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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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OFFICE OF PESTICIDE PROGRAMS
Health Effects Division

MEMORANDUM

SUBJECT: Review of Additional Data of a Carcinogenicity Study with Diuron in the Diet of Mice (original MRID#421595-01 and 421595-02)

TO: Linda Propst, Peg Perrealt PM-73
SRRD/Reregistration (7508W)

FROM: David S. Liem, Ph.D. 4/11/94
Section II, Toxicology Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head *K. Clark Swentzel 4/11/94*
Section II, Toxicology Branch II/HED (7509C)
and
Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II/HED (H7509C) *M van Gemert 4/12/94*

MRID No.: 421595-01 and -02 (original) DP Barcode No.: D198141
Submission #: S456163 Caswell No.: 410

ACTION REQUESTED

To review two additional submissions from the Registrant dated July 25 and September 16, 1993, in response to deficiencies noted in a study entitled "Diuron: Study for Chronic Toxicity and Carcinogenicity with NMRI Mice (Administration in Diet for 24 Months)" (original MRID#421595-01), that was classified as core-supplementary (original DER dated 4/1/92; doc#009486).

BACKGROUND

The study was reviewed and classified by the Toxicology Branch II as core-supplementary (DER dated April 1, 1992, #009486) because of numerous deficiencies in the study report. The registrant submitted additional information to the Agency dated on July 15 and September 16, 1993 (MRID#s were not provided). This attached supplemental D&R is an evaluation of the additional information and a rebuttal of some histopathological conclusions on this study, submitted by the registrant. After evaluation of the additional information, the mouse oncogenicity with diuron is now upgraded to core-minimum. The revised executive summary for this study (original MRID# 421595-01 and original HED document #009486 dated 4/1/92) is presented below.

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AMENDED EXECUTIVE SUMMARY

The following amended executive summary was based on data presented in the original report (MRID#421595-01), and the additional data submitted by the registrant on July 25 and September 16, 1993 (MRID# not supplied) in response to TOXII request.

Technical diuron was administered in the diet of four groups of 60 NMRI mice/sex per group at 0, 25, 250, and 2500 ppm ($\sigma = 5.4, 50.8, \text{ and } 640.1 \text{ mg/kg/day}$ and $\rho = 7.5, 77.5 \text{ and } 869.0 \text{ mg/kg/day}$) for 24 months. Administration of technical diuron in the diet of NMRI mice at these dose levels for 24 months resulted in the following treatment-related effects:

- o A body weight decrease in the 2500 ppm (640.1 mg/kg/day) males.
- o Increased spleen and liver weight in the 2500 ppm (640.1 mg/kg/day) males.
- o Elevated leucocyte and reticulocyte counts in the 2500 ppm ($\sigma = 640.1 \text{ mg/kg/day}$; $\rho = 867.0 \text{ mg/kg/day}$) mice of both sexes.
- o Elevated mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the 2500 ppm mice ($\sigma = 640.1 \text{ mg/kg/day}$; $\rho = 867.0 \text{ mg/kg/day}$) of both sexes.
- o An elevated bilirubin in the 2500 ppm ($\sigma = 640.1 \text{ mg/kg/day}$; $\rho = 867.0 \text{ mg/kg/day}$) mice of both sexes.
- o Increased incidence of intracellular golden brown pigments in the cortical renal tubules in the 2500 ppm (867.0 mg/kg/day) females, and in the spleen of the 2500 ppm ($\sigma=640.1 \text{ mg/kg/day}$; $\rho = 867.0 \text{ mg/kg/day}$) males and females
- o An increased incidence of hemosiderin deposits in liver cells of the 2500 ppm (640.1 mg/kg/day) males.
- o Increased incidence of liver single cell necrosis and cell mitosis in the 2500 ppm ($\sigma=640.1 \text{ mg/kg/day}$; $\rho= 867.0 \text{ mg/kg/day}$) mice of both sexes.
- o An increased incidence of enlarged degenerative liver cells in the 2500 ppm (867.0 mg/kg/day) females,
- o Increased incidence of hepatopathy and Kupffer cells in the 2500 ppm (640.1 mg/kg/day) males.
- o Increased incidence of urinary bladder edema, epithelial hyperplasia, and thickened mucosa in the 2500 ppm (867.0 mg/kg/day) females.
- o An increased incidence of enlarged (with > 2 mm diameter) uterine horn in the 2500 ppm (867.0 mg/kg/day) females.

