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Chemical:	**Diuron**
PC Code:	**035505**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

014692

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

DATE: October 4, 2001

MEMORANDUM

SUBJECT: **DIURON**: Updated DERs and Executive Summaries for Diuron

FROM: Yung G. Yang, Ph.D. Yung G. Yang 10/4/2001
Reregistration Branch 2
Health Effects Division (7509C)

THROUGH: Alan Nielsen, BSS A. Nielsen 10/4/2001
Reregistration Branch 2
Health Effects Division (7509C)

TO: Roberta Farrell / Kathy Monk
PM 52
Special Review and Reregistration Division (7508W)

DP Barcode:	D274490	**Submission:**	S596407
Chemical:	Diuron	**Case:**	818790
PC Code:	035505	**CAS No.:**	330-54-1

Action: Prepare a toxicology database for diuron RED.

Response: The toxicology database for diuron has been prepared and reviewed by the Reregistration Branch 2. The database has undergone peer reviewed by the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) on May 29, 2001 and by the HED FQPA Safety Factor Committee on June 18, 2001. The toxicology RED chapter for diuron has been prepared under a separate memorandum to support a Reregistration Eligibility Decision (RED).

The updated DERs and executive summaries are as follows and are attached.

A. Data Evaluation Records (DERs)

MRID#	Study Type
40886501	Chronic Toxicity/Carcinogenicity Study in Rats.
42159501	Carcinogenicity Study in Mice
00091192	Chronic Toxicity Study in Dogs

B. Updated Executive Summaries

MRID#	Study Type
40886502	Subchronic Oral Toxicity in Rats
42718301	Twenty-one Day Dermal Toxicity Study in Rabbits
41957301	Multigeneration Reproduction Study in Rats
40228802	Developmental Toxicity Study in Rabbits
40228801	Developmental Toxicity Study in Rats
00146608	Mutagenicity- Reverse Mutation Assay in Bacteria
00146609	Mutagenicity- Forward Mutaion *in vitro*
00146611	Mutagenicity- *in vivo* Cytogenetic Assay
44350301	Mutagenicity- *in vivo* Cytogenetic Assay
00146610	Mutagenicity- UDS Assay in Mammalian Cells

DATA EVALUATION RECORD

DIURON

014692

STUDY TYPE: CHRONIC TOXICITY/ONCOGENICITY ORAL STUDY - RAT
[OPPTS 870.4300 (§83-5)]
MRID 40886501, 43871901, 43804501, and 44302003

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-96

Primary Reviewer: Sylvia Milanez, Ph.D., D.A.B.T.	Signature:	Sylvia Milanez
	Date:	4-16-01
Secondary Reviewers: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature:	Carol S. Forsyth
	Date:	APR 17 2001
Robert H. Ross, Group Leader, M.S.	Signature:	Robert H. Ross
	Date:	APR 17 2001
Quality Assurance: Donna L. Fefee, D.V.M.	Signature:	Robert H. Ross for D. L. Fefee
	Date:	APR 17 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: Yung G. Yang, Ph.D. Yung G. Yang, Date 5/8/2001
Toxicology Branch (7509C)
EPA Secondary Reviewer: Joycelyn Stewart, Ph.D. Joycelyn Stewart, Date 5/10/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD

This is an updated DER of MRID 40886501 (HED#011912 and 008160) to include MRIDs 43871901, 43804501, and 44302003. The final conclusion has not been changed.

STUDY TYPE: Combined Chronic/Oncogenicity Feeding – Rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D272128 SUBMISSION CODE: S591433
P.C. CODE: 035505 TOX. CHEM. NO.: not reported

TEST MATERIAL (PURITY): Diuron (98.7% a.i.)

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

CITATIONS: Schmidt, W. 1985. Diuron: study for chronic toxicity and carcinogenicity with Wistar rats (administration in diet for up to two years). Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Performing laboratory project number (Bayer AG) T 8010647, October 29, 1985 (translation completed July 14, 1986). MRID 40886501. Unpublished.

Rossberg, W. (1995) Volume 1 of supplementary data supporting the diuron 2-year feeding study in rats. Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Registrant's report D/Tox 17 Supplement No. 1, July 1995. MRID 43804501. Unpublished.

Rossberg, W. and U. Wirnitzer. (1995) Addendum 1 supporting the diuron 2-year feeding study in rats. Bayer AG Institute of Toxicology, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany. Report No. 13962A, July 1995. MRID 43871901. Unpublished.

Malek, D. (1997) Volume 2 of supplementary data supporting the diuron two-year feeding study in rats. E.I. du Pont de Nemours and Company, DuPont Agricultural Products, Barley Mill Plaza, Wilmington DE, 19880-0038, USA. DuPont project identification D/TOX 17, Supplement no. 2, June 18, 1997. MRID 44302003. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company

EXECUTIVE SUMMARY: In a chronic toxicity/oncogenicity study (MRID 40886501; supplementary data provided in MRIDs 43871901, 43804501, and 44302003), diuron (98.7% a.i.; batch no. 232114080) was administered to groups of 60 male and 60 female Wistar rats at dietary concentrations of 0, 25, 250, or 2500 ppm (0, 1.0, 10, or 111 mg/kg/day, respectively, for males and 0, 1.7, 17, or 202 mg/kg/day for females, respectively) for up to 24 months. At 12 months, 10 animals/sex/group were sacrificed for interim evaluation.

Treatment with diuron did not affect the survival of rats. The only reported treatment-related clinical sign was reddish discolored or bloody urine in high-dose males. A significant decrease in body weight was seen in both sexes of high-dose rats (12-15% for males; 6-14% for females, $p<0.01$) throughout the study. Body weight gains were similarly depressed, the total gains for high-dose males and females were 82 and 79% of controls, respectively. The slight decreases in body weights and weight gains of mid-dose males (4-6%; $p<0.05$ or 0.01) were not biologically significant. Food consumption was unaffected but overall food efficiency was lowered for high-dose males and females (86% and 76% of controls, respectively).

The hematopoietic system and urinary bladder (and renal pelvis) are the primary diuron target organs. Exposure to diuron causes erythrocyte damage resulting in hemolytic anemia and compensatory hematopoiesis, which are manifested as significantly decreased ($p< 0.05$ or 0.01) erythrocyte counts, hemoglobin levels, and hematocrit and increased MCV, MCH, abnormal erythrocyte forms, reticulocyte counts, and leukocyte counts (with no effect on differential counts) in mid- and/or high-dose males and females, and in low-dose females ($\leq$25% change for most parameters; 3-fold increase for reticulocytes). Hemolysis also led to increased (39-50%) plasma bilirubin in high-dose males and females. Consistent with erythrocyte damage, post-mortem gross examination showed a dose-related increase (18-220%) in spleen weight (absolute and relative to body) for all test groups at 12 and/or 24 months, and an increased incidence of spleen dark discoloration and/or swelling in mid and high-dose males and females after 12 and/or 24 months. Morphometric analysis of spleen sections to determine the percentage area of hemosiderin revealed an increase at $\geq$250 ppm in both sexes at 12 months and in all groups at 24 months ($p<0.05$ or 0.01), with the females being affected more severely. The chronic overburden of spleen function led to an increased incidence of spleen fibrosis in 2500 ppm males and females ($p<0.01$). Bone marrow activation occurred in both sexes at all test doses at 24 months ($p<0.05$ or 0.01 for all but low-dose females). This was evident morphometrically as an increase in hematopoietic (red) bone marrow for mid- and high-dose rats at 12 and/or 24 months (possibly in low-dose males at 12 months) with a concomitant decrease in fat marrow at 12 months (not evaluated at 24 months).

Gross pathology showed that the incidence of urinary bladder wall thickening was elevated at 24 months for low- and high-dose males and high-dose females ($p<0.05$ or 0.01). Microscopic evaluation showed that epithelial focal hyperplasia of the urinary tract and renal pelvis increased in severity in both sexes at 12 and/or 24 months, and increased in incidence ($p<0.01$) in high-dose males at 12 months and in mid and high-dose females at 12 and/or 24 months. Some gross and/or microscopic changes were also seen in the liver (increased weight, swelling, discoloration, vacuolar cell degeneration, round cell infiltration, hyperemia) although these effects were not clearly primary effects of treatment.

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Under the conditions of this study, the LOAEL is 25 ppm for both sexes of rats (1.0 and 1.7 mg/kg/day for males and females, respectively) based on evidence of hemolysis and compensatory hematopoiesis (decreased erythrocyte count, increased reticulocyte counts, increased spleen weight and bone marrow activation). A NOAEL is not established.

This study showed conclusive evidence for the carcinogenicity of Diuron in male and female rats. The incidence of urinary bladder carcinoma was increased at 2500 ppm in both sexes (males: 33/49 vs. 1/50 for controls; females: 11/50 vs. 0/48 for controls; $p<0.01$). The malignancies were usually characterized as transitional epithelial carcinomas. The slight increase (NS) in the incidence of urinary bladder papilloma and the 3 neoplasms in the renal pelvis in high-dose males (one papilloma and two carcinomas) were also considered treatment-related. Dosing was adequate based on numerous toxic effects (hematological, microscopic, etc.) observed in the animals at all tested doses.

This chronic toxicity /oncogenicity study, together with the subsequently submitted supplementary materials, is **Acceptable/Guideline** and does satisfy the guideline requirement for a chronic toxicity/oncogenicity oral study [OPPTS 870.4300 (§83-5)] in the rat. There were some noted deficiencies but none that would invalidate the study.

COMPLIANCE:

MRID 40886501: Signed and dated GLP (OECD Principles), Quality Assurance, and Data Confidentiality statements were present but a Flagging statement was lacking. This study was conducted before current DER reporting requirements were established.

MRID 43804501: Signed and dated Data Confidentiality and GLP statements were present, the latter stating that GLP standards "do not apply to the supplemental information provided in this report." Quality Assurance and Flagging statements were not provided.

MRID 43871901: Signed and dated GLP (OECD Principles), Quality Assurance, Data Confidentiality, and Flagging statements were present.

MRID 44302003: Signed and dated Quality Assurance, Data Confidentiality, and Flagging statements were present. Two GLP statements were included: one (signed 6/18/97) stated that GLP regulations were not applicable, and a second (signed 1/4/97 and 1/16/97) stated that the study and amendment were conducted by GLP (OECD Principles).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Diuron

   Description: solid at room temperature (color not specified)
   Batch No.: 232114080
   Purity: 98.7% a.i.

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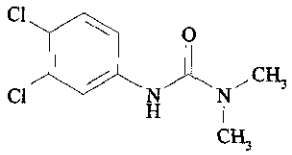
Stability of compound: stored under refrigeration during study; stability not specifically addressed but it was stated that "the sample was checked by analysis and approved."
CAS No.: 330-54-1
Structure:



2. Vehicle and/or positive control

The test material was administered continuously in the diet (Altromin 1321); no positive control was used.

3. Test animals

Species: rat
Strain: Wistar [BOR: WISW (SPF Cpb)]
Age and weight at study initiation: 6-7 weeks old; males: mean of 85 g (65-105g); females; mean of 84 g (63-103g).
Source: Winkelmann, Borchen
Housing: Singly in Makrolon type II cages on dust-free wood granules.
Diet: Altromin 1321 (made by Altromin GmbH, Lage), *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: ~22°C
Humidity: ~50%
Air changes: not reported
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 8-9 days

B. STUDY DESIGN

1. In life dates

Start: September 1, 1981 (males) and September 2, 1981 (females)
End: September 9, 1983 (last necropsy)

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2. Animal assignment

Animals were sorted into two weight groups (light, heavy) and randomly assigned to the test groups in Table 1. The main study rats received treated or control diet continuously for up to 2 years and the satellite study rats for 12 months, at which time they were sacrificed.

TABLE 1: Study design

Test Group	Conc. in diet (ppm)	Dose to animal[1] (mg/kg/day)		Main study (24 months)		Satellite study (12 months)	
		Male	Female	Male	Female	Male	Female
1 – Control	0	0	0	50	50	10	10
2 – Low	25	1.0	1.7	50	50	10	10
3 – Mid	250	10	17	50	50	10	10
4 – High	2500	111	202	50	50	10	10

Data taken from pp. 13 and 21, MRID 40886501.
[1]Doses are from MRID 40886501; similar doses were provided in MRID 43871901 (0, 1.02, 10.5, 111 mg/kg/day for males and 0, 1.69, 16.9, and 203 mg/kg/day for females given 0, 25, 250, and 2500 ppm, respectively).

3. Dose selection rationale

Dose selection was based on a previously conducted chronic feeding rat study (Hodge, H.C. et al., Fd. Cosmet. Toxicol. 5: 513, 1967), as well as a pilot 2-week rat feeding study (Eiben, R., 1981, Institute of Toxicology, Bayer AG, not published). No further details of these two studies were provided.

4. Diet preparation and analysis

Diet was prepared weekly throughout the study by mixing appropriate amounts of test material with feed, and was stored at room temperature. No further details were provided. Homogeneity was examined by determining the percent of theoretical recovery in three 25 ppm and three 2500 ppm samples. The stability of the test compound in the diet was determined over a 9-day storage period at room temperature in 25 ppm and 2500 ppm samples (appeared to be one sample per concentration). The test concentration was evaluated in 25, 250, and 2500 ppm samples on 8/81 (before study initiation) and on 11/81, 3/82, 6/82, 8/82, 11/82, 3/83, 5/83, and 8/83.

Results –
Homogeneity Analysis: The triplicate 25 ppm and 2500 ppm samples yielded recoveries of 85-86% and 99-100% of theoretical, respectively, indicating that the samples were homogenous.

Stability Analysis: Notable differences were not seen in the test material recovery in the 25 and 2500 ppm samples. For the 25 ppm samples, recoveries for days 1-9 ranged from 102-114% of theoretical, compared to 104% at day 0. For the 2500 ppm

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samples, recoveries for days 1-9 ranged from 111-123% of theoretical, compared to 102% at day 0.

Concentration Analysis: Test concentrations for the 25, 250, and 2500 ppm diets prior to the study initiation were 95, 107, and 89% of nominal, respectively. Over the 2-year treatment period, the test concentrations as a percent of nominal were 92-125%, 93-115%, and 95-108% for the 25, 250, and 2500 ppm diets, respectively.

Analysis of the test diets indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Significant differences between control and treated groups were determined using the Mann and Whitney U-test (Ann. Math. Stat. 18:50, 1947) and Wilcoxon's method (Biomehtrics 1:80, 1948) at the 5% and 1% levels of significance. Differences between treated and control groups in morphometric analysis were evaluated by the two-sided t-test if the distribution of values was normal otherwise by the U-test: group comparisons for the spleen were made as follows: groups 1 & 2, 2 & 3, 3 & 4, 1 & 4 (12 months) or 1 & 3 (24 months); group comparisons for the bone marrow were as follows: for males and females, groups 1 & 2, 1 & 3, and additionally 2 & 3 in 12-month males, 3 & 4 in 12-month females; 1 & 4 and 3 & 4 in 24 month males; 1 & 3 and 3 & 4 in 24 month females. Postmortem incidence data were analyzed by the reviewer using Fisher's Exact test.

