor EL-620 (a polyoxyethylated vegetable oil, GAF Corp., NY, NY) will be administered to rats at 10 mg/kg (NOEL) (radioactivity ca. 25 μCi/rats; Emulphor ca. 2 ml/kg).

b. High dose level: A single oral dose of ^{14}C-prodiamine in Emulphor EL-620 will be administered at 400 mg/kg (Produce some hematological alterations and liver weight gains) (radioactivity ca. 25 μCi/rat; Emulphor ca. 2 ml/kg).

c. Consecutive dose level: A single oral dose of ^{14}C-prodiamine in Emulphor will be administered to rats at 10 mg/kg (NOEL) (radioactivity ca. 25 μCi/rat; Emulphor ca. 2 ml/kg) within 24 hr after pretreatment with 14 consecutive daily oral doses of unlabeled prodiamine in Emulphor (ca. 2 ml/kg) at 10 mg/kg (NOEL).

4. Observation period: Animals will be kept in individual cages for 4 days after radioactive dose at which time all of the animals will be killed.

The following items must be observed:

1) Check the radiochemical purity of ^{14}C-prodiamine dissolved in Emulphor prior to administration to ascertain the stability of prodiamine in Emulphor.
2) Weigh each rat before compound administration and sacrifice.
3) Administer prodiamine by intubation.
4) Measure daily food consumption and water intake.
5) Observe clinical sign.

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6) House the treated rats individually in metabolism cages to collect urine and feces separately.

7) Add 1 ml of saturated mercuric chloride solution to urine collection cup to prevent microbial degradation.

8) Collect urine and feces from each rat at 7 hr, 1 day and thereafter each day after ^{14}C-prodiamine dosing.

9) Measure urine volume and feces weight at each sampling time.

10) Freeze urine and feces at - 20°C prior to analysis.

ANALYSIS

a) ^{14}C recovery: For each of all rats of the three dose groups, the radiocarbon in the urine and feces samples will be measured. The following analytical techniques should be observed:

1) Radioassay each sample in duplicate.

2) Radioassay ^{14}C in urine by addition of aliquots of urine to vials containing suitable scintillation fluid.

3) Radioassay ^{14}C in feces by combustion of a known weight of the samples.

b) ^{14}C tissue residues: For each rat of the three dose groups, the radiocarbon in bone, brain, fat, gonad, heart, kidney, liver, lung, blood, muscle, spleen and residual carcass will be measured after termination of the experiment.

c) ^{14}C metabolites: Metabolite extraction, isolation, identification and quantitation will be conducted on excreta and tissue samples containing enough radioactivity. Suitable extraction, cleanup and instrumental procedures will be used. Authentic model metabolites will be synthesized and used as references.

RESULTS

a) ^{14}C recovery: Data will be expressed as percent of the dosed radiocarbon for each rat and also as mean values ± SD of five rats.

b) ^{14}C tissue residues: Data will be expressed as μg prodiamine equivalents/g wet tissue and percent recovery of the administered dose for each rat and also as mean values ± SD of five rats.

c) ^{14}C metabolites: Data will be expressed as percent of the administered dose for urinary and fecal metabolites of each rat and also as mean values ± SD of five rats for each urinary and fecal metabolites.

Data will be summarized in tabular forms with statistical evaluations. As shown in the EPA guideline, all data including the counting efficiency, combustion efficiency and radioautogram should be retained.

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Nietschmann, D. A. 1985. Comparative metabolism of prodiamine, a dinitroaniline herbicide in rats and goats. M.S. thesis. Illinois Institute of Technology, Chicago, IL.

APPROVAL

Craig Chen Jr. August 6, 1986

Study Director Date

L H Patterson Aug. 6, 1986

Director, Environmental Sciences Date