2

- o Increased incidence of ovarian luteoma and mammary gland adenocarcinoma in the 2500 ppm (867.0 mg/kg/day) females.
- o TOXII concurs with the registrant that this study was conducted to evaluate the carcinogenic potential of Diuron in mice (Guideline §83-2) and not considered as a "Chronic Toxicity and Carcinogenicity Study in Mice" (Guideline §83-1 and §83-2) as originally submitted by the registrant (MRID#421595-01 & -02). Thus, this study is now considered as a mouse carcinogenicity study (Guideline §83-2)

Based on the data presented in the study report, the systemic LOEL for diuron is determined to be 2500 ppm ($\sigma=640.1$ mg/kg/day; $\rho=867.0$ mg/kg/day) based on the following treatment-related effects: decreased body weight, and increased spleen and liver weight in males; elevated leucocyte and reticulocyte counts, mean corpuscular volume and mean corpuscular hemoglobin, and bilirubin values in both sexes; increased incidence of intracellular golden brown pigments in renal tubules in females and in the spleen of males and females; increased incidence of hemosiderin deposits in liver cells in males; increased incidence of liver single cell necrosis and cell mitosis in both sexes; increased incidence of enlarged degenerative cells in females and of hepatopathy and Kupffer cells in males; increased incidence of urinary bladder edema and epithelial hyperplasia, thickened mucosa and an enlarged (with > 2 mm diameter) uterine horn in females. The systemic NOEL for diuron is 250 ppm ($\sigma = 50.8$ mg/kg/day; $\rho = 77.5$ mg/kg/day) for both sexes of NMRI mice.

The doses employed in this study were sufficient to produce a compound-related systemic effect and appear to be adequate to test the carcinogenic potential of the test material, however, this will be determined by the HED Cancer Peer Review Committee.

This study is considered to be a 6(a)2 study, because diuron affected the hematopoietic system and there is evidence of a carcinogenic effect.

The data of this study together with the two rat chronic feeding/oncogenicity studies (MRID# 408865 and 00017764) will be presented to the HED Cancer Peer Review Committee.

CLASSIFICATION: This study satisfies USEPA's Guideline 83-2 requirements for a carcinogenicity study in mice, and it is now upgraded to core-minimum.

Primary Reviewer: David S. Liem, Ph.D.
Section II, Toxicology Branch II/HED
Secondary Reviewer: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II/HED

010102
H. Clark Swentzel
4/11/94

SUPPLEMENTAL DATA EVALUATION REPORT

Original MRID#421595-01/02
Original Document #009486

Study Type: Carcinogenicity Study in NMRI Mice (§83-2)

EPA Identification No.s: Original MRID#: 421595-01 and 421595-02
Additional Submissions: July 25 and September 16, 1993
DP Barcode#: D198141 Caswell No.:410
Submission #: S456163 PC#: 035505

Test Material: Diuron with a purity of 98.7%; batch No.23114080



Synonym: N'-(3,4-dichlorophenyl)-N,N-dimethyl urea

Dosages: 0, 25, 250, and 2500 ppm

Sponsor: Agricultural Products Department,
E.I. du Pont de Nemours & Co., Inc.

Study Number: Bayer AG T 4010922; Du Pont Report No. DIUR/TOX9

Study Period: October 1981 and October 1983

Testing Facilities: Institute of Toxicology-Industrial Chemicals
(in-life study) and the Institute of
Toxicology-Pharmacology (Clinical laboratory
tests and pathology studies), Fachbereich
Toxikologie, Bayer AG, Friedrich-Ebert-
Strasse 217-333, Wuppertal, Germany.

Title of Report: Diuron: Study for Chronic Toxicity and
Carcinogenicity with NMRI Mice (Administration
in diet for 24 Months)

Author: Dr. R. Eiben

4
4

Report Issued: The study was completed in October 29, 1983; Study Director signature on the report dated May 24, 1990; Translation was completed in January 1991; Final Report submission date is December 19, 1991.

Background: The study was reviewed and classified by the Toxicology Branch II as core-supplementary (DER dated May 7, 1992, #009486) because of numerous deficiencies in the study report. The registrant submitted additional information to the Agency dated on July 15 and September 16, 1993 (MRID#s were not provided). This Supplemental DER is an evaluation of the additional information and a rebuttal of some histopathological conclusions on this study submitted by the registrant. The revised executive summary for this study (original MRI # 421595-01 and original HED document #009486) is presented below.

Amended Executive Summary

The following amended executive summary was based on data presented in the original report (MRID#421595-01), and the additional data submitted by the registrant on July 25 and September 16, 1993 (MRID# not supplied) in response to TOXII request.

Technical diuron was administered in the diet of four groups of 60 NMRI mice/sex per group at 0, 25, 250, and 2500 ppm (σ = 5.4, 50.8, and 640.1 mg/kg/day and ρ = 7.5, 77.5 and 869.0 mg/kg/day) for 24 months. Administration of technical diuron in the diet of NMRI mice at these dose levels for 24 months resulted in the following treatment-related effects:

- o A body weight decrease in the 2500 ppm (640.1 mg/kg/day) males.
- o Increased spleen and liver weight in the 2500 ppm (640.1 mg/kg/day) males.
- o Elevated leucocyte and reticulocyte counts in the 2500 ppm (σ = 640.1 mg/kg/day; ρ = 867.0 mg/kg/day) mice of both sexes.
- o Elevated mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the 2500 ppm mice (σ = 640.1 mg/kg/day; ρ = 867.0 mg/kg/day) of both sexes.
- o An elevated bilirubin in the 2500 ppm (σ = 640.1 mg/kg/day; ρ = 867.0 mg/kg/day) mice of both sexes.
- o Increased incidence of intracellular golden brown pigments in the cortical renal tubules in the 2500 ppm (867.0 mg/kg/day) females, and in the spleen of the 2500 ppm (σ =640.1 mg/kg/day; ρ = 867.0 mg/kg/day) males and females

5

5

- o An increased incidence of hemosiderin deposits in liver cells of the 2500 ppm (640.1 mg/kg/day) males.
- o Increased incidence of liver single cell necrosis and cell mitosis in the 2500 ppm (σ =640.1 mg/kg/day; ρ = 867.0 mg/kg/day) mice of both sexes.
- o An increased incidence of enlarged degenerative liver cells in the 2500 ppm (867.0 mg/kg/day) females,
- o Increased incidence of hepatopathy and Kupffer cells in the 2500 ppm (640.1 mg/kg/day) males.
- o Increased incidence of urinary bladder edema, epithelial hyperplasia, and thickened mucosa in the 2500 ppm (867.0 mg/kg/day) females.
- o An increased incidence of enlarged (with > 2 mm diameter) uterine horn in the 2500 ppm (867.0 mg/kg/day) females.
- o Increased incidence of ovarian luteoma and mammary gland adenocarcinoma in the 2500 ppm (867.0 mg/kg/day) females.
- o TOXII concurs with the registrant that this study was conducted to evaluate the carcinogenic potential of Diuron in mice (Guideline §83-2) and not considered as a "Chronic Toxicity and Carcinogenicity Study in Mice" (Guidelines §83-1 and §83-2) as originally submitted by the registrant (MRID#421595-01 &-02). Thus, this study is now considered as a mouse carcinogenicity study (Guideline §83-2)

Based on the data presented in the study report, the systemic LOEL for diuron is determined to be 2500 ppm (σ =640.1 mg/kg/day; ρ = 867.0 mg/kg/day) based on the following treatment-related effects: decreased body weight, and increased spleen and liver weight in males; elevated leucocyte and reticulocyte counts, mean corpuscular volume and mean corpuscular hemoglobin, and bilirubin values in both sexes; increased incidence of intracellular golden brown pigments in renal tubules in females and in the spleen of males and females; increased incidence of hemosiderin deposits in liver cells in males; increased incidence of liver single cell necrosis and cell mitosis in both sexes; increased incidence of enlarged degenerative cells in females and of hepatopathy and Kupffer cells in males; increased incidence of urinary bladder edema and epithelial hyperplasia, thickened mucosa and an enlarged (with > 2 mm diameter) uterine horn in females. The systemic NOEL for diuron is 250 ppm (σ = 50.8 mg/kg/day; ρ = 77.5 mg/kg/day) for both sexes of NMRI mice.

The doses employed in this study were sufficient to produce a compound-related systemic effect and appear to be adequate to test the carcinogenic potential of the test material. The HED Cancer Peer Review Committee will evaluate these data further.

This study is considered to be a 6(a)2 study, because diuron affected the hematopoietic system and there is evidence of a carcinogenic effect.

The data of this study together with the two rat chronic feeding/oncogenicity studies (MRID# 408865 and Doc# 00017764) will be presented to the HED Cancer Peer Review Committee.

The tumor data as presented in the original study report are attached in Appendix B.

CLASSIFICATION: This study satisfies USEPA's Guideline 83-2 requirements for a carcinogenicity study in mice, and it is now upgraded to core-minimum.

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EVALUATION OF THE ADDITIONAL DATA SUBMITTED BY THE REGISTRANT IN RESPONSE TO THE AGENCY'S REQUEST

A. Body weight gains were not presented in the study report.

Registrant's Response: The Registrant submitted the mean body weight gains over various intervals as well as the overall body weight gain throughout the study period. Data of selected intervals are summarized as follows (only the percent body weight gains for 2500 ppm groups were calculated):

Intervals Weeks	0 ppm ♂ / ♀	25 ppm ♂ / ♀	250 ppm ♂ / ♀	2500 ppm ♂ / ♀
0 - 18	13.4 / 8.0	12.2 / 8.0	13.1 / 7.3	10.9 / 8.4 (-19% / +5%)
0 - 26	17.0 / 10.1	16.4 / 9.9	16.1 / 8.8	13.4 / 9.9 (-21% / -2%)
0 - 52	19.2 / 13.4	18.3 / 13.5	18.6 / 12.6	15.5 / 12.0 (-19% / -5%)
0 - 78	19.2 / 15.4	17.5 / 15.8	17.7 / 15.4	15.1 / 13.5 (-21% / -12%)
0 - 102	15.0 / 14.7	14.3 / 16.8	15.9 / 15.8	13.9 / 12.9 (-7% / -12%)

TOXII Evaluation: The data showed that the mean body weight gain in the high-dose males was less than the controls; the percent body weight gain reductions in the high-dose males were more than 10% at various intervals, except for the overall (weeks 0-102) value. The mean percent body weight gain in the high-dose females did not differ greatly as compared to the control. These additional data confirmed TOXII's previous conclusions (DER dated 4/1/92 doc#009486) that the body weight reduction in the high-dose males was judged to be related to treatment.