C. METHODS

1. Observations

Animals were inspected at least twice daily during the week and once daily on week-ends and holidays for signs of toxicity and mortality. Detailed individual examinations were conducted once a week.

2 Body weight

Animals were weighed individually at weekly intervals for weeks 0-27, every other week for weeks 28-104, and immediately before necropsy at 12 and 24 months.

3. Food consumption and compound intake

Food consumption (g food/rat/day) for each group of animals was determined at weekly intervals throughout the study, by determining the amount of food left over from the previous weekly feeding and taking into account the number of live animals per day. Food efficiency was not calculated in the original study report (MRID 40886501) but was provided in a subsequent addendum (MRID 43871901). The

overall food efficiency was calculated as the [mean daily body weight gain (g) per animal divided by the mean daily food intake per animal] x 100. Compound intake (mg/kg/day) was calculated based on food consumption, body weight, and nominal concentration of test substance in the diet.

4. Water consumption

No mention was made of water consumption monitoring during the study.

5. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted. Changes (i.e., opacity) in the eye cornea and lens were evaluated during general examinations of the animals.

6. Blood was collected from a maximum of 10 rats/sex/dose after 6, 12, 18, and 24 months for hematology and clinical chemistry analysis. For examining the plasma glucose concentration, blood was collected without anesthesia from one of the venae caudale, and for the other parameters (in plasma) blood was collected from the retro-orbital venous plexus under ether anaesthesia. Reticulocytes were counted only after 12 and 24 months and the thromboplastin time was measured only after 24 months. The study authors did not indicate whether the animals were fasted. The CHECKED (X) parameters were examined. Note that several clinical chemistry parameters were not evaluated because they were not required by guidelines at the time the study was conducted (albumin, chloride, inorganic phosphate, calcium, potassium, and sodium).

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic toxicity/oncogenicity based on Subdivision F Guidelines.

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b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*		Albumin*
	Chloride*		Albumin:globulin ratio
	Magnesium	x	Blood creatinine*
	Phosphorus*	x	Blood urea nitrogen*
	Potassium*	x	Total Cholesterol
	Sodium*		Globulins
		x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Plasma alanine aminotransferase*		
x	Plasma aspartate amino-transferase*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.

7. Urinalysis

Urine was collected over a 16-hour period (from the same 10 male and 10 female rats per group as used for blood sampling) after 6, 12, 18, and 24 months. The animals were given water *ad libitum* but were fasted during collection. The CHECKED (X) parameters were examined. The urinary glucose, blood, protein, bilirubin, ketone bodies, and pH were determined semi-quantitatively with Bili-Labstix test strips. Urobilinogen was measured semi-quantitatively with Urobilistix. Protein was also measured quantitatively (method reference was cited). Several urinalysis parameters were not evaluated because they were not required by guidelines at the time the study was conducted (urine color, volume, appearance and specific gravity).

X		X	
	Appearance*	x	Glucose*
	Volume*	x	Ketones*
	Specific gravity*	x	Bilirubin
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.

8. Sacrifice and pathology

All animals that died and those sacrificed *in extremis* or on schedule (10 rats/sex at 12 months and the remainder of survivors at 24 months) were subjected to gross pathological examination. The animals were anesthetized with diethyl ether and killed by exsanguination. The CHECKED (X) tissues were collected from all animals and fixed in 10% buffered formalin. The CHECKED (XX) tissues were additionally embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically in all main study animals and in some interim sacrifice animals (all

interim dose groups: lungs, liver, spleen, bone marrow, kidneys, renal pelvis, urinary bladder, sternum, femur, and "macroscopically conspicuous tissues"; only control and 2500 ppm animals: all other tissues). The cecum and rectum were harvested, if possible, and evaluated as part of the large intestine in only the main study groups (stated in MRID 43804501). Results of the histopathological examination of the six sections of the intestine were sometimes reported as part of the small or large intestines. Kidney sections from rats sacrificed after 12 months were stained with the periodic acid Schiff reaction and frozen liver sections were stained with Oil Red O for fat content evaluation for all dose groups. Sections of lung, liver, spleen, and kidneys were also stained with Prussian blue to detect hemosiderin. To quantitate hemosiderin deposits in the spleen and hematopoietic activity in the bone marrow (femur), Prussian blue-stained samples were examined morphometrically using an automatic imaging system (Zeiss, IBAS II) for all interim sacrifice groups (4-10 animals/group; 40-100 measurements/group) and main study animal groups (25 animals/group; 100-250 measurements/group). The [XXX] organs were weighed after 12 and 24 months for all dose groups (brain and ovaries were not weighed because this was not a guideline requirement at the time).

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	x	Brain*+
x	Salivary glands*	xxx	Heart*	x	Periph. nerve*
x	Esophagus*	xx	Bone marrow* (femur)	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xxx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		
x	Ileum*				**GLANDULAR**
x	Cecum*		**UROGENITAL**	xxx	Adrenal gland*
x	Colon*	xxx	Kidneys*+		Lacrimal gland
x	Rectum*	xx	Renal pelvis	xx[b]	Mammary gland*
xxx	Liver*+	xx	Urinary bladder*	x	Parathyroids*
x	Pancreas*	xxx	Testes*+	x	Thyroids*
		x	Epididymides		
	RESPIRATORY	x	Prostate		**OTHER**
x	Trachea*	x	Seminal vesicle	xx	Bone*
xxx	Lung*	x	Ovaries*	x	Skeletal muscle*
	Nose	x	Uterus*		Skin*
	Pharynx		Oviduct		Ears
x	Larynx		Vagina	xx	All gross lesions and masses*

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.
+Organ weight required in chronic toxicity/oncogenicity studies.
[b]Mammary tissue was not routinely collected and evaluated in MRID 40886501 (only if there were visible lesions), but was re-sectioned from salivary gland tissue samples and evaluated in MRID 44302003.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

Clinical signs were not systematically tabulated in the original study report (MRID 40886501) although it was stated that high-dose males tended to have reddish

discolored urine during the second study year. After a subsequent review of the data by the study director, the incidences of reddish or bloody urine were subsequently presented in MRID 43804501 (supplementary data in response to EPA review) and MRID 43871901 (addendum to 2-year study). The results are shown in Table 2. The discrepancy between the results reported in the two documents (MRID 43804501 and 43871901) may be due to the fact that different findings are reported (memo, 5/10/96, L. Taylor, DP barcode D221892 and D221893).

TABLE 2. Incidence of reddish discolored and/or bloody urine in rats administered Diuron in the diet for 2 years.

Sex	Dietary concentration (ppm)			
	0	25	250	2500
Incidence of reddish discolored urine (p. 19, MRID 43804501)				
Males (n=50)	0	0	1	17
Females (n=50)	0	0	1	1
Incidence of reddish discolored urine/ bloody urine (p. 13, MRID 43871901)				
Males (n=50)	1	0	2	28
Females (n=50)	7	6	2	7

2. Mortality

No treatment-related effect was observed for survival of rats receiving the test material compared with that of the controls. The survival rates at study termination were 96, 84, 90, and 86% for males and 78, 82, 94 and 78% for females administered 0, 25, 250, and 2500 ppm Diuron, respectively.

B. BODY WEIGHT

Mean body weight and body weight gain data (as well as food consumption and food efficiency) of each group are summarized in Table 3. A significant decrease in body weight was seen in both sexes of high-dose rats (12-15% for males and 6-14% for females, $p<0.01$) throughout the study. Body weight gains were similarly depressed, the total gains for high-dose males and females being 82 and 79% of controls, respectively. Body weights of mid-dose males were also decreased (4-6%; $p<0.05$ or 0.01), however, the small magnitude of the decrease and the animals' appreciable overall body weight gain (95% or controls) indicated that this change was not biologically significant. No effects on body weights or body weight gains were seen in mid-dose females or low-dose males and females. [Body weight gains were not provided in the original study, i.e. MRID 40886501, but weekly body weight changes were subsequently given in the addendums MRID 43804501 and 43871901 to satisfy this deficiency noted by EPA.]

TABLE 3. Selected mean body weights, body weight gains, food consumption, and food efficiency data for male and female rats fed Diuron for up to 2 years.

Week	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
Mean body weights (g) (p.75-86, MRID 40886501)								
0	86	85	85	85	84	82	84	84
12	314	318	294** (94)[1]	266** (85)	182	185	187	171** (94)
26	364	364	346** (95)	318** (87)	206	209	208	192** (93)
51	397	396	381** (96)	351** (88)	219	223	218	196** (89)
73	419	412	400* (95)	359** (86)	240	245	242	212** (88)
103	418	415	401* (96)	357** (85)	249	260	257	215** (86)
Mean body weight gains (g)[2] (p. 16, MRID 43871901; p.75-86, MRID 40886501)								
0-26	278	279	261 (94)	233 (84)	122	127	124	108 (89)
0-51	311	311	296 (95)	266 (86)	135	141	134	112 (83)
0-73	333	327	315 (95)	274 (82)	156	163	158	128 (82)
51-103	21	19	20 (95)	6 (29)	28	37	39	19 (68)
0-103	332	330	316 (95)	272 (82)	165	178	173	131 (79)
Food consumption (g) (p . 21, MRID 40886501; p. 17, MRID 43871901)								
0-103	11,902	11,727	11,616 (98)	11,305 (95)	11,362	11,117	11,055	11,922 (105)
Food efficiency [(g body weight gain/g food consumed) × 100] (p. 17, MRID 43871901)								
0-103	2.79	2.81	2.72 (97)	2.41 (86)	1.45	1.60	1.56	1.10 (76)

[1]Numbers in parentheses are percent of control, calculated by the reviewer.
[2]Body weight gain calculated by the reviewer; total weight gain also given on p. 16, MRID 43871901.
*p<0.05, **p<0.01, statistically significant treated groups compared with the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Total food consumption was similar for all dose groups of males and females, as shown in Table 3. The greatest difference from controls was for high-dose males, which consumed 5% less food overall than the respective control group. All other groups consumed from 3% less to 5% more food than controls.

2. Compound consumption

The time-average-weighted dose for each treatment group is presented in Table 1.

3. Food efficiency

The overall food efficiency was affected by treatment for only the high-dose rats: values for males and females were 86% and 76% of controls, respectively. Food efficiency for the other groups was similar to that of the controls, as shown in Table 3.

D. WATER CONSUMPTION

No water consumption monitoring was reported for the study.

E. OPHTHALMOSCOPIC EXAMINATION

Although ophthalmoscopic examinations were not conducted, it was stated that no treatment-related alterations were seen in the rats' eyes. A tabulation of the incidences of corneal and lenticular opacity in the study addendum (MRID 43871901) showed a similar incidence of corneal opacity and lenticular opacity in treated and control rats.

F. BLOOD WORK

1. Hematology

A number of hematologic parameters were significantly altered ($p < 0.05$ or 0.01) in a dose-related manner in both male and female rats. Leukocyte counts were increased at 6, 12, 18, and 24 months in high-dose males and females (125-219% of controls) and in mid-dose males at 6, 12, and 24 months (115-128% of controls). There was no effect on the differential counts. Erythrocyte counts, hemoglobin levels, and hematocrit were decreased (75-96% of controls) and the MCV, MCH, and reticulocyte counts were increased (105-121%; reticulocytes up to 424% of controls) in high-dose males and all groups of females at most/all time points, and at some time points in mid-dose males. These changes are suggestive of treatment-induced hemolysis. The decrease in MCHC in mid-and high-dose males and for all doses of females was small ($\leq$4%) and generally not dose-related. The altered hematology parameters are summarized in Table 4. Additionally, abnormal erythrocyte forms (Jolly and Heinz bodies, anisocytosis, polychromasia, and anulocytosis) were seen at increased incidences at all time points for high-dose males and females. Severity was generally greater in females than males. The forms were seen at a lower incidence and/or severity in low- and mid-dose rats starting at 6 months in females and 12 months in males.

TABLE 4. Selected hematology parameters in male and female rats fed Diuron for up to 104 weeks [1].

Study month	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
Leukocytes (10^9 /L)								
6	8.9	9.2	10.2* (115)	11.1** (125)	7.2	6.7	6.4* (89)	11.1** (154)
12	7.1	8.0	8.3** (117)	11.7** (165)	6.7	9.0	7.5	12.8** (191)
18	7.0	7.6	7.6	10.3** (147)	6.4	5.9	7.1	14.0** (219)
24	6.7	6.1	8.6** (128)	11.3** (169)	7.3	7.3	6.1	13.4** (184)
Erythrocytes (10^{12}/L)								
6	8.34	8.32	7.47** (90)	6.42** (77)	7.83	7.37* (94)	6.50** (83)	5.91** (75)
12	8.86	8.59	8.74	7.21** (81)	7.93	6.94** (88)	6.71** (85)	5.99** (76)
18	8.42	8.22	8.02	6.97** (83)	7.77	7.17* (92)	6.63** (85)	5.95** (77)
24	8.24	8.14	7.90	6.82** (83)	7.69	6.86* (89)	6.40** (83)	5.95** (77)
Hemoglobin (gram/L)								
6	158	160	146** (92)	140** (89)	151	147	133** (88)	131** (87)
12	163	164	165	152** (93)	152	140** (92)	140** (92)	137** (90)
18	156	153	149* (96)	144** (92)	150	144	139** (93)	132** (88)
24	155	158	151	143* (92)	155	144	141** (91)	138** (89)
MCV (10^{-15} L)								
6	58	60* (103)	61	70** (121)	64	66	70** (109)	75** (117)
12	57	59* (104)	58	67** (118)	61	64* (105)	67** (110)	72** (118)
18	61	62	63	70** (115)	60	64* (107)	68** (113)	70** (117)
24	57	58	59	65** (114)	59	63* (107)	65** (110)	67** (114)
Reticulocyte (0/00)								
6	–	–	–	–	–	–	–	–
12	16	19	24* (150)	41** (256)	17	38	32** (188)	66** (388)
18	–	–	–	–	–	–	–	–
24	17	22	21	72** (424)	17	23* (135)	30** (176)	62** (365)
Hematocrit (L/L)								
6	0.49	0.50	0.45* (92)	0.45** (92)	0.50	0.49	0.46** (92)	0.45** (90)
12	0.51	0.50	0.50	0.48** (94)	0.48	0.44** (92)	0.45** (94)	0.43** (90)
18	0.51	0.51	0.50	0.48* (94)	0.47	0.46	0.45* (96)	0.41** (87)
24	0.47	0.47	0.46	0.44	0.45	0.43	0.42** (93)	0.40** (89)
MCH (10^{-12} gram/erythrocyte)								
6	18.9	19.2	19.6	21.9** (116)	19.2	20.0	20.6** (107)	22.1** (115)
12	18.4	19.0*(103)	18.9	21.1** (115)	19.1	20.4* (107)	21.0** (110)	22.9** (120)
18	18.5	18.7	18.6	20.7** (112)	19.3	20.1* (104)	21.0** (109)	22.1** (115)
24	18.8	19.5	19.1	20.9** (111)	20.2	21.2* (105)	22.1** (109)	23.2** (115)
MCHC (gram/L)								
6	324	321	323* (100)	312** (96)	302	300	292** (97)	293** (97)
12	322	326	326	317	316	318	315	319
18	305	302	297** (97)	297* (97)	320	314* (98)	310** (97)	317
24	332	336	326	325	344	336* (98)	338	346

Data taken from pp. 24-25 and 87-94, MRID 40886501.
*p<0.05, **p<0.01, statistically significant, treated groups compared with the control.
[1]Numbers in parentheses are percent of control, calculated by the reviewer.

2. Clinical chemistry

Treatment-related alterations (p<0.05 or 0.01) occurred in plasma bilirubin and urea concentrations in both sexes of rats. Bilirubin levels were 150% of controls in high-dose males at 24 months, and were 139-148% of controls in high-dose females at 6, 18, and 24 months. Urea levels were increased in mid-dose males after 6 and 12

months and in mid-dose females at 24 months (112% of controls), as well as in high-dose males at 6-24 months and females at 12-24 months (127-163% and 124-139% of controls, respectively). Cholesterol levels were decreased in high-dose males (6, 18, and 24 months; 69-79% of controls) and transiently in mid-dose females (12 months; 88% of controls); the change in males may have been related to the poor body weight gain of these animals. These alterations are summarized in Table 5. Note that a number of clinical chemistry parameters were not evaluated because they were not required by guidelines at the time the study was conducted (albumin, chloride, inorganic phosphorus, calcium, potassium, sodium). It is unlikely that this omission would notably alter the interpretation this bioassay because the kidneys and liver did not appear to be target organs.

Other clinical chemistry parameters were statistically significantly different from controls, although they did not appear to be treatment-related and/or toxicologically significant because the magnitude of the changes was small (not biologically significant), there was no dose-response, and/or the change was transient. These changes occurred at one or more time points in one or more dose groups, and include slightly decreased alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, and glucose, and increased creatinine.

TABLE 5. Selected clinical chemistry parameters in male and female rats fed Diuron for up to 104 weeks [1].

Study month	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
Bilirubin (micromol/L)								
6	3.4	3.3	3.1	3.6	2.7	2.7	2.9	4.0** (148)
12	2.5	3.0	2.9	3.1	4.3	2.8	3.5	4.1
18	4.2	4.4	3.8	4.4	4.0	4.4	4.4	5.6** (140)
24	2.8	2.6	3.0	4.2** (150)	3.3	3.2	2.6* (79)	4.6** (139)
Urea (mmol/L)								
6	6.19	6.77	6.95* (112)	8.10** (131)	7.41	6.68	6.98	7.47
12	6.49	7.16	7.25* (112)	8.22** (127)	7.42	7.94	8.28	10.34** (139)
18	6.90	7.12	7.08[2]	11.23** (163)	7.48	8.25	8.07	9.47** (127)
24	5.68	5.88	6.44	7.68** (135)	6.86	7.07	7.65* (112)	8.49** (124)
Cholesterol (mmol/L)								
6	1.66	1.59	1.59	1.21** (73)	1.05	1.21	0.99	0.93
12	2.97	2.78	3.13	2.39	2.72	2.83	2.38* (88)	2.45
18	3.53	3.33	3.60	2.80** (79)	2.49	2.32	2.25	2.30
24	3.83	3.49	3.97	2.66** (69)	2.38	2.32	2.20	2.69

Data taken from pp. 29-30 and 103-110, MRID 40886501.
*p<0.05, **p<0.01, statistically significant, treated groups compared with the control.
[1]Numbers in parentheses are percent of control, calculated by the reviewer.
[2]Mean was given as 7.09 in MRID 40886501 but subsequent recheck of the study data as reported in MRID 43871901 (pp. 29-30) indicated that the mean was 7.08.

G. URINALYSIS

The only notable urinalysis finding was a dose-related increase in the incidence of blood in the urine of males at 12 and 24 months and of females at 18 months. The overall group incidence of blood in the urine (6-24 months) could not be determined because all the same animals were not tested at the different time points. The severity of the findings was similar across dose groups. The incidence results are shown in Table 6. The failure to evaluate the urine color, volume, appearance and specific gravity did not likely alter the study conclusions since the liver and kidneys did not appear to be target organs.

TABLE 6. Incidence of blood in urine of male and female rats fed Diuron for up to 104 weeks [1].

Study month	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
6	0/10	0/10	1/10	1/10	0/10	1/10	0/10	0/10
12	0/10	2/10	2/10	8/10**	1/10	1/10	2/10	1/10
18	4/10	4/10	1/10	1/10	1/10	2/10	2/10	4/10
24	1/10	3/10	6/10*	8/10**	2/10	1/10	1/10	3/10

Data taken from pp. 295-310, MRID 40886501.
*p<0.05, **p<0.01: Significantly different from controls, determined by reviewer using the Fisher exact test.
[1]Most, but not all, of the same 10 animals were used for urinalysis at 6, 12, 18, and 24 months.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

Treatment caused a dose-related increase in spleen absolute and relative (to body) weights in all doses of males and females at 12 and 24 months. The increase for males was significant (p<0.01) at all doses at 12 months (118-242% of controls) and at the mid- and high doses at 24 months (111-227% of controls). Spleen weight increases of females were significant (p<0.01) for the mid- and high doses at 12 months (156-320% of controls) and for all doses at 24 months (139-285% of controls). Relative liver weights were increased (p<0.01) in mid- and high-dose males at 24 months (107-115% of controls) and in high-dose females at 12 months (114% of controls). At 24 months, all female dose groups had elevated absolute (111-120% of controls) and relative liver weights (107-134% of controls). It is unclear whether the increases in liver weights in either sex were treatment-related because there was not a clear dose-response and the increased relative weights may be due to the animals' lower body weights. The spleen and liver weights are shown in Table 7. Weights of the other organs were altered at 12 and/or 24 months but the changes were not considered treatment-induced because they lacked dose-response and/or could be attributed to decreased animal body weights. The failure to weigh the brain and ovaries did not appear to negatively impact the interpretation of this bioassay since these organs have not been shown to be target organs for Diuron in previous studies.

TABLE 7. Spleen and liver weights (absolute and relative) in male and female rats fed Diuron for up to 104 weeks [1].

Organ	Dietary concentration (ppm)			
	0	25	250	2500
Males – 12 months				
Body wt. (g)	384	393	373	355
Spleen (g)	0.598	0.724** (121)	0.707** (118)	1.329** (222)
% body weight	0.155	0.185** (119)	0.190** (123)	0.375** (242)
Liver (g)	13.370	14.127	12.979	13.395
% body weight	3.480	3.596	3.447	3.771
Males – 24 months				
Body weight (g)	421	418	401* (95)	354** (84)
Spleen (g)	0.791	0.841	0.877** (111)	1.514** (191)
% body weight	0.188	0.201	0.218** (116)	0.427** (227)
Liver (g)	15.007	14.884	15.362	14.509
% body weight	3.575	3.559	3.813** (107)	4.104** (115)
Females – 12 months				
Body weight (g)	211	220	210	190** (90)
Spleen (g)	0.407	0.460	0.634** (156)	1.167** (287)
% body weight	0.192	0.212	0.300** (156)	0.615** (320)
Liver (g)	7.757	8.649	7.699	7.973
% body weight	3.673	3.968	3.659	4.205** (114)
Females – 24 months				
Body weight (g)	249	256	257	220** (88)
Spleen (g)	0.493	0.717** (145)	0.797** (162)	1.265** (257)
% body weight	0.200	0.277** (138)	0.311** (156)	0.570** (285)
Liver (g)	8.276	9.925** (120)	9.225** (111)	9.847** (119)
% body weight	3.345	3.900** (117)	3.590** (107)	4.490** (134)

Data taken from pp. 33-34, MRID 40886501.
*p<0.05, **p<0.01, statistically significant, treated groups compared with the control.
[1]Numbers in parentheses are percent of control, calculated by the reviewer.

2. Gross pathology

The gross pathology data were not tabulated in MRID 40886501. The reviewer compiled group data for organ lesions with elevated incidences (determined using the Fisher Exact test) from the individual animal data provided on pp. 634-1472 of MRID 40886501. A similar compilation but excluding the kidney results was made by the original EPA reviewer of MRID 40886501 (memo, D.S. Liem; November 15, 1990; EPA Record No. 268,431, Caswell No. 410, Identifying No. 0046).

At the 12-month interim sacrifice, the incidence of spleen discoloration (dark) was elevated (p<0.05 or 0.01) in high-dose males and females, and was increased slightly in mid-dose males. The incidence of swollen/enlarged or darkly discolored liver was increased (p<0.01) in only the high-dose females.

The main study animals (rats sacrificed at 24 months or *in extremis*, and rats that died on study) had similar liver and spleen lesions. The incidence of spleen lesions was

increased in a dose-related manner in both sexes, the increase being statistically significant (p<0.01) in all mid- and high-dose groups. The incidence of liver lesions was increased at 25 and 250 ppm in males and 25 and 2500 ppm in females. The lack of dose-response leaves unclear whether the liver lesions were treatment-induced. Urinary bladder wall thickening and kidney surface alterations (changes in the texture and shape, and color) were also elevated in the main study animals, the incidence being increased for all groups of males (p<0.05 or 0.01 at 25 and 2500 ppm) and for the high-dose females (p<0.01). The macroscopic changes are summarized in Table 8.

TABLE 8. Incidence of gross pathology findings in male and female rats fed Diuron for up to 104 weeks.

Organ: lesion	Study group	Dietary concentration (ppm)							
		0	25	250	2500	0	25	250	2500
		Males				Females			
Spleen: Enlarged/ discolored[1]	Interim	0/10	0/10	3/10	10/10**	0/10	0/10	4/10*	10/10**
	Main	0/50	3/50	30/50**	45/49**	2/48	8/50	24/50**	40/50**
Liver: Enlarged/ discolored[1]	Interim	0/10	1/10	1/10	0/10	0/10	1/10	0/10	6/10**
	Main	12/50	28/50**	30/50**	9/49	8/48	18/50*	7/50	22/50**
Urinary bladder: Wall thickening	Interim	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	Main	1/50	8/50*	5/50	28/49**	1/48	0/50	0/50	12/50**
Kidneys: Surface alterations[2]	Interim	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
	Main	5/50	13/50*	12/50	18/49**	5/48	4/50	3/50	14/50*

Data taken from pp. 634-1472, MRID 40886501.
*p<0.05, **p<0.01: Significantly different from controls, determined by reviewer using Fisher exact test.
[1]Spleen and kidney were swollen, enlarged, and/or darkly discolored.
[2]Kidney surface alterations include changes in the texture, shape, and color.

3. Microscopic pathology

a. Non-neoplastic

At the interim (12 month) sacrifice, a positive reaction for iron staining was seen in the spleen, liver, lungs, and kidneys of both sexes. In the spleen, iron staining occurred in all control and test animals. The treated animals (all groups) had a significantly higher (p<0.01) incidence of spleen hemosiderin storage than controls. Spleen hemosiderin and iron deposits were seen as early as 9 months in one high-dose male. The incidence of bone marrow activation did not differ from controls.

Morphometric analysis of the spleen to determine the percentage area of hemosiderin revealed an increase at 250 ppm in both sexes and 2500 ppm in females (p<0.05; see Section I.B.5 for list of group comparisons). Morphometric analysis of bone marrow (femur) showed an increase in red marrow and a decrease in fat (i.e., yellow) marrow at ≥ 250 ppm in both sexes (p< 0.05; see Section I.B.5 for list of group comparisons) [marrow not examined in high-dose males due to specimen damage]. The area of red bone marrow in low-dose males was also

elevated slightly ($p<0.05$; see Section I.B.5 for list of group comparisons) but the study authors stated that the increase was equivocal due to inconsistencies in sample preparation (i.e. thickness of tissue sections) and because a correlating decrease was not seen in the proportion of fat marrow. The degree of change for both the spleen and bone marrow was greater in females than males.

The incidence of iron staining was also increased in a dose-related manner in the livers of mid- and high-dose females ($p<0.05$) and kidneys of high-dose males ($p<0.05$), but in the lungs it decreased with dose for both sexes ($p<0.05$ or 0.01 in mid- and high-dose males). [Note that the kidney and renal pelvis histological data for male #7 were included in the summary tables but inadvertently omitted during the translation from German in the individual animal findings; this was clarified in MRID 43804501 and the translation was provided in MRID 43871901.] Urinary tract epithelial focal or papillary hyperplasia increased in incidence and severity in high-dose males and mid- and high-dose females ($p<0.05$ or 0.01). The incidence of renal pelvis focal epithelial hyperplasia increased in a dose-related manner in both sexes, although the increase was statistically significant in only high-dose males ($p<0.01$). High-dose males had an increased incidence of lung marginal emphysema ($p<0.01$). The 12-month sacrifice results are summarized in Table 9.

TABLE 9. Incidence of selected non-neoplastic microscopic pathology findings and morphometric evaluation of the spleen and liver of interim sacrifice rats (fed Diuron for 12 months).

Organ: lesion	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
Spleen:+ Iron stain	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Hemosiderin storage	0/10	10/10**	9/10**	10/10**	3/10	10/10**	10/10**	10/10**
Urinary bladder:	4/10	5/10	5/10	10/10**	0/10	3/10	5/10*	9/10**
Epithelial hyperplasia	(1.25)[1]	(1.00)	(1.20)	(2.80)		(1.00)	(1.00)	(1.78)
Renal pelvis:	5/10	4/10	6/10	10/10**	1/10	3/10	4/10[2]	4/10
Epithelial hyperplasia	(1.00)	(1.25)	(1.00)	(1.50)	(1.00)	(1.33)	(1.00)	(1.00)
Liver: + Iron stain	5/10	8/10	0/10*	10/10*	5/10	7/10	10/10*	10/10*
Lungs:								
Iron stain	8/10	8/10	3/10*	1/10**	10/10	9/10	9/10	7/10
Marginal emphysema	1/10	2/10	0/10	10/10**	3/10	2/10	2/10	1/10
Kidneys: + Iron stain	4/10	5/10	4/10	9/10*	0/10	3/10	0/10	0/10
Morphometric evaluation (% area):								
Spleen	11.66	13.16	15.14‡	12.97	17.79	17.94	33.14‡	28.92‡
Bone marrow (red)	38.67	41.55‡[3]	45.07‡	NE	45.16	43.96	58.09‡	60.72‡
Bone marrow (fat)	33.55	31.94	22.98‡	NE	29.19	29.70	11.28‡	5.36‡

Data taken from pp. 361, 369-376 and 470-633, MRID 40886501.
NE=not examined due to specimen damage.
*p<0.05, **p<0.01: Significantly different from controls, determined by the reviewer using the Fisher exact test.
‡p<0.05 or 0.01: Significantly different from other groups by the t-test using the group means or by the U-test using group medians (not shown) (see Statistics section I.B.5.).
[1]Numbers in parentheses are mean group severities for hyperplasia calculated by reviewer using only affected rats, based on 1= approximately 4-6 layers of cells with normal structure;
2= approximately 6-9 layers of cells and clear predominance of small cells;
3= more than 9 layers of cells and barely apparent or absent covering layer, often nodular proliferation of urothelia with sinuate arrangement of basal membrane.
[2]The incidence was given as 4/10 on p. 375 and 5/10 on p. 51 of MRID 40886501.
[3]The significance is questionable because of irregularities in the thickness of cut tissue sections.