B. The mean food intake efficiency data (body weight gain divided by food consumed multiplied by 100) were not calculated nor were they discussed in the report.

Registrant's Response: The requested feeding efficiency data were submitted.

TOXII Evaluation: The mean food intake efficiency data as presented by the registrant did not showed feeding efficiency differences between the treated groups as compared to the controls and no dose-related trends were evident. This confirmed the previous evaluation (DER dated 4/1/92; doc#009486).

C. The oviduct and the mesenteric lymph node were not harvested and were not subjected to histopathological evaluations.

8

Registrant's Response: Histopathological evaluations of the mesenteric lymph node and oviduct were not required by the OECD guidelines.

TOXII Evaluation: Both OECD guidelines 451 (oncogenicity study) and 453 (combined chronic toxicity/oncogenicity study), as well as EPA guidelines (§83-2 and §83-5), call for histopathological evaluation of accessory genital organs, including the oviduct. This missing data did not compromise the integrity of the study. Evaluation of the mandibular instead of mesenteric lymph node is acceptable.

- D. No explanation was given whether the summary gross macroscopic data presented on p. 422-429 of the study report were findings of all mice on study throughout the 24 month period or findings of mice after the 12 month study period. Separate summary tables are required for interim sacrificed mice and for those that were found dead or sacrificed in extremis within the first 12 months of study.

Registrant's Response: Gross macroscopic findings on p. 422-429 were from found dead, moribund and terminal sacrificed mice, excluding autolyzed and the 12-month interim sacrificed mice. The registrant submitted a summary table of gross macroscopic evaluation of tissues of the interim sacrificed mice.

TOXII Evaluation: The data confirmed the previous assessment (DER dated 4/1/92; doc#009486), that no treatment-related macroscopic findings were evident in mice during the first 12 months of the study.

- E. The brain and ovaries were not weighed.

Registrant's Response: The weighing of ovaries are not required by OECD guideline 453 for a combined chronic/oncogenicity study.

TOXII Evaluation: OECD guideline 453 and EPA guidelines §83-2 and §83-5 require that the brain and gonads (including ovaries) be weighed. Oversight for not weighing the brain did not compromise the integrity of the study, since no significant macro- and microscopic findings were evident. Oversight for not weighing the ovaries did not compromise the integrity of the study, because the microscopic evaluation conducted on the ovaries has been adequate to determine the possible ovarian effects. Therefore, previous conclusions on the organ weight (DER dated 4/1/94; Doc#009486) remain unchanged, namely, only the increased mean absolute spleen and liver weights in the high-dose males are considered to be related to treatment based on the data presented.

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F. The reticulocyte count at the 6 month interval was not provided in the summary Table on p. 44 of the study report, and no explanation was given.

Registrant's Response: Evaluation of reticulocytes was conducted in the mouse study after it was apparent that a hematotoxic effect was evident in a rat study conducted in parallel with the mouse study.

TOXII Evaluation: The omission of reticulocyte counts at the sixth month interval did not affect the interpretation of a treatment-related elevation of the reticulocyte count in high-dose (2500 ppm) male and female mice. Thus, the previous conclusion (DER dated 4/1/92; doc#009486) on the treatment-related elevation of the reticulocyte count in the high-dose (2500 ppm) males and female mice, remains the same.

G. The following clinical chemistry parameters were not determined: albumin, inorganic phosphate, calcium, sodium, potassium, chloride, and creatine phosphokinase.

Registrant's Response: The registrant noted that due to limited blood sample volume available, not all parameters were evaluated.

TCXII Evaluation: Since it was determined that the mouse study with diuron is a carcinogenicity study, evaluation of these clinical chemistry parameters are not required.

H. Urinalysis was not determined.

Registrant's Response: The registrant noted that since small amount of urine is excreted, urinalysis was not practical.

TOXII Evaluation: Since it was determined that the mouse study with diuron is a carcinogenicity study, urinalyses is not required.

I. The number of animals in the historical controls on hematology and clinical chemistry data (p. 81-82) were not provided. Only the historical control values after 1981 (the year when this study was conducted) were provided.

Registrant's Response: The registrant provided the requested historical control data.

TOXII Evaluation: The additional information submitted did not change TOXII's previous conclusions (DER dated 4/1/94; Doc#009486), that treatment-related effects were elevation of leucocyte, mean corpuscular volume, mean corpuscular hemoglobin, and bilirubin, all noted in the 2500 ppm mice of both sexes.