For the main study animals (including rats that died spontaneously or were sacrificed in extremis or at study termination at 24 months), the incidence of iron staining was not reported. The incidence of spleen fibrosis was increased in 2500 ppm males and females (p<0.01), as was the incidence of spleen hyperemia in high-dose males. A dose-related increase in bone marrow activation occurred in both sexes, the incidences were significantly increased relative to controls (p<0.05 or 0.01) for all groups except the low-dose females. Spleen hemosiderin storage was seen in most of the animals (90-100% of each group), including controls, by conventional microscopy. However, morphometric evaluation revealed a significant increase in the percent hemosiderin area of the spleen and hematopoietic (red) bone marrow for mid- and high-dose rats and an increase in spleen hemosiderin area in low-dose rats of both sexes (p<0.05 or 0.01; see Section I.B.5 for list of group comparisons). The females had a higher percentage surface area of hemosiderin deposits than males in the spleen at 24 months, but the area of hematopoietic bone marrow was comparable for the two sexes. The spleen and

bone marrow changes are consistent with the macroscopic and interim pathology results and the hematology evaluations, and are considered treatment-related.

Also considered treatment-related were the dose-related increases in the incidence and/or severity of epithelial focal hyperplasia of the urinary tract epithelium of the bladder and renal pelvis in both sexes. The incidence of the two lesions was increased in mid and/or high-dose females ($p<0.01$). The findings in both sexes are consistent with the interim and gross pathology results.

Other histopathological findings were statistically increased in the treated groups ($p<0.05$ or 0.01) but did not appear to be treatment-related because either there was not a clear dose-response or the increase was marginal, comparable to controls of the other sex, decreased with dose, and/or lacked supporting pathological correlates. These lesions occurred in the liver (increases in incidences of vacuolar cell degeneration in mid-dose males and high-dose females, round cell infiltration in high-dose females, and hyperemia in all treated groups of females), lungs (marginal emphysema in high-dose females), larynx (round-cell infiltration in high-dose males), the thyroid (c-cell hyperplasia in high-dose males and mid- and high-dose females), and the kidneys (round cell infiltration in low and high-dose males and high-dose females). Some of these changes may be attributed to the age of the animals. The study authors state that thyroid hyperplasia is a strain-specific spontaneous alteration. Selected non-neoplastic histopathology results are summarized in Table 10.

TABLE 10. Incidence of selected non-neoplastic microscopic pathology findings and morphometric evaluation of the spleen and liver of main study rats (fed Diuron up to 24 months).[1]

Organ: lesion	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
Spleen:								
Fibrosis	0/50	0/50	3/50	18/49**	0/48	0/50	0/50	17/50**
Hemosiderin storage	49/50	45/50	47/50	46/49	44/48	46/50	50/50	49/50
Hyperemia	0/50	0/50	1/50	15/49**	0/48	1/50	0/50	2/50
Bone marrow: Activated	0/50	5/50*	7/50*	42/49**	5/48	12/50	22/50**	42/50**
Urinary bladder:	13/50	5/50	16/50	15/49	11/48	7/49	17/50	30/50**
Epithelial hyperplasia	(1.15)[2]	(1.00)	(1.06)	(2.67)	(1.09)	(1.00)	(1.76)	(2.17)
Renal pelvis:	37/50	37/50	45/50	43/47	23/48	25/50	46/50**	42/47**
Epithelial hyperplasia	(1.19)	(1.19)	(1.64)	(2.33)	(1.13)	(1.12)	(1.83)	(1.98)
Liver:								
Hyperemia	36/50	26/50	35/50	36/49	19/48	32/50*	33/50**	36/50**
Vacuolar degeneration	12/50	10/50	32/50**	14/49	0/48	1/50	3/50	11/50**
Round cell infiltration	15/50	10/50	9/50	17/49	4/48	9/50	10/50	13/50*
Kidneys: Round cell infiltr.	3/50	12/50*	9/50	31/49**	1/48	3/50	2/50	7/50*
Thyroid: C-cell hyperplasia	29/50	27/48	37/50	39/49*	17/47	23/49	30/50*	28/48*
Morphometric evaluation (% area):								
Spleen	6.79	7.55‡	13.97‡	12.87‡	9.22	12.70‡	17.74‡	18.03‡
Bone marrow (red)	56.83	57.43	60.96‡	81.38‡	50.39	51.30	71.08‡	81.26‡
Bone marrow (fat)	NE	NE	NE	NE	NE	NE	NE	NE

Data taken from pp. 369-376 and 470-605, MRID 40886501.
NE=not examined
*$p<0.05$, **$p<0.01$: Significantly different from controls, determined by reviewer using Fisher exact test.
‡$p<0.05$ or 0.01: Significantly different from other groups by the t-test using the group means or by the U-test using group medians (not shown) (see Statistics section I.B.5.).
[1]Includes rats that died spontaneously or were sacrificed in extremis or at study termination (24 months).
[2]Numbers in parentheses are mean group severity ratings for hyperplasia, calculated by reviewer as in Table 9.

In the original 2-year bioassay (MRID 40886501), the skin, mammary glands, and oviducts were not routinely collected and preserved or examined histologically, but only if there were gross lesions present because it was not required by guidelines in effect at the time (1981-1983). In its review of the bioassay, the EPA RfD Peer Review Committee determined that the failure to examine mammary tissue in female rats was a serious study deficiency (10/14/96 memo from G.Z. Ghali). To rectify this deficiency, the registrant retrieved and recut paraffin blocks of the salivary glands because mammary gland tissue can often be found next to the salivary glands in rodents (MRID 44302003). The majority of the samples examined contained either "sufficient" or "only little" mammary gland tissue in the section and were evaluated (interim: 6/10 controls, 0/3 at 2500 ppm; main study: 35/48, 35/50, 27/50, 23/50 at 0, 25, 250, and 2500 ppm). Lesions found were: focal hyperplasia in one control and one low-dose female, and focal dilated alveoli in one mid-dose female. Therefore there were no treatment-related effects on mammary glands in females.

b. Neoplastic

The earliest neoplastic histological finding was a mammary adenoarcinoma in a low-dose female that died 5 days before the interim sacrifice. For males, the earliest detected neoplasm was a pituitary carcinoma in a mid-dose male at study month 16. At the interim sacrifice no males had tumors and one 2500 ppm female had a benign ovarian tumor.

In the main study animals, the most notable finding was an increased incidence of urinary bladder carcinoma at 2500 ppm in both sexes ($p<0.01$). The malignancies were usually characterized as transitional epithelial carcinomas. One high-dose male had a papilloma and two had carcinomas in the renal pelvis. Of the animals that died or were sacrificed prematurely, the most common neoplasms were urinary bladder carcinomas in males and malignant uterine neoplasms in females. Hemangioendotheliomas (benign) of the blood-forming system were reported only for one mid-dose male (in right hind leg) and in two control males (in mesenteric lymph nodes) (in MRID 40886501). However, in MRID 43871901, the hemangioendothelioma in the mid-dose male was re-classified as originating in soft tissue and not in the blood-forming system. Benign or malignant tumors were found in numerous other organs, although the incidence was comparable between the treated and control groups. The incidence of uterine adenocarcinoma was increased slightly in high-dose females, although the increase was not statistically significant and according to the study authors, was within the historical control range (2-20%).

The total number of tumors as well as the number of animals with tumors was clearly increased for the high-dose males (not statistically analyzed). Both sexes of 2500 ppm rats had an increase in the total number of malignant tumors as well as in the number of rats with malignant tumors, with a concomitant decrease in the number of benign tumors. Urinary bladder carcinoma accounted for a large portion of the malignant neoplasms in both sexes. The study authors used the convention of only listing primary tumors in the tumor incidence in the summary tables, so the sites of metastasis were not listed (e.g. in mesenteric lymph nodes); this point was clarified in MRID 43804501. Dosing was considered adequate based on numerous toxic effects (hematological, microscopic, etc.) observed in the animals at one or more doses. Selected neoplastic findings are shown in Table 11.

TABLE 11. Selected neoplastic findings of main study rats (fed Diuron up to 24 months).[1]

Organ: lesion	Dietary concentration (ppm)							
	0	25	250	2500	0	25	250	2500
	Males				Females			
Number animals examined	50	50	50	49	48	50	50	50
Urinary bladder:	1	0	1	33**	0	0	1	11**
Carcinoma Papilloma	0	0	0	3	1	0	2	2
Renal pelvis:	0	0	0	2[2]	0	0	0	0[2]
Carcinoma Papilloma	0	0	0	1[2]	0	0	0	0[2]
Uterus: Adenocarcinoma	–	–	–	–	5	5	5	9 (NS)
Total neoplasms[3]	21	16	19	57	31	31	31	39
Benign[3]	18	12	11	18	23	20	19	19
Malignant[3]	3	4	8	39	8	11	12	29
No. rats with neoplasms	19	14	16	41**	26	27	22	29
Benign only	17	10	8*	4**	18	16	10*	4**
Malignant only	2	2	6	25**	6	10	6	19**
Benign and malignant	0	2	2	12**	2	1	6	6

Data taken from pp. 42-47, 50-51, and 444-461, MRID 40886501.
*p<0.05, **p<0.01: Significantly different from controls, determined by reviewer using Fisher exact test.
[1]Includes rats that died spontaneously or were sacrificed in extremis or at study termination (24 months).
[2]Total number of animals = 47 for each sex.
[3]Not statistically analyzed.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The investigators indicated that the primary nonneoplastic effect of Diuron was to damage erythrocytes, causing hemolytic anemia and thereby stimulating hematopoiesis. Diuron-induced erythrocyte damage was seen at all tested doses; a no-effect level was not identified. The investigators also concluded that Diuron carcinogenicity was demonstrated in both sexes at 2500 ppm as transitional epithelial carcinoma of the urinary bladder, and occasionally, of the renal pelvis in males. The no-effect level with respect to urothelium-specific effects, i.e., severe urothelial hyperplasia as a possible precursor of a neoplastic alteration, was between 25 and 250 ppm.

B. REVIEWER'S DISCUSSION/CONCLUSIONS

Treatment with Diuron did not affect the survival of rats. The only reported treatment-related clinical sign was reddish discolored or bloody urine in high-dose males. A significant decrease in body weight was seen in both sexes of high-dose rats (12-15% for

males and 6-14% for females, $p<0.01$) throughout the study. Body weight gains were similarly depressed, the total gains for high-dose males and females were 82 and 79% of controls, respectively. Body weights of mid-dose males were also decreased (4-6%; $p<0.05$ or 0.01), however, the small magnitude of the decrease and the animals' appreciable overall body weight gain (95% of controls) indicated that this change was not biologically significant. Total food consumption was similar for all dose groups of males and females but overall food efficiency was decreased for high-dose males and females, being 86% and 76% of controls, respectively.

The erythrocytes and urinary bladder (and renal pelvis) were the primary Diuron target organs. Erythrocyte damage resulted in hemolytic anemia and compensatory hematopoiesis, which were manifested hematologically as significantly decreased ($p<0.05$ or 0.01) erythrocyte counts, hemoglobin levels, and hematocrit and increased MCV, MCH, abnormal erythrocyte forms, reticulocyte counts, and leukocyte counts (with no effect on differential counts). The changes occurred at one or more time points in mid- and/or high-dose males and females, and in low-dose females. Hemolysis also led to increased plasma bilirubin levels in high-dose males and females.

Consistent with erythrocyte damage, post-mortem gross examination showed a dose-related increase in spleen weight (absolute and relative to body) for all test doses of both sexes at 12 and/or 24 months, and an increased incidence of spleen dark discoloration and/or swelling in mid- and high-dose males and females after 12 and/or 24 months. Microscopic analysis revealed a positive reaction for iron staining in the spleen, liver, lungs, and kidneys of both sexes at 12 months, although the incidences were not dose-related for one or both sexes. In the spleen, iron staining occurred in all control and test animals, although morphometric analysis to determine the percentage area of hemosiderin revealed an increase at 250 ppm in both sexes and in 2500 ppm females ($p<0.01$; see Section I.B.5 for list of group comparisons). At 24 months, the incidence of iron staining was not reported but spleen hemosiderin storage was seen in most animals (including controls) by conventional microscopy. However, morphometric evaluation revealed a significant dose-related increase in spleen hemosiderin area at all doses of both sexes ($p<0.05$ or 0.01; see Section I.B.5 for list of group comparisons). Females had a greater spleen hemosiderin percentage area than males at 12 and 24 months. The chronic overburden of spleen function led to an increased incidence of spleen fibrosis in 2500 ppm males and females ($p<0.01$).

The compensatory increase in hematopoiesis was also evident microscopically as a dose-related increase in the incidence of bone marrow activation in both sexes of low-, mid- and high-dose rats at 24 months ($p<0.05$ or 0.01 for all but low-dose females). Morphometric evaluation revealed a significant increase in hematopoietic (red) bone marrow for mid- and high-dose rats at 12 and 24 months and an equivocal increase in low-dose males at 12 months (high-dose males not evaluated at 12 months) ($p<0.05$ or 0.01; see Section I.B.5 for list of group comparisons). The area percentage of hematopoietic bone marrow was higher in females at 12 months but was comparable for the two sexes at 24 months.

Concomitant with the increase in hematopoetic bone marrow, the area percentage of fat marrow decreased in both sexes at 12 months (not evaluated at 24 months).

Gross and microscopic pathology findings showed that the urinary bladder and renal pelvis were also Diuron target organs. Gross lesions consisted of an increased incidence of urinary bladder wall thickening at 24 months for low- and high-dose males and high-dose females ($p<0.05$ or 0.01). Microscopic evaluation showed dose-related increases in the severity of epithelial focal hyperplasia of the urinary bladder and renal pelvis in both sexes at 12 and/or 24 months. The incidence of urinary bladder epithelial hyperplasia was increased significantly in high-dose males and mid- and high-dose females and in high-dose females at 24 months ($p<0.05$ or 0.01). The incidence of renal pelvis epithelial hyperplasia was increased significantly in high-dose males at 12 months and in mid- and high-dose females at 24 months ($p<0.01$). The increase in plasma urea concentration in mid and/or high-dose rats at one or more time points may be due to the urothelial alterations, although the levels of urea were comparable to control reference levels for Wistar rats provided by the study authors. The lesions in the urinary bladder (including neoplasia) may have led to blood in the urine of mid- and high-dose males.

Gross and/or microscopic changes were also seen in the liver but it was equivocal whether they directly resulted from Diuron treatment. The increases in absolute and relative liver weights and of swollen/enlarged or darkly discolored livers in both sexes (at 12 and/or 24 months) were not clearly dose-related and could have been secondary effects of hemolysis (discoloration) or due to the animals' lower body weights (relative weight increases), or be non-specific adaptive responses. The microscopic liver lesions (increased incidences of vacuolar cell degeneration in mid-dose males and high-dose females, round cell infiltration in high-dose females, and hyperemia in all groups of females) also lacked a clear dose-response or were comparable in incidence to controls of the other sex, and were may be attributed to age. There were no correlated increases in plasma liver enzymes to confirm a significant toxic effect on the liver.

Several parameters were not evaluated in the original study (MRID 40886501) because it was not required by guidelines in effect at the time. These included some clinical chemistry parameters (albumin, chloride, inorganic phosphorus, calcium, potassium, sodium), urinalysis parameters (color, volume, appearance and specific gravity), organ weights (brain, ovaries), and post-mortem analyses (mammary glands, the skin, and oviducts were not routinely collected and preserved or examined histologically ; subsequent analysis of mammary tissue by recutting paraffin blocks of the salivary glands revealed no treatment-related effects.). These omissions did not likely affect the interpretation of this bioassay because the data in this study as well as in previously conducted Diuron studies indicate that no other organs are targets for Diuron. Aditionally, the LOAEL would be unaffected.

Under the conditions of this study the LOAEL is 25 ppm for both sexes of rats (1.0 and 1.7 mg/kg/day for males and females, respectively) based on evidence of hemolysis and compensatory hematopoiesis (decreased erythrocyte count, increased

reticulocyte counts, increased spleen weight and bone marrow activation). A NOAEL is not established.

This study showed conclusive evidence for the carcinogenicity of Diuron in male and female rats. The incidence of urinary bladder carcinoma was significantly increased at 2500 ppm in both sexes (males: 33/49 vs. 1/50 for controls; females: 11/50 vs. 0/48 for controls; $p<0.01$). The malignancies were usually characterized as transitional epithelial carcinomas. A slight increase (NS) was also seen in the incidence of urinary bladder papilloma. Three neoplasms in the renal pelvis in high-dose males (one papilloma and two carcinomas) were also considered treatment-related. The neoplasms in the urinary bladder and renal pelvis appeared to be a continuation of the epithelial hyperplasia. Both sexes of 2500 ppm rats had a marked increase in the number of malignant tumors as well as in the number of rats with malignant tumors. Dosing was considered adequate based on numerous toxic effects (hematological, microscopic, etc.) observed in the animals at all tested doses.

This chronic toxicity /oncogenicity study (MRID 40886501), together with the subsequently submitted supplementary materials (MRID 43871901, 43804501, and 44302003), is **Acceptable/Guideline** and does satisfy the guideline requirement for a chronic toxicity/oncogenicity oral study [OPPTS 870.4300 (§83-5)] in the rat. There were some noted deficiencies but none that would invalidate the study. A similar conclusion was reached in a previous EPA review of MRID 43871901 and 43804501 (memo, 5/10/96, L. Taylor, DP barcode D221892 and D221893).

C. STUDY DEFICIENCIES

Numerous study deficiencies were identified in the EPA's original review of this chronic/oncogenicity study (memo, D.S. Liem; November 15, 1990; EPA Record No. 268,431, Caswell No. 410, Identifying No. 0046). These included (1) poor translation from German to English, (2) illegible body weight curves, and failure to provide (3) the pathologist's signature, (4) adequate description of the test material, (5) the animal source, (6) all environmental conditions, (7) clinical signs data, (8) some clinical chemistry, urinalysis, and hematology parameters (latter refers to reticulocyte counts at 6 and 18 months), (9) body weight gains, (10) food efficiency, (11) tabulated gross pathology results, (12) histological evaluation of the cecum, rectum, skin, mammary glands, and oviduct, (13) the weight of the brain and ovaries, (14) histological analysis of some interim sacrifice tissues at the low and mid-dose, especially the thyroid, (15) a key to blanks results for numerous entries in the non-neoplastic histopathological summary table, (16) kidney histological evaluation for one interim sacrifice male rat, (17) a compilation of iron staining results for the spleen, lung, liver, and kidney, (18) morphometric evaluation of the percent surface area of the fat marrow at 24 months, and (19) an explanation for discrepancies in the study tables and text with respect to the incidences of hemangioandothelioma and neoplastic lesions in the blood-forming system. Due to these deficiencies, this study was initially classified by EPA as Core-Supplementary.

In response to the EPA's findings, the registrant addressed the vast majority of the above deficiencies in MRID 43804501, 43871901, and 44302003. These results are incorporated in the present review of the chronic/oncogenicity study on Diuron. The deficiencies which still remain outstanding in relation to the present guidelines, and that have some potential of affecting the interpretation of the study include # 8 (missing clinical chemistry, urinalysis, and hematology parameters) and #13 (missing weight of brain and ovaries). However, the results of the chronic/oncogenicity study, together with previous results from other studies, indicate that no significant pathological findings were missed due to these omissions. One still troubling deficiency is the failure to tabulate gross pathology results, which is much more easily done by the registrant than manually by the reviewer, and could have resulted in a missed effect (but would not change the LOAEL).

DATA EVALUATION RECORD

DIURON

014692

STUDY TYPE: ONCOGENICITY FEEDING – MOUSE
[OPPTS 870.4200 (§83-2b)]

MRID 42159501
MRID 43349301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 01-96B

Primary Reviewer: A. A. Francis, M.S., D.A.B.T.	Signature:	for A. A. Francis
	Date:	APR 19 2001
Secondary Reviewers: Tim Borges, Ph.D., D.A.B.T	Signature:	T Borges
	Date:	APR 19 2001
Robert H. Ross, M.S., Group Leader	Signature:	Robert H. Ross
	Date:	APR 19 2001
Quality Assurance: Donna L. Fefee, D.V.M	Signature:	for Robert H. Ross for D. L. Fefee
	Date:	APR 19 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: Yung G. Yang, Ph.D. Yung G. Yang, Date 5/8/2001
Toxicology Branch (7509C)
EPA Work Assignment Manager: J. Stewart, Ph.D. Joycelyn Estlewood, Date 5/10/2001
Toxicology Branch (7509C)

014692

DATA EVALUATION RECORD

This is an updated DER of MRID 421595-01/02 (HED# 009486) to include MRID 43349301. The final conclusion has not been changed

STUDY TYPE: Oncogenicity Feeding - Mouse [OPPTS 870.4200 (§83-2b)]

DP BARCODE: D272128 SUBMISSION CODE: S591433
P.C. CODE: 035505 TOX. CHEM. NO.: 410

TEST MATERIAL (PURITY): Diuron (purity, 98.7% a.i.)

SYNONYMS: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

CITATION: Eiben, R. (1983) Diuron: study for chronic toxicity and carcinogenicity with NMRI mice (administration in diet for 24 months). Bayer Agricultural Institute of Toxicology, Wuppertal, Friedrich - Ebert - Strasse 271-333, West Germany. Study no. T4010922, October 1983 (Translation completed January 1991). MRID 42159501. Unpublished.

Hardesty, P. and C. van Pelt (1994) Volume I of supplementary data supporting the diuron 2-year feeding study in NMRI mice. E.I. Du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Delaware 19714. Report No. MFS-1, July 1994. MRID 43349301. Unpublished

SPONSOR: E.I. Du Pont de Nemours and Company, Inc.

EXECUTIVE SUMMARY: In an oncogenicity study (MRID 42159501), Diuron (98.7% a.i., batch no. 232114080) was administered to groups of 60 male and 60 female NMRI (SPF HAN) mice in the diet at concentrations of 0, 25, 250, or 2500 ppm. The test diets were given for 24 months except for 10 mice/sex/group which were sacrificed after 12 months for an interim study. The concentrations of 25, 250, and 2500 ppm resulted in mean daily compound intakes for males of 5.4, 50.8, or 640.13 mg/kg/day; and for females of 7.5, 77.5, or 867.0 mg/kg/day, respectively. A supplementary document (MRID 43349301) provided additional summary data from the original study on body weight gain, food efficiency, macroscopic findings at 12 months, ovarian and mammary gland tumors; and also provided historical control tumor frequencies.

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No significant treatment-related effects were seen in clinical signs or survival. Body weights after 78 weeks of treatment were 7% ($p \leq 0.01$) and 4% (NS) less than the controls for high-dose males and females, respectively; and cumulative body weight gains were 21% and 12% less than the controls at 78 weeks. The overall food intake was about 17% greater for high-dose males and 12% greater for high-dose females than the controls. Food efficiency for the 2-year study was decreased in high-dose males and females by 21-22% compared to the controls.

Small, but statistically significant increases of 4-10% in group mean erythrocyte cell volume and mean cell hemoglobin were seen in males and females at various times during the study. Reticulocyte counts were increased in high-dose males by 9-62% and in females by 24-63% compared to the controls. These hematology changes were accompanied by increased absolute and relative (to body) spleen weights, increased serum bilirubin, and increased iron deposits (hemosiderin) in the spleens of high-dose males and females. These observations are consistent with a treatment-related compensated hemolytic anemia at 2500 ppm. Total leukocyte counts were increased by 48% and 51% ($p \leq 0.01$) in high-dose males and females, respectively, at 18 months and by 95% ($p \leq 0.01$) in high-dose females at 24 months. Differential counts were within normal parameters for both sexes at all doses.

Serum alanine aminotransferase activity was increased by 95% in males and by 66% in females at 2500 ppm compared to the controls after 24 and 6 months of treatment, respectively. The absolute and relative (to body) liver weights were increased by 9% and 11 %, respectively in high-dose males at 24 months compared to the control. Microscopic evidence of liver toxicity at 2500 ppm included increased incidences of increased mitosis in both sexes, centrilobular hypertrophy in males, Kupffer cell clusters in males, enlarged/degenerative liver cells in females, and single cell necroses in females.

Increased incidences of urinary bladder edema, thickened mucosa, and epithelia hyperplasia were seen in high-dose females after 24 months of treatment compared to the control. The epithelia hyperplasia incidence was increased after 12 months in high-dose females. There was also an increased incidence of uterine horn diameters measuring greater than 2 mm in females at 2500 ppm, but no adverse microscopic findings were noted.

The LOAEL is 2500 ppm in the diet for males (640.13 mg/kg/day) and females (867.0 mg/kg/day), based on hemolytic anemia and liver toxicity in both sexes, and urinary bladder toxicity in females. The NOAEL is 250 ppm for males (50.8 mg/kg/day) and females (77.5 mg/kg/day).

Treatment of up to 102 weeks with 2500 ppm Diuron resulted in a significant increase in the incidences of mammary adenocarcinomas (control, 4%; 2500 ppm, 12%, $p \leq 0.05$) and ovarian luteomas (control, 6%; 2500 ppm, 14%, $p \leq 0.01$) in female NMRI (SPF HAN) mice under the conditions of this study. However, the incidence of mammary adenocarcinoma in high-dose females was at or near the high range of incidences seen in historic controls.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200 (§83-2b)] in mice. There were no serious deficiencies found in the study.

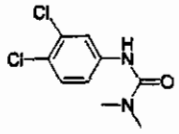
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided in the original study (MRID 42159501).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Diuron (IPC) technical

   Description: whitish solid with a slight odor
   Batch #: 232114080
   Purity: 98.7% a.i.
   Stability of compound: stable for the duration of the study.
   CAS #: 330-54-1
   Structure:



2. Vehicle and/or positive control: The test material was mixed with feed; a positive control was not included in this study.

3. Test animals: Species: mouse

   Strain: Bor strain NMRI (SPF HAN)
   Age and weight at study initiation: age: approximately 7 weeks; mean initial body weight: males, 27 g (19 - 33 g); females, 23 g (19 - 27 g)
   Source: Winkelmann, Borchen
   Housing: animals were housed in Makrolon® type I cages. Low-dust wood granulate bedding (Buntenback, Solingen) and cages were changed weekly. Bedding was randomly checked for contaminants.
   Diet: Altromin® 1321 meal from Altromin GmbH, Lage, *ad libitum*
   Water: tap water, *ad libitum*
   Environmental conditions:
   Temperature: range, 22 ± 2°C
   Relative humidity: approximately 50%
   Ventilation: approximately 10 air changes / hour
   Light cycle: 12 hours light: 12 hours dark
   Acclimation period: not supplied

B. STUDY DESIGN:

1. In life dates - Start: October, 1981 end: October, 1983
2. Animal assignment

Animals were assigned to the test groups listed in Table 1 using computer-generated random lists.

TABLE 1. Study design

Test group	Dietary concentration (ppm)	Dose to animals (mg/kg/day)		Number of animals	
		Male	Female	Male	Female
1 - Control	0	0	0	60[a]	60
2 - Diuron	25	5.4	7.5	60	60
3 - Diuron	250	50.8	77.5	60	60
4 - Diuron	2500	640.13	867.0	60	60

Data taken from p. 10, and p. 17, MRID 42159501.
[a]Ten animals per sex per group were sacrificed after 12 months of treatment for an interim study.

3. Dose selection:

The dose selection was based on the results observed in a previous 4-week study (Study No. T8010403) in which groups of 20 male mice were fed diets containing 0, 1000, 2500, or 5000 ppm diuron. Dose dependent spleen enlargement was observed at 2500 and 5000 ppm. No adverse effects were noted at 1000 ppm.

4. Diet preparation and analysis

Test diets were prepared approximately weekly by mixing appropriate amounts of diuron with the standard diet using a Loedige mixing granulator. The homogeneity of the 25 and 2500 ppm dietary mixtures was determined by analyzing 3 samples taken from different locations in the mixture container. The stability of the 25 and 2500 ppm samples was tested by measuring the concentration of diuron in the mixtures after storage for 17 days (storage temperature was not supplied). All dietary concentrations were analyzed for diuron content prior to study initiation and at approximately 3 month intervals during the study.