J. Historical control data for neoplastic lesions in the strain of mouse were not provided with the study report.

Background: TOXII requested the historical control tumor data mice in order to determine whether statistically significant increased incidences of ovarian luteoma and mammary gland adenocarcinoma in the 2500 ppm females were related to treatment, as was concluded in the previous evaluation (DER 4/1/92; Doc#009486).

Registrant's Response: The registrant provided historical control data of neoplastic lesions derived from 18 NMRI mouse studies conducted from 1981 to 1988 at Bayer's Laboratories, and from a published paper on 12 mouse studies conducted in three different laboratories (Bomhard and Mohr, 1989), as well as data from other publications (see Appendix A). Based on these data, the registrant did not consider the increased incidence of ovarian luteoma and mammary gland adenocarcinoma in the mouse carcinogenicity study with diuron to be related to treatment, because of the following reasons:

o Since the ovarian luteoma is classified as sex cord stromal tumor, ovarian luteoma should be combined with other sex cord stromal tumors (i.e. arrhenoblastoma, androblastoma, and thecoma, granulosa, lipid, Sertoli, and Leydig cell tumors), when evaluating an increased incidence of ovarian tumors. When all types of sex cord stromal tumors were combined, the increase of the overall ovarian tumor incidences in the diuron treated groups were within the historical control range of 0 - 35.5% reported in published papers (see further TOXII discussions below).

o Although the mammary gland adenocarcinoma increase was statistically significant (increased by 12%) in the 2500 ppm females, this increase was within the historical control range for carcinoma reported in published papers (9%-14% for ad libitum and 2%-9% for restricted fed mice). The registrant considered that the observed increase of 12% in the 2500 ppm diuron treated females to be within the historical control range and concluded that diuron is not carcinogenic for the mammary gland.

TOXII EVALUATION: Spontaneous tumors incidence is unpredictably variable among groups of concurrent controls as well as among control groups of the same strain from different studies and laboratories. It can vary due to various factors such as the age when the animals were necropsied, feed, animal supplier, husbandry conditions, when or where studies were conducted, and the uniformity of pathological evaluations. Thus, it is essential that these factors should be considered when using historical control data.

To address these variables, pertinent supporting documents submitted by the registrant are summarized below.

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Table A: Ovarian and mammary gland tumors incidence/percentages in NMRI mice carcinogenicity study with Diuron (MRID#421595-01)

Tumor	0 ppm(# / %)	25 ppm(# / %)	250 ppm(# / %)	2500 ppm(# / %)
Total # Mice Examined	50	47	49	50
OVARIAN TUMORS				
-Granulosa/Theca Cell Tumor				
+ Unilateral (b)	7 / 14%	4 / 9%	11 / 22%	5 / 10%
+ Bilateral (b)	1 / 2%	1 / 2%	2 / 4%	2 / 4%
+ Unilateral (m)	0 / 0%	1 / 2%	0 / 0%	0 / 0%
-Luteoma				
+ Unilateral (b)	3 / 6%	0 / 0%	2 / 4%	7** / 14%**
+ Bilateral (b)	0 / 0%	1 / 2%	0 / 0%	0 / 0%
-Tubular Cystadenoma (b)	2 / 4%	1 / 2%	1 / 2%	0 / 0%
-Leiomyoma - Unilateral (b)	0 / 0%	0 / 0%	0 / 0%	1 / 2%
-Teratoma - Unilateral (b)	0 / 0%	1 / 2%	0 / 0%	0 / 0%
-All sex cord stromal tumors\$	11 / 22%	7 / 15%	14 / 29%	14 / 28%
-All Ovarian Tumors	13 / 26%	9 / 17%	16 / 31%	15 / 30%
MAMMARY GLAND TUMORS				
-Adenocarcinoma (m)	2 / 4%	1 / 2%	1 / 2%	6* / 12%*
-Carcinoma, Anaplastic (m)	0 / 0%	1 / 2%	0 / 0%	0 / 0%
-Adenocarcinoma Type A & B combined	2 / 4%	2 / 4%	1 / 2%	6* / 12%*

b = benign; m = malignant; * = P ≤ 0.05, ** = P ≤ 0.01; derived from p. 73-74 of the study report; Source of mice: Winkelmann, Borchen; Strain of Mice: BOR:NMRI(SPF Ha.) Mice; \$ = Combined Granulosa/Theca Cell and Luteoma Tumors.