Results

Homogeneity – The concentrations of 3 samples taken from various locations in the 25 and 2500 ppm mixture containers had maximum deviations from the mean value of 0.8% and 0.7%, respectively. The relative standard deviations for the 25 and 2500 ppm mixture concentrations were 0.7% and 0.6%, respectively.

Stability – Analysis of 25 and 2500 ppm mixtures after storage for 17 days (storage conditions not supplied) showed concentrations of 96% and 110% of the original concentrations, respectively.

Concentration analysis – The concentration analyses showed the maximum deviations from the target concentrations of samples were 20% in the 25 ppm mixture, 12% in the 250 ppm mixture, and 8% in the 2500 ppm mixture. The sample means were 96%, 100%, and 101% of the 25, 250, and 2500 ppm target concentrations, respectively.

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Fisher's exact test was utilized to analyze mortality data. The body weights, organ weights, and laboratory results were compiled and the group means, standard deviations and upper and lower confidence limits ($1-\alpha = 95\%$ and 99%) were calculated. Organ weights that differed by as much as a factor of 5 from the group mean value due to the presence of a tumor were not included in the statistical calculations. The findings in treated animals were compared with those of the controls using the Mann Whitney U test and Wilcoxon's method. Microscopic findings during necropsy were tested for trend according to Peto.

Neoplastic incidences were tested utilizing the Cochran-Armitage trend test and a trend test according to Peto.

Findings were flagged as significant at $p \leq 0.01$ and $p \leq 0.05$.

C. METHODS

1. Observations

Animals were inspected for clinical signs twice daily during the week and once during weekends and holidays. The animals were given a detailed weekly examination. All mice that were obviously ill or had developed neoplasms were segregated and inspected more frequently.

2. Body weight

Animals were weighed before treatment initiation, at weekly intervals through week 27, then every 2 weeks up to week 37. After week 37, the body weights were measured every week again, and at study termination.

3. Food consumption and compound intake

The food consumption was measured by weighing the food added to or removed from each cage for the test week. Food consumption for each study group was determined weekly through week 103. Group mean values were calculated as g food/mouse/day and g food/kg body weight/day. Food efficiency values [mean body weight gained (g)/food consumed (g)] × 100 were calculated by the study authors over various periods of time during the study (1 to 12 weeks), and the mean values were reported for each measurement interval. The compound intake (mg/kg/day) was calculated for each concentration from the food intake and body weight data using the nominal dietary concentration of the test material.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not required and were not performed.

5. Blood was collected from the tail vein of 10 non-fasted unanesthetized mice per sex per group for blood glucose determinations. For all other blood parameter measurements, blood was collected from the retro-orbital sinus of 10 non-fasted mice per sex per group that were anesthetized with ether. The blood samples used to measure hematology parameters and clinical chemistry parameters were taken a week apart after 6, 12, 18, and 24 months of treatment. Reticulocytes were not counted at 6 months. Smears were stained with Wright's stain for differential blood counts. Reticulocytes were stained with brilliant cresyl blue. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Red and white blood cell and platelet morphology
	(Thromboplastin time)		Red cell distribution width (RCDW)
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for oncogenicity studies unless effects are observed, based on Subdivision F Guidelines.

b. Clinical chemistry*

	ELECTROLYTES		OTHER
	Calcium		Albumin
	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorus	X	Total Cholesterol
	Potassium		Globulins
	Sodium	X	Glucose
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)		
X	Serum aspartate amino-transferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Not required for oncogenicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urinalysis tests were not conducted and are not required for oncogenicity studies based on Subdivision F guidelines.

7. Sacrifice and pathology

Ten animals per sex per group were killed after 12 months of treatment, and all surviving animals were killed at the end of the 24-month treatment period by exsanguination under ether anesthesia. Necropsies were done on all animals in the study. The CHECKED (X) tissues were collected for histopathological examination. Organs and tissues were fixed in 10% buffered formalin. Tissue samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All listed tissues that were available from animals that died or were killed at unscheduled times during the study and from all animals in the control and high-dose groups were examined microscopically. The kidneys, liver and lungs from all animals and all tissues found to be abnormal upon macroscopic examination were processed and examined. Spleens were also examined at 250 ppm and spleen sections from males were stained with Turnbull's blue stain for optical densitometer measurements. Connective tissue was stained according to Van Gieson and Masson. The (XX) organs from all animals killed at 12 months and at treatment termination were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	X	Brain*+
	Oral tissue	XX	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary*
X	Stomach*	XX	Spleen*	X	Eyes*
X	Duodenum*	X	Thymus*		
X	Jejunum*				**GLANDULAR**
X	Ileum*		**UROGENITAL**	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal/Harderian glands
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	Liver*+	X	Epididymides	X	Thyroids*
X	Gall bladder*	X	Prostate		Auditory sebaceous gland
X	Pancreas*	X	Seminal vesicle		(Zymbal's gland)
			Coagulating gland		
	RESPIRATORY		Preputial gland		**OTHER**
X	Trachea*	X	Ovaries*+	X	Bone*
XX	Lung*	X	Uterus*	X	Skeletal muscle*
	Nose		Cervix	X	Skin* and subcutis
	Pharynx		Oviduct		Mediastinal tissue
	Larynx		Vagina		Mesenteric tissue
				X	All gross lesions and masses*

* Required for oncogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no treatment-related increases in the incidences of clinical observations in treated animals compared to the control groups. Weekly palpations revealed masses or swellings in all groups with cumulative incidences generally higher in the controls than in the treated group (controls, 33/60 and 13/60; 2500 ppm, 11/60 and 9/60 for males and females, respectively; p.34, MRID 42159501). The time of onset of palpable masses was not dose-related. The first appearance in males was during week 22 at 250 ppm; and, in females, during week 24 at 2500 ppm. At treatment week 52 the incidences were 7/58, 5/56, 6/54, and 2/55 for males at 0, 25, 250, and 2500 ppm, respectively; no palpable masses were seen in females at week 52 at any dose. During week 78, the incidences were 17/42, 13/37, 17/41, and 7/36 for males and 3/31, 4/32, 1/35, and 0/33 for females at 0, 25, 250, and 2500 ppm, respectively (MRID 42159501, Vol II, pg. no. not legible).

2. Mortality

The percent survival at selected times for mice in the 2-year study is given in Table 2. There were no significant decreases in the survival of treated compared to control

animals. The survival of females at 250 ppm was significantly higher after 52 weeks of treatment than the control group (control, 80%; 250 ppm, 98%, p≤0.01). However, by week 78 the survival of females at 250 ppm was comparable to the control group. The survival at study termination in high-dose males was 42% (NS) compared to 36% in the control group. Survival in high dose females was 30% compared to 22% in the female control group.

TABLE 2. Percent survival of male and female mice fed Diuron for 102 weeks

Study interval	Dietary concentration (ppm)			
	0	25	250	2500
Males (n = 50)				
25 Weeks	98	100	100	96
52 Weeks	96	92	88	90
78 Weeks	84	74	84	72
102 Weeks	36	46	50	42
Females (= 50)				
25 Weeks	96	98	98	96
52 Weeks	80	86	98**	94
78 Weeks	62	64	70	70
102 Weeks	22	22	22	30

Data calculated from Table 2, p. 36, MRID 42159501
**p≤0.01, significantly different from the control.

B. BODY WEIGHT

Group mean body weights and body weight gains from the study initiation, and at selected intervals throughout the study are summarized in Table 3. The group mean body weights of males at 2500 ppm were significantly (p≤0.05 or 0.01) lower than that of the control group in 71 of 82 measurements from treatment week 16 to study termination. The largest differences in group mean body weights in high dose males compared to the control group were seen from week 25 through week 63 during which all measurements were significant (p≤0.01). High-dose male body weights were generally decreased by less than 10% compared to the control weights. The group mean body weight gains were discussed in the study supplement (MRID 43349301) and reflected the differences seen in the body weights. The cumulative body weight gains in high-dose males were about 21% less than the control for the first 26 weeks of treatment, 21% less through 78 weeks, but only 7% less than the control for the entire treatment period.

The group mean body weights of females were also decreased at 2500 ppm compared to the control group, but the differences were not as consistent as for males and were statistically significant in only 22 of 82 measurements from week 16 throughout the remainder of the study. The weight differences in high-dose females compared to the control group appeared later in the study than in males with the differences becoming statistically significant primarily after week 37 and the greatest differences (up to about 11%) occurring after week 74. The cumulative group mean body weight gain for high-dose females was comparable to the control for the first 26 weeks, but decreased by up to

12% of the control weight gain by 78 weeks. There were no treatment-related effects on body weights or weight gains at 250 or 25 ppm in either sex.

TABLE 3. Group mean body weights and body weight gains in male and female mice fed Diuron for up to 102 weeks (g)

Body weight or weight gain measured on test week	Dietary concentration (ppm)			
	0	25	250	2500
Males				
Body wt., week 0	27.3 ± 2.8[a]	27.8 ± 2.5	28.1 ± 2.6	28.0 ± 2.6
Body wt., week 16	40.2 ± 0.5	39.9 ± 3.3	40.9 ± 2.7	39.0 ± 2.6*
Body wt., week 52	46.5 ± 5.6	46.1 ± 4.2	46.7 ± 3.8	43.5 ± 3.7**
Body wt., week 78	46.5 ± 4.7	45.3 ± 3.9	45.8 ± 4.8	43.1 ± 4.6**
Body wt., week 102	42.3 ± 4.5	42.1 ± 3.9	44.0 ± 3.8	41.9 ± 3.6
Wt. gain, weeks 0-26	17.0	16.4	16.1	13.4 (-21%)
Wt. gain, weeks 0-52	19.2	18.3	18.6	15.5
Wt. gain, weeks 0-78	19.2	17.5	17.7	15.1 (-21%)
Wt. gain, weeks 0-102	15.0	14.3	15.9	13.9 (-7%)
Females				
Body wt., week 0	22.9 ± 1.3	23.0 ± 1.5	23.1 ± 1.9	23.1 ± 1.8
Body wt., week 16	30.7 ± 2.7	30.8 ± 3.1	29.8 ± 2.6	30.7 ± 3.1
Body wt., week 52	36.3 ± 4.2	36.5 ± 4.8	35.7 ± 4.1	35.1 ± 4.3
Body wt., week 78	38.3 ± 5.7	38.8 ± 6.6	38.5 ± 6.1	36.6 ± 3.9
Body wt., week 102	37.6 ± 3.5	39.0 ± 5.6	38.9 ± 4.2	36.0 ± 5.1
Wt. gain, weeks 0-26	10.1	9.9	8.8	9.9
Wt. gain, weeks 0-52	13.4	13.5	12.6	12.0
Wt. gain, weeks 0-78	15.4	15.8	15.4	13.5 (-12%)
Wt. gain, weeks 0-102	14.7	16.8	15.8	12.9 (-12%)

Data taken from Part 2, Body Weights Table, pp. not legible, MRID 42159501, and Table 1, p. 7, MRID 43349301.
[a]Mean ± Standard deviation
*$p \leq 0.05$, **$p \leq 0.01$, significantly different from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Selected food consumption values are summarized in Table 4. The food consumption in high-dose males and females was higher (overall, males, 17%; females 12%) than that of the control groups throughout the study, and the differences were greater in the later months of the study than during the first year of treatment. The food consumption differences in the 25 and 250 ppm dose groups compared to the control groups (overall, 6% and -2% in males, 0.7% and 2% in females, respectively, are not likely to be treatment-related.

TABLE 4. Group mean food consumption (g/mouse/day) and food efficiency in male and female mice fed Diuron for up to 102 weeks

Food consumption and efficiency / study period	Dietary concentration (ppm)			
	0	25	250	2500
Males				
Food consumption/ weeks 15-18	8.7	9.0	8.4	9.5
Food consumption/ weeks 40-52	8.0	8.1	7.8	9.2
Food consumption/ weeks 66-78	8.4	9.6	8.6	11.5
Food consumption/ weeks 1-102	9.1	9.4	8.9	10.7
Overall food consumption (g/mouse)	6593	6798	6444	7717
Overall food efficiency/ weeks 0-102[a]	0.228	0.210	0.247	0.180
Females				
Food consumption/ weeks 15-18	9.7	9.8	9.9	10.3
Food consumption/ weeks 40-52	8.9	9.1	9.0	10.1
Food consumption/ weeks 66-78	10.9	11.3	11.3	13.2
Food consumption/ weeks 1-102	10.4	10.5	10.7	11.7
Overall food consumption (g/mouse)	7547	7599	7718	8464
Overall food efficiency/ weeks 0-102[a]	0.195	0.221	0.205	0.152

Data taken from Table 7, p. 41, MRID 42159501; and Table 2, p. 8 and Table 3, p. 9, MRID 43349301.
[a]The overall food efficiency (g weight gained/g food consumed X 100) was calculated by the reviewer from the overall weight gain and the food consumption supplied by the study author.

2. Compound consumption

The compound consumption was calculated by the study authors from the food consumption and body weight data. The results are given in Table 1.

3. Food efficiency

The food efficiency is summarized in Table 4. The food efficiency was calculated by the study authors for selected weighing intervals and was generally, but not always lower for both sexes at 2500 ppm diuron than in the control and other dose groups. The overall food efficiency was estimated by the reviewer from the overall body weight gains and the food consumption values supplied in the study. The food efficiency values for weeks 1-102 for males as a percent of the control value were 92%, 108%, and 79% at 25, 250, and 2500 ppm, respectively, and in females, were 113%, 105%, and 78%, respectively.

D. BLOOD WORK

1. Hematology

Selected hematology variables are summarized in Table 5. The mean cell volume was increased by about 3% ($p \leq 0.05$) in males at 25 and 250 ppm and by 6% at 2500 ppm after 6 months of treatment, but was not significantly different from the control group at 12, 18, or 24 months. In high-dose females, the mean cell volume was increased by

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about 2% at 12 months and by 6 % at 18 and 24 months ($p \leq 0.05$), but was not increased at 6 months compared to the control group. The mean cell hemoglobin was increased in high-dose males by 5% and 7% at 6 and 12 months, respectively, compared to the control, but was not significantly affected at 18 and 24 months. The mean cell hemoglobin in high-dose females was not significantly increased at 6 months, but was increased by 3%, 3% ($p \leq 0.05$), and 10% ($p \leq 0.01$) at 12, 18, and 24 months, respectively, compared to the control group. Leukocyte counts in males were increased by 32%, 22%, ($p \leq 0.05$) and 66% ($p \leq 0.01$) after 6 months at 25, 250, and 2500 ppm, respectively, compared to the control, and were increased at 2500 ppm by 32% (NS), 48% ($p \leq 0.01$), and 29% (NS) after 12, 18, and 24 months treatment. The leucocyte counts in females were increased at 2500 ppm by 29% ($p \leq 0.05$), 18% (NS), 51%, and 95% ($p \leq 0.01$) after 6, 12, 18, and 24 months compared to the control group. Differential leukocyte counts on the male and female mice did not show any changes outside the normal range. Reticulocyte counts were increased in high-dose males compared to the controls by 62%, 50% ($p \leq 0.01$), and 9% ($p \leq 0.05$) and in high-dose females by 46% ($p \leq 0.01$), 24% ($p \leq 0.05$) and 63% (NS) after 12, 18, and 24 months of treatment, respectively. Increases in reticulocyte counts of 23% ($p < 0.05$) were also seen at 250 ppm in males and of 3% and 11% in females at 25 and 250 ppm, respectively, after 18 months of treatment.