Table B: Spontaneous Ovarian and Mammary Gland Tumors in NMRI Mice Reported in Published Papers

TUMOR	Rehm et al (1984) Source of Mice: CILAB/ Hanover Laboratory: Hanover Total # Mice: a=43; b=4339 Strain: Han: NMRI Mice ^d	Rehm et al (1985) Source of Mice: CILAB, Hanover Laboratory: Hanover Total # Mice: a=1478; b=1459 Strain: Han: NMRI Mice	Lohris et al (1984) ^e Source of Mice: Tübingen Laboratory: Haidelberg Total # Mice: a=150 Strain: Sw: NMRT Mice
OVARY			
-Tubular Adenocarcinoma	a = 0% b = 8%	ni	ni
-Tubular Adenoma	a = 2.7% b = 49%	ni	ni
-Granulosa Cell Tumor	a = 1.9% b = 25%	ni	a = 6.7%
-Luteoma (b)	a = 3% b = 3%	ni	ni
-Sertoli Cell tumor (b)	a = 9% b = 9%	ni	ni
-Teratoma	a = 0.7% b = 0%	ni	ni
-Mesenchymal tumors	a = 0% b = 0.7%	ni	ni
All sex cord stromal tumors\$	a = 30% b = 37%	ni	a = 6.7%
All Ovarian Tumors	a = 69.7% b = 94.7%	a = 52% b = 65%	a = 6.7%
MAMMARY GLAND			
-Carcinoma Type A and B	ni	a = 9.5% b = 2.1%	ni
-Carcinoma Type C	ni	a = 2.0% b = 0.7%	ni
-Carcinosarcoma	ni	a = 0% b = 1.4%	ni
-Adenoma	ni	ni	a = 4.0%
-Adenoacanthoma	ni	a = 2.7% b = 1.4%	ni
-Adenocarcinoma	ni	a = 4.0% b = 2.0%	a = 6.7%
-Sarcoma	ni	a = 0% b = 2.0%	a = 0.7%
Adenocarcinoma type A & B ^f	ni	a = 13.5% b = 4.1%	a = 10.7%
All Mammary Gland Tumors	ni	a = 18% b = 9.6%	a = 11.4%

a = ad libitum feeding; b = restricted feeding; ni = not indicated; B = controls with 16.7% body fat; @ = untreated mice kept until they died; \$ = luteoma, and granulosa and Sertoli cell tumor; # = Carcinoma types A and B, Adenoma, and Adenocarcinoma

Table C: Historical Control of Spontaneous Ovarian and Mammary Gland Tumor Incidence in NMRI Mice Conducted at Bayer

TUMOR	From 12 Studies (1974-1979) ^o			From 18 Studies (1981-1988) ^{oo}		
	#Obs.	Total#	%	#Obs.	Total#	%
OVARY						
-Granulosa/Theca Cell Tumor (b)	108	591	18.3%	60	862	7.0%
-Granulosa/Theca Cell Tumor (m)	5	591	0.9%	9	862	1.0%
-Interstitial Cell Tumor (b)	-	591	-	1	862	0.1%
-Adenoma (b)	-	591	-	1	862	0.1%
-Tubular Adenoma (b)	-	591	-	9	862	1.0%
-Papillary Adenoma (b)	1	591	0.2%	5	862	0.6%
-Luteoma (b)	-	591	-	15	862	1.7%
-Luteal Cell tumor (b)	-	591	-	2	862	0.2%
-Sertoli Cell tumor (b)	-	591	-	2	862	0.2%
-Cystadenoma	9	591	1.5%	-	-	-
-Adenocarcinoma, papillary	3	591	0.5%	-	-	-
All sex-cord stromal tumors^s	113	591	19.1%	88	862	10.2%
All Ovarian Tumors	121	591	20.3%	104	862	12.1%
MAMMARY GLAND						
-Adenoma (b)	5	591	0.9%	2	717	0.2%
-Carcinoma (m)	-	591	-	22	717	3.1%
-Carcinosarcoma (m)	-	591	-	1	717	0.1%
-Adenocanthoma (m)	1	591	0.2%	4	717	0.6%
-Adenocarcinoma	14	591	2.4%	-	-	-
Adenocarcinoma Type A & B&	19	591	3.3%	24	717	3.3%
All Mammary Gland Tumors	20	591	3.5%	29	717	4.0%

OBS. = Total # of lesions observed; TOTAL# = Total # of mice examined; % = Percentage of lesions; b = benign; m = malignant; - = none observed; @ = From Bomhard and Mohr (1989) - variable study durations, 1 study was 132 weeks, 1 study was 147 weeks, 1 study was 126 weeks, 4 studies were 108 weeks, and 5 studies for 104 weeks; @@ = From the registrant submission (9/16/93); S = combined arrhenoblastoma, androblastoma, and granulosa, thecoma, Sertoli, Leydig, and lipid cell tumor; & = Adenoma, carcinoma, and adenocarcinoma; r = ranging from 5% - 35.5%.