TABLE 5. Selected hematology variables in mice fed Diuron for up to 102 weeks

Variable/time period	Exposure concentration (ppm)			
	0	25	250	2500
Males				
Mean cell volume/ 6 mo. (fL)	62	64*	64*	66**
Mean cell volume/ 12 mo. (fL)	51	51	51	53
Mean cell volume/ 18 mo. (fL)	51	53	52	52
Mean cell volume/ 24 mo. (fL)	50	50	50	50
Mean cell hemoglobin/ 6 mo. (pg)	17.7	18.5	17.9	18.5**
Mean cell hemoglobin/ 12 mo. (pg)	17.2	17.6	17.6	18.4**
Mean cell hemoglobin/ 18 mo. (pg)	17.7	17.7	17.6	18.2
Mean cell hemoglobin/ 24 mo. (pg)	16.3	16.3	16.0	15.9
Leukocyte count/ 6 mo. ($X10^3/mm^3$)	4.1	5.4*	5.0*	6.8**
Leukocyte count/ 12 mo. ($X10^3/mm^3$)	6.3	7.0	4.2*	8.3
Leukocyte count/ 18 mo. ($X10^3/mm^3$)	6.1	6.2	6.1	9.0**
Leukocyte count/ 24 mo. ($X10^3/mm^3$)	4.9	5.0	5.1	6.3
Reticulocyte count/ 12 mo. (0/00)	21	22	23	34**
Reticulocyte count/ 18 mo. (0/00)	30	30	37*	45**
Reticulocyte count/ 24 mo. (0/00)	33	38	31	36*
Females				
Mean cell volume/ 6 mo. (fL)	62	63	61	63
Mean cell volume/ 12 mo. (fL)	54	53	52	55*
Mean cell volume/ 18 mo. (fL)	52	53	51	55**
Mean cell volume/ 24 mo. (fL)	48	51	49	51*
Mean cell hemoglobin/ 6 mo. (pg)	18.8	20.2*	18.6	19.6
Mean cell hemoglobin/ 12 mo. (pg)	18.4	18.2	17.6*	18.9*
Mean cell hemoglobin/ 18 mo. (pg)	17.9	18.8*	17.9	18.5*
Mean cell hemoglobin/ 24 mo. (pg)	15.4	16.1	16.0	17.0**
Leukocyte count/ 6 mo. ($X10^3/mm^3$)	4.1	4.2	3.5	5.3*
Leukocyte count/ 12 mo. ($X10^3/mm^3$)	4.9	4.9	4.2	5.8
Leukocyte count/ 18 mo. ($X10^3/mm^3$)	4.7	4.6	4.5	7.1**
Leukocyte count/ 24 mo. ($X10^3/mm^3$)	4.3	4.6	3.8	8.4**
Reticulocyte count/ 12 mo. (0/00)	24	22	20	35**
Reticulocyte count/ 18 mo. (0/00)	38	39*	42*	47*
Reticulocyte count/ 24 mo. (0/00)	35	40	32	57

Data taken from Table 4, p. 44, MRID 42159501.
*$p \leq 0.05$, **$p \leq 0.01$, Significantly different from control.

2. Clinical chemistry

Selected blood clinical chemistry values are summarized in Table 6. The serum alanine aminotransferase activity was significantly increased in high-dose males after 24 months of treatment (control, 74.5 U/L; 2500 ppm, 145.1 U/L, $p \leq 0.05$) compared to the control group, and was also increased at 6 and 18 months, but the increases were not significantly different from the control. In high-dose females, the serum

alanine aminotransferase activity was significantly increased only at 6 months (control, 37.3 U/L; 2500 ppm, 61.8 U/L, $p \leq 0.05$). The activity was also increased in high-dose females at 24 months, but the increase was not statistically different from the control and did not show dose-dependency. Serum bilirubin levels were increased in high-dose males by 38 to 58% ($p \leq 0.05$ or 0.01) at all time intervals tested compared to the controls. Bilirubin was also significantly increased in females by 46% and 49% ($p \leq 0.01$) at 250 and 2500 ppm, respectively, compared to the controls after 12 months of treatment, and by 24% at 25 ppm after 24 months. The bilirubin levels were not significantly affected at the other time points and dose levels.

TABLE 6. Selected clinical chemistry variables in mice fed Diuron for up to 102 weeks

Variable/time period	Exposure concentration (ppm)			
	0	25	250	2500
Males				
Alanine aminotransferase/ 6 mo. (U/L)	41.1	42.7	54.6	67.0
Alanine aminotransferase/ 18 mo. (U/L)	57.4	80.9	63.5	97.5
Alanine aminotransferase/ 24 mo. (U/L)	74.5	72.8	51.9*	145.1*
Bilirubin/ 6 mo. (μM/L)	1.3	1.5	1.5	1.9*
Bilirubin/ 12 mo. (μM/L)	4.7	4.1	4.5*	6.5**
Bilirubin/ 18 mo. (μM/L)	2.4	3.0	3.2	3.7*
Bilirubin/ 24 mo. (μM/L)	2.6	3.2	2.9	4.1**
Females				
Alanine aminotransferase/ 6 mo. (U/L)	37.3	37.6	40.1	61.8*
Alanine aminotransferase/ 18 mo. (U/L)	51.9	61.4	55.6	55.9
Alanine aminotransferase/ 24 mo. (U/L)	66.7	105.8	58.8	87.5
Bilirubin/ 6 mo. (μM/L)	2.2	2.1	2.2	2.8
Bilirubin/ 12 mo. (μM/L)	3.5	3.9	5.1**	5.2**
Bilirubin/ 18 mo. (μM/L)	2.9	4.1	3.2	3.8
Bilirubin/ 24 mo. (μM/L)	3.7	4.6*	4.0	4.1

Data taken from Table 6, p. 47, MRID 42159501.
*$p \leq 0.05$, **$p \leq 0.01$, Significantly different from control.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

The final body weights and selected absolute and relative weights to body weights are summarized in Table 7. The group mean absolute liver weight was increased in high-dose males at 24 months by about 9% ($p \leq 0.05$) and the liver weight relative to body weight (mg liver weight/100 g body weight) was increased about 11% ($p \leq 0.01$) compared to the control group. The absolute liver weights of treated mice did not show a significant change compared to the control group at 12 months; however, the relative liver weight to body weight was increased in high-dose males by 15% ($p \leq 0.01$). The mean absolute spleen weight was increased in high-dose males at

12 months by 47% ($p \leq 0.01$) and the spleen weight relative to body weight was increased by 55% ($p \leq 0.01$). The spleen weights were not increased in either sex at any dose after 24 months of treatment compared to the controls. The absolute and relative organ weights were not significantly changed in treated females compared to the control group. The relative liver weight of high-dose females at 24 months was slightly (6%, NS) increased, and the absolute and relative spleen weights were increased by 6% and 9% (NS), respectively, after 12 months compared to the controls.

TABLE 7. Group mean absolute and relative[b] organ and terminal body weights in male and female mice fed Diuron for 52 or 102 weeks

Organ/ treatment period	Dietary concentration (ppm)			
	0	25	250	2500
Males				
Terminal body, 12 mo. (g)	48 ± 6[a]	48 ± 5	46 ± 4	45± 4
Terminal body, 24 mo. (g)	44 ± 3	44 ± 5	46 ± 3	43± 4
Absolute liver, 12 mo. (mg)	2352 ± 327	2632 ± 385	2676 ± 1188	2563± 231
Absolute liver, 24 mo. (mg)	2459 ± 1113	2273 ± 383	2320 ± 540	2685± 813* (+9%)
Liver/body[b], 12 mo.	4956 ± 359	5486 ± 594	5812 ± 2599	5684 ± 431** (+15%)
Liver/body[b], 24 mo.	5588 ± 2514	5134 ± 654	5063 ± 1032	6180 ± 1421** (+11%)
Absolute spleen, 12 mo. (mg)	96 ± 20	111 ± 34	104 ± 19	141± 24** (+47%)
Spleen/body[b], 12 mo.	202 ± 32	230 ± 56	227 ± 51	314 ± 61** (+55%)
Females				
Terminal body, 12 mo. (g)	35 ± 4[a]	37 ± 4	38 ± 5	35 ± 5
Terminal body, 24 mo. (g)	38 ± 3	38 ± 4	40 ± 5	37 ± 6
Absolute liver, 24 mo. (mg)	2393 ± 370	2708 ± 1337	2364 ± 658	2448 ± 713
Liver/body[b], 12 mo.	6241 ± 674	7109 ± 3569	5873 ± 902	6640 ± 1461
Absolute spleen, 12 mo. (mg)	156 ± 84	121 ± 30	152 ± 51	165 ± 54
Spleen/body[b], 12 mo.	432 ± 186	329 ± 68*	407 ± 146	469 ± 112

Data taken from Part 2, Absolute and Relative Organ Weights Tables, pp. not legible and Table 7, pp. 50-51, MRID 42159501.

[a]Mean ± Standard deviation

[b]Calculated as mg organ weight/100g body weight.

*$p \leq 0.05$, **$p \leq 0.01$, Significantly different from the control.

2. Gross pathology

Selected macroscopic observations from the 2-year study are summarized in Table 8. There were no macroscopic changes seen in the 12-month interim study or in animals that died at unscheduled times during the first 12 months that were attributed to treatment with Diuron (see Enclosure 1, p. 19, MRID 43349301). Changes in the liver that could be treatment-related included clear lobule demarcation in 13% (NS) of high-dose males compared to 4% of the controls and changes in liver color in 27% ($p<0.05$) of high-dose females compared to 11% of controls. Lung color changes were seen in 16%, 27%, 30% (NS), and 35% ($p \leq 0.05$) of males and in 39%, 35%, 58% ($p \leq 0.05$), and 49% (NS) of females at 0, 25, 250, and 2500 ppm, respectively.

The uteri appeared enlarged in a higher number of females at 2500 ppm than in the control group. Measurements of the uterine horn diameters served to quantitate this observation. The incidences of uterine horn diameters 2 mm or greater were higher at 2500 ppm (24/45, $p \leq 0.05$) than in the control group (16/46).

TABLE 8. Macroscopic findings in male and female mice fed Diuron for 102 weeks

Organ or tissue/ finding	Dietary concentration (ppm)			
	0	25	250	2500
Males				
Liver/ clear lobule demarcation	2/45[a]	4/48	4/47	6/46
Liver/ change in color	7/45	15/48	5/47	5/46
Lung/ change in color	7/45	13/48	14/47	16/46*
Females				
Liver/ clear lobule demarcation	1/46	5/37	2/48	3/45
Liver/ change in color	5/46	10/37	12/48	12/45*
Lung/ change in color	18/46	13/37	28/48*	22/45
Uterus/ enlarged (uterine horn diameter ≥ 2 mm)	16/46	15/37	14/48	24/45*

Data taken from Part 3, Incidence of Macroscopic Findings, pp. 422-429; and from Part 1, Table 15, p. 66, MRID 42159501.

[a]Number of animals with finding/number of animals examined

*$p \leq 0.05$, Significantly different from controls, Fisher's exact test by the reviewer.

3. Microscopic pathology

a. Non-neoplastic

Selected microscopic findings are summarized in Table 9. An increase in urinary bladder epithelial hyperplasia incidence was seen in high-dose females at 12 months (control, 0/10; 2500 ppm, 5/10, $p \leq 0.05$), and the incidences of iron deposits in the spleen were increased at the high dose in both sexes after 12 months, but the increase was statistically significant only in females (males: control, 1/10, 2500 ppm, 5/10, NS; females: control, 0/10; 2500 ppm, 6/10, $p<0.01$). Slight increases in the incidences of fatty infiltration of the liver in treated males compared to the control and in uterine cysts in high-dose females were seen, but the changes were not significantly different from the controls ($p>0.05$).

After 24 months of treatment, there were increases in several liver findings in high-dose animals compared to the control groups including incidences of single cell necroses (males: control, 3/45; 2500 ppm, 7/46, $p \leq 0.05$; females: control, 12/46; 2500 ppm, 19/46, $p \leq 0.01$), increased mitosis (males: control, 1/45; 2500 ppm, 8/46, $p \leq 0.01$; females: control, 0/46; 2500 ppm, 4/46, $p \leq 0.05$), hypertrophy in high-dose males only (control, 1/45; 2500 ppm, 15/46, $p \leq 0.01$), Kupffer cell clusters in high-dose males (control, 6/45; 2500 ppm, 11/46, $p<0.05$), enlarged cells apparently in the process of degeneration in high-dose females (control, 0/46; 2500 ppm, 10/46, $p \leq 0.01$), and increased percent of hepatocytes and phagocytes

with hemosiderin deposits graded 2-4 and 2-3, respectively, in high-dose males (controls, 14% and 3%; 2500 ppm, 27% and 18% for hepatocytes and phagocytes, respectively). Increased incidences of golden brown pigment (hemosiderin) deposits in spleens were seen in high-dose males (control, 1/45, 2500 ppm, 14/46, $p \leq 0.01$) and females (control, 6/46; 2500 ppm, 19/46, $p < 0.01$). An increased incidence of golden brown pigment (hemosiderin) deposits was also seen in the kidney tubule epithelia of high-dose females compared to the control group (control, 0/46; 2500 ppm, 5/46, $p \leq 0.01$). Urinary bladder epithelia hyperplasia incidences were increased in high-dose females confirming the observation made after 12 months of treatment (control, 5/46; 2500, 23/44, $p \leq 0.01$). Additional findings in the urinary bladder of high-dose females at 24 months compared to the controls include increased incidences of edema (control, 0/46; 2500 ppm, 17/44, $p \leq 0.01$) and thickened mucosa (control, 0/46; 2500 ppm, 5/44, $p \leq 0.01$). No increases in the incidences of uterine cysts were seen in females after 24 months of treatment compared to the control group.

The hemosiderin deposits in the spleens and kidneys of high-dose animals were noticed as accumulations of golden brown pigment with hematoxylin and eosin staining. The presence of iron-containing hemosiderin was confirmed by staining sections with Turnbull's blue stain, but the differences between the high-dose and control groups could only be seen after the slides were read by optical densitometry. Other than showing the presence of iron, the specialized staining did not clearly quantify the increased pigment deposits in treated versus control organs. The Turnbull's blue staining of phagocytes and hepatocytes did allow more specific grading of the amount of hemosiderin deposited in the cells increasing from 0 to 4.