Tables B and C show variations of spontaneous ovarian and mammary gland tumor incidence. For example, the ovarian luteoma for 18 mice studies conducted in 1981 - 1988 at the Bayer's laboratory was only 1.7%, while Bomhard and Mohr (1989) did not report any luteoma occurrence from their 12 studies (Table C). Rehm et al (1984) noted that spontaneous ovarian luteoma incidence in NMRI mice was rare and gave a 3% (for *ad libitum* and restricted fed control mice) spontaneous occurrences (Table B). The concurrent controls in the diuron mouse carcinogenicity study showed a 6% spontaneous ovarian luteoma incidence (Table A). When all ovarian sex cord stromal tumors (i.e. granulosa/theca cell tumors and luteoma) in the diuron mouse carcinogenicity study are combined, the tumor incidence increase in the 2500 ppm diuron dose level was not significantly higher than the concurrent controls (22% in control versus 28% in the 2500 ppm dose level; see Table A). The combined ovarian sex cord stromal tumor historical control incidence for studies conducted at the

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13

Bayer laboratories (1981-1988) was 10.2% and Bomhard and Mohr (1989) cited a 19.1% (with a range of 5.0% - 35.5%) spontaneous incidence for these tumors in 12 studies (Table C). Rehm et al (1984) reported a 30% (for ad libitum fed control mice) and 86.7% (for restricted fed control mice) spontaneous sex cord stromal tumor incidence in a NMRI mouse diet study, while Lohrke et al (1984) noted a 6.7% spontaneous combined sex cord stromal tumor incidence for the control mice fed ad libitum in a diet study with outbred Sut:NMRT mice (Table B).

These observations suggest the effects of the various factors influencing the spontaneous tumor occurrences discussed above. Therefore, only selected historical control data could be used as an adjunct aid to the interpretation of the data and should not replace the concurrent controls. Some of the published historical control data presented above may be of limited use for inferring background spontaneous tumor incidence in the diuron mouse study.

Since the Bayer's historical control data (18 NMRI mouse studies tabulated in Table C) were derived from studies conducted at the same Bayer laboratories, using the same mouse stock from the same animal supplier and were conducted within an overlapping time frame with the diuron mouse carcinogenicity study, this data is considered to be the most appropriate (among the historical control data presented above) to gauge spontaneous tumor incidence in the mouse study with diuron. The other historical data presented are of limited use because they were not derived from the same animal supplier, were not conducted in the same laboratory under the same husbandry conditions and were not done within the same time frame as the diuron mouse carcinogenicity study.

Ovarian Tumors: A statistically significant increase (14% at $p \leq 0.01$) of ovarian luteoma was noted in mice fed at 2500 ppm diuron as compared to the concurrent controls (6%; Table A). This value was higher than the ovarian luteoma tumor historical control of 1.7% reported for the Bayer Laboratory (Table C).

When all ovarian sex cord stromal tumors (i.e. granulosa/theca cell tumors and luteoma) in the diuron mouse carcinogenicity study were combined, the tumor incidence increase of 28% at the 2500 ppm diuron dose level was not significantly higher than the concurrent controls of 22% (Table A). The historical control of the combined sex cord stromal tumor incidence reported for the Bayer laboratory was 10.2% (Table C).

Some pathologists believe that ovarian luteoma develop from granulosa cell tumors, i.e. they represent a spectrum of differentiation of the same cell type. However, since the ovarian granulosa tumors are relatively common, and ovarian luteoma are rare and it occurs at later stages than the ovarian granulosa cell tumors, the rare occurrence of ovarian luteoma should

considered separately. Thus, the statistically significant increased incidence of the rare ovarian luteoma at 14% in the 2500 ppm females fed with diuron is still considered to be related to treatment as was previously concluded in a DER dated on April 1, 1992 (doc#009486). The HED Cancer Peer Review Committee will evaluate these data further.

Mammary Gland Tumors

The observed mammary gland tumors (adenocarcinoma type A and B) in mice treated with diuron were statistically significantly increased in the 2500 ppm diuron treated females (12% at $P \leq 0.05$) as compared to the concurrent control (4%; Table A). The historical control data of mammary gland adenocarcinoma type A and B in the Bayer Laboratory was reported at 3.3% (24 out of 717 mice; Table C). Thus, the increased incidence of mammary gland adenocarcinoma in the 2500 ppm diuron treated females is considered to be related to treatment as it was previously concluded in the DER dated on May 7, 1992 (doc#009486).

Conclusions

Based on the data presented in the original report (MRID#421595-01) and the additional data submitted on July 25 and September 16, 1993 (MRID# not supplied), administration of technical diuron at 25, ($\sigma = 5.4$ mg/kg/day; $\rho = 7.5$ mg/kg/day), 250 ($\sigma = 50.8$ mg/kg/day; $\rho = 77.5$ mg/kg/day), and 2500 ppm ($\sigma = 640.1$ mg/kg/day; $\rho = 867.0$ mg/kg/day) in the diet of NMRI mice for 24 months resulted in the following treatment-related effects:

- o Body weight decrease in the 2500 ppm (640.1 mg/kg/day) males.
- o Increased spleen and liver weight in the 2500 ppm (640.1 mg/kg/day) males.
- o Elevated leucocyte and reticulocyte counts in the 2500 ppm ($\sigma = 640.1$ mg/kg/day; $\rho = 867.0$ mg/kg/day) mice of both sexes.
- o Elevated mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the 2500 ppm mice ($\sigma = 640.1$ mg/kg/day; $\rho = 867.0$ mg/kg/day) of both sexes.
- o An elevated bilirubin in the 2500 ppm ($\sigma = 640.1$ mg/kg/day; $\rho = 867.0$ mg/kg/day) mice of both sexes.
- o Increased incidence of intracellular golden brown pigments in the cortical renal tubules in the 2500 ppm (867.0 mg/kg/day) females, and in the spleen of the 2500 ppm ($\sigma = 640.1$ mg/kg/day; $\rho = 867.0$ mg/kg/day) males and females.

- o An increased incidence of hemosiderin deposits in liver cells of the 2500 ppm (640.1 mg/kg/day) males.
- o Increased incidence of liver single cell necrosis and cell mitosis in the 2500 ppm (σ =640.1 mg/kg/day; ρ = 867.0 mg/kg/day) mice of both sexes.
- o An increased incidence of enlarged degenerative liver cells in the 2500 ppm (867.0 mg/kg/day) females,
- o Increased incidence of hepatopathy and Kupffer cells in the 2500 ppm (640.1 mg/kg/day) males.
- o Increased incidence of urinary bladder edema, epithelial hyperplasia, and thickened mucosa in the 2500 ppm (867.0 mg/kg/day) females.
- o An increased incidence of enlarged (with > 2 mm diameter) uterine horn in the 2500 ppm (867.0 mg/kg/day) females.
- o Increased incidence of ovarian luteoma and mammary gland adenocarcinoma in the 2500 ppm (867.0 mg/kg/day) females.
- o TOXII concurs with the registrant that this study was conducted to evaluate the carcinogenic potential of Diuron in mice (Guideline §83-2) and not considered as a "Chronic Toxicity and Carcinogenicity Study in Mice" (Guidelines §83-1 and §83-2) as originally submitted by the registrant (MRID#421595-01 & -02). Thus, this study is now considered as a mouse carcinogenicity study (Guideline §83-2)

Based on the data presented in the study report, the systemic LOEL for diuron is determined to be 2500 ppm (σ =640.1 mg/kg/day; ρ = 867.0 mg/kg/day) based on the following treatment-related effects: decreased body weight, and increased spleen and liver weight in males; elevated leucocyte and reticulocyte counts, mean corpuscular volume and mean corpuscular hemoglobin, and bilirubin values in both sexes; increased incidence of intracellular golden brown pigments in renal tubules in females and in the spleen of males and females; increased incidence of hemosiderin deposits in liver cells in males; increased incidence of liver single cell necrosis and cell mitosis in both sexes; increased incidence of enlarged degenerative cells in females and of hepatopathy and Kupffer cells in males; increased incidence of urinary bladder edema and epithelial hyperplasia, thickened mucosa and an enlarged (with > 2 mm diameter) uterine horn in females. The systemic NOEL for diuron is 250 ppm (σ = 50.8 mg/kg/day; ρ = 77.5 mg/kg/day) for both sexes of NMRI mice.

The doses employed in this study were sufficient to produce a compound-related systemic effect and appear to be adequate to test the carcinogenic potential of the test material. The HED Cancer Peer Review Committee will evaluate carcinogenic effects further.

This study is considered to be a 6(a)2 study, because diuron affected the hematopoietic system and there is evidence of a carcinogenic effect.

The data of this study together with the two rat chronic feeding/oncogenicity studies (MRID# 408865 and 00017764) will be presented to the HED Cancer Peer Review Committee.

CLASSIFICATION: This study satisfies USEPA's Guideline §83-2 requirements for a carcinogenicity study in mice, and it is now upgraded to core-minimum.

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APPENDIX A: CITES REFERENCES

Bomhard, E., and U. Mohr. Spontaneous Tumors in NMRI Mice from Carcinogenicity Studies. Exp. Pathol., 36:129-145, 1989.

Lohrke, H., Hesse, B., and G. Goerttler. Spontaneous Tumors and Lifespan of Females NMRI mice of the Outbred Stock Sut:NMRT During a Lifetime Study. J. Cancer Res. Clin. Oncol., 108:192-196, 1984.

Rehm, S. Dierksen, D., and F. Deerberg. "Spontaneous Ovarian Tumors in Han:NMRI Mice: Histologic Classification, Incidence, and Influence of Food Restriction". JNCI, 72:1383-1395, 1984.

Rehm. S., Rapp, K. and F. Deerberg. Influence of food Restriction and Body Fat on Life Span and Tumour Incidence in Female Outbred Han:NMRI Mice and Two Sublines. Z. Versuchstierk, 27:249-283, 1985.

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APPENDIX B: TUMOR INCIDENCE TABLES

1. Tumor Incidence Tables for Interim Sacrificed Mice (copied from p.70 of the study report).
2. Tumor Incidence Tables for Moribund and Terminal Sacrificed Mice (copied from p.72-74 of the study report).

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