TABLE 9. Histopathology findings in male and female mice fed Diuron for up to 102 weeks

Organ, treatment period / finding	Dietary concentration (ppm)			
	0	25	250	2500
Males				
Liver, 12 mo. / fatty infiltration	6/10[a]	8/10	9/10	9/10
Liver, 24 mo. / single necroses	3/45	2/48	5/46	7/46*
Liver, 24 mo. / increased mitosis	1/45	2/48	0/46	8/46**
Liver, 24 mo. / centrilobular hepatopathy	1/45	0/48	0/46	15/46**
Liver, 24 mo. / Kupffer cell clusters	6/45	6/48	8/46	11/46*
Liver, 24 mo. / enlarged & degenerative cells	0/45	0/48	0/46	0/46
Liver, 24 mo. / hemosiderin in hepatocytes (gd. 2-3), %	14	ne[b]	17	27
Liver 24 mo. / hemosiderin in phagocytes (gd. 2-3), %	3	ne	7	18
Spleen, 12 mo. / iron deposits	1/10	ne	0/10	5/10
Spleen, 24 mo. / golden brown pigment (hemosiderin) deposits	1/45	0/48	1/46	14/46**
Kidney, 24 mo. / golden brown pigment (hemosiderin) deposits in tubular epithelia	0/45	0/48	0/47	0/46
Bladder, 12 mo. / epithelial hyperplasia	0/10	ne	ne	0/10
Bladder, 24 mo. / epithelial hyperplasia	14/44	12/47	13/47	12/46
Females (n = 50)				
Liver, 12 mo. / fatty infiltration	10/10	8/10	9/10	10/10
Liver, 24 mo. / single necroses	12/46	7/38	10/48	19/46**
Liver, 24 mo. / increased mitosis	0/46	3/38	0/48	4/46*
Liver, 24 mo. / centrilobular hepatopathy	0/46	0/38	0/48	0/46
Liver, 24 mo. / Kupffer cell clusters	9/46	9/38	9/48	9/46
Liver, 24 mo. / enlarged & degenerative cells	0/46	1/38	3/48	10/46**
Spleen, 12 mo. / iron deposits	0/10	ne	0/10	6/10++
Spleen, 24 mo. / golden brown pigment (hemosiderin) deposits	6/46	2/38	6/48	19/46**
Kidney, 24 mo. / golden brown pigment (hemosiderin) deposits in tubular epithelia	0/46	1/38	1/48	5/46**
Bladder, 12 mo. / epithelial hyperplasia	0/9	ne	ne	5/10+
Bladder, 24 mo. / epithelial hyperplasia	5/46	5/36	3/45	23/44**
Bladder, 24 mo. / edema	0/46	0/36	0/45	17/44**
Bladder, 24 mo. / thickened mucosa	0/46	0/36	0/45	5/44**
Uterus, 12 mo. / cyst	1/10	0/1	1/3	4/10

Data taken from Part 3, Pathology Report, Table of Microscopic Findings, pp. 468-483; Part 1, Table 9, p. 55, Table 10, p. 56, Table 11, p. 58, Table 13, p. 61, and Table 14, p. 62, MRID 42159501

[a]Number of animals with lesion / number of animals examined

[b]ne = not examined

*$p \leq 0.05$, **$p \leq 0.01$, Trend test by study authors

+$p \leq 0.05$, ++$p \leq 0.01$, Fisher's exact test by the reviewer.

b. Neoplastic

A summary of the incidences of commonly found neoplasms seen in the 78-week study is given in Table 10. There were no treatment-related increases in the incidences of neoplasms in males. Increased incidences of mammary gland adenocarcinoma (control, 2/50; 2500 ppm, 6/50, $p \leq 0.01$) and luteoma in the ovaries (control, 3/50; 2500 ppm, 7/50, $p \leq 0.05$) were statistically significant for

trend at 2500 ppm according to the method of Peto and/or Cochran Armitage trend test calculated by the study authors. These changes were not significantly different from the controls with the Fisher's exact test by the reviewer. Most of the mammary adenocarcinomas (control, 2/2; 2500 ppm, 5/6) and ovarian luteomas (control, 1/3; 2500 ppm, 5/7) were found in animals that died or were killed prior to study termination. However, the deaths all occurred after 400 days of treatment and the time of tumor onset was not different from that of the other groups.

TABLE 10. Total neoplastic incidences in male and female mice fed Diuron for up to 102 weeks

Organ / neoplasm	Dietary concentration (ppm)			
	0	25	250	2500
Males				
Liver/ adenoma	2/49[a]	5/50	5/49	6/49
Lung/ adenoma	14/49	14/50	13/49	12/49
Lung/ carcinoma	4/49	4/50	9/49	4/49
Hemolymphoreticular system/ lymphoma	4/49	14/50	7/49	5/49
Total number of mice with neoplasms	33/49	34/50	34/49	37/49
Total number of benign neoplasms observed	37	33	30	31
Total number of malignant neoplasms observed	13	25	22	12
Total number of neoplasms combined	50	58	52	43
Females				
Liver/ adenoma	0/50	0/47	1/49	0/50
Lung/ adenoma	4/50	4/47	9/49	4/50
Lung/ carcinoma	2/50	2/47	2/49	1/50
Hemolymphoreticular system/ lymphoma	19/50	16/47	26/49	18/50
Mammary gland/ adenocarcinoma	2/50	1/47	1/49	6/50*
Ovaries/ luteoma	3/50	1/47	2/49	7/50**
Total number of mice with neoplasms	32/50	30/47	36/49	38/50
Total number of benign neoplasms observed	26	22	38	34
Total number of malignant neoplasms observed	29	26	32	33
Total number of neoplasms combined	55	48	70	67

Data taken from Table 17, pp. 72-74, and Table 18, p. 76, MRID 42159501.
[a]Number of animals with lesion / number of animals examined
*$p \leq 0.05$, **$p \leq 0.01$, Significant by the Cochran-Armitage trend test by study authors (see study supplement, p. 14, MRID 43349301).

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that Diuron treatment resulted in increased incidences of erythrocyte decomposition at 2500 ppm in both sexes that was compensated for by the animals. High leukocyte counts in both sexes at 2500 ppm could possibly be treatment-related, but the lack of treatment-related changes in the differential counts compared to the controls makes the source of the high counts questionable. Blood enzyme activity levels and gross and microscopic evidence indicate increased incidences of liver injury at

2500 ppm in both sexes. Increased incidences of urinary bladder epithelial hyperplasia and mucosal edema and thickening were found in females at 2500 ppm compared to the controls. These changes were thought to be treatment-related. No treatment-related effects were seen at 250 ppm.

Slight increases in ovarian luteomas in females at 2500 ppm compared to the control group were thought to be likely due to minimal shifts in the hormone balances of the organ and a shift of the incidence of sex cord stromal tumors toward luteomas. The increased incidence of mammary tumors seen at 2500 ppm compared to the control group was within the normal occurrence of this tumor in mice of this strain. The study authors concluded that Diuron has no carcinogenic effects.

B. REVIEWER'S DISCUSSION

No treatment-related clinical signs were observed in the study, and survival was not significantly affected by treatment in either sex.

The group mean body weights and body weight gains were slightly decreased at 2500 ppm in both sexes compared to the control groups. The body weights of high-dose males were decreased about 7% ($p \leq 0.01$) after 78 weeks and high-dose females body weights were decreased about 4% (NS). The decreases in individual weekly body weight measurements at 2500 ppm were consistently significantly different from the controls in males and sporadically significant in females from treatment week 16. The cumulative group mean body weight gains were about 21% less in high-dose males and 12% less in high-dose females than in the control groups for week 0-78 treatment period. The group mean body weights and body weight gains were not affected by treatment with Diuron at 250 ppm. The overall food intake was about 17% higher in males and 12% higher in females at 2500 ppm than in the controls. This and the slightly decreased body weight gains allowed for a decreased overall food efficiency at 2500 ppm (males: control, 0.228; 2500 ppm, 0.180; females: control, 0.195; 2500 ppm, 0.152). Decreased food efficiency is consistent with a toxic effect of diuron treatment at 2500 ppm.

No treatment-related changes were seen in the differential white cell counts in treated males and females compared to the control groups, although total white cell counts were increased by 48% and 51% ($p \leq 0.01$) in high-dose males and females, respectively, at 18 months and by 95% ($p \leq 0.01$) in high-dose females at 24 months compared to the control groups. No explanation for the increased counts was found and the toxicological significance of the observation is doubtful.

Slight increases were seen in group erythrocyte mean cell volume of about 6% in high-dose males ($p \leq 0.01$) at 6 months and in high-dose females at 18 and 24 months ($p \leq 0.05$ or 0.01) compared to the controls. Similar increases in mean cell hemoglobin were seen in high-dose males compared to the controls of 5% and 7% ($p \leq 0.01$) at 6 and 12 months, respectively and in high-dose females of 3%, 3% ($p \leq 0.05$), and 10% ($p \leq 0.01$) at 12, 18, and 24 months, respectively. The hematocrit and erythrocyte counts at 2500 ppm were

comparable to the control; however, increased reticulocyte counts were seen at 2500 ppm in both sexes, especially at 12 months. The reticulocyte counts in high-dose males were increased by about 62% at 12 months, 50% ($p \leq 0.01$) at 18 months and 9% ($p \leq 0.05$) at 24 months compared to the control group. In females, the reticulocyte count was increased by 46% ($p \leq 0.01$) at 12 months, 24% ($p \leq 0.05$) at 18 months, and 63% (NS) at 24 months. Serum bilirubin concentration was increased by 38 - 58% in high-dose males at all time points compared to the controls ($p \leq 0.05$ or 0.01), and in females by 49% at 12 months ($p \leq 0.01$). These changes together with increased absolute and relative (to body weight) spleen weights at 12 months (males: 47% and 55%, $p \leq 0.01$; females: 6% and 9%, NS, for absolute and relative weights, respectively) at 2500 ppm compared to the controls, and microscopic findings of increased iron deposits in the spleens of both sexes at 2500 ppm are indicative of a compensated hemolytic anemia in both sexes. The number of mice with increased iron deposits (hemosiderin), seen as accumulations of golden brown pigment, in the spleen increased from 1/45 controls to 14/46 ($p \leq 0.01$) high-dose males and from 6/46 controls to 19/46 ($p \leq 0.01$) high-dose females after 24 months of treatment. Increased incidences of iron deposits in the kidney tubule epithelia of high-dose females were seen, and increased incidences of iron deposits (graded 2 - 4 or 2 - 3) in hepatocytes and liver phagocytes in high-dose males were also seen after 24 months of treatment.

The observed liver toxicity was secondarily related to the hemolytic anemia. Serum alanine aminotransferase activity was increased by 95% in high-dose males at 24 months and in high-dose females by 66% at 6 months compared to the controls ($p \leq 0.05$). The absolute and relative (to body weight) liver weights were increased in high-dose males by 9% and 11%, respectively, at 24 months and the relative liver weight was increased by 15% at 12 months ($p \leq 0.05$ or 0.01). The liver weights in treated females were not significantly increased. Microscopic findings after 24 months of treatment indicate liver damage in both sexes. The incidences of increased mitosis were increased at the high dose in both sexes (males: control, 2%; 2500 ppm, 17%, $p \leq 0.01$; females: control, 0%; 2500 ppm 9%, $p \leq 0.05$). The incidences of centrilobular hypertrophy (control, 2%; 2500 ppm, 32%, $p \leq 0.01$) and the appearance of Kupffer cell clusters (control, 13%; 2500 ppm, 24%, $p \leq 0.05$) were increased in high-dose males. In high-dose females, the incidences of enlarged/degenerative liver cells (control, 0%; 2500 ppm, 22%, $p \leq 0.01$) and single cell necroses (control, 26%; 2500 ppm, 41%, $p \leq 0.01$) were increased compared to the control group.

Other effects that appeared to be treatment-related include increased incidences of urinary bladder edema (control, 0%; 2500 ppm, 39%, $p \leq 0.01$) and thickened mucosa (control, 0%; 2500 ppm, 11%, $p \leq 0.01$) in high-dose females compared to the controls after 24 months of treatment. Increased incidences of urinary bladder epithelial hyperplasia were also seen in high-dose females at 12 months (control, 0%; 2500 ppm, 50%, $p \leq 0.05$) and 24 months (control, 11%; 2500 ppm, 52%, $p \leq 0.01$). On gross examination, the uteri appeared enlarged in larger number of high-dose females than in the control group. Measurement of the uterine horn diameters showed an increased incidence of uterine horn diameter over 2 mm at the high dose. No adverse microscopic uterine changes in treated females were found that would account for the macroscopic finding. The study authors

attribute the changes to hormonal variations, which could also partially account for the neoplastic changes seen at 2500 ppm.

The LOAEL is 2500 ppm for both sexes (640 mg/kg/day for males and 867 mg/kg/day for females) based on erythrocyte destruction and microscopic liver changes in both sexes and microscopic bladder changes in females. The NOAEL is 250 ppm (50.8 mg/kg/day for males and 77.5 mg/kg/day for females).

Treatment of NMRI (SPF HAN) mice for up to 102 weeks at 2500 ppm diuron resulted in statistically significant increases in the incidences of mammary gland adenocarcinomas (control, 4%; 2500 ppm 12%, $p \leq 0.05$) and ovarian luteomas (control, 6%; 2500 ppm, 14%, $p \leq 0.01$) when tested by the Cochran-Armitage trend test. No significant increases in neoplasms were seen in treated males compared to the control group. Most of the mammary gland and ovarian neoplasms were found in animals that died or were killed prior to the study termination, but all were under treatment for over 400 days and there were no observed differences in the time to tumor development.

The study authors presented data from historic controls performed from 1981 to 1988 using mice of the same strain from the same source, that showed mammary gland adenocarcinoma incidences that ranged from 0% to 13% with the average frequency being 3.2%. This same source showed ovarian luteoma frequencies ranging from 0% to 7% [Bomhard, E. (1992) Historical control data showing the frequency of tumors in NMRI-mice taken from 18 long-term studies over 21 months., Bayer Report No. 21534]. An additional reference provided historic control data collected from studies done from 1974 through 1981 [Bomhard, E. and Mohr, U. (1989) Spontaneous tumors in NMRI mice from carcinogenicity studies. Exp. Pathol. 36: 129-145]. In the latter reference, the mammary adenocarcinoma average incidence was 3.9% with a range of 0% to 10.8% and the ovarian granulosa cell tumor average frequency was 19.1% with a range of 5.0% to 35.5%. Ovarian luteomas develop from granulosa cells, but were not specifically identified in the reference. Compared to the historic data, the mammary adenocarcinoma incidences seen in the control group in the current study agree well, but the incidences in the high-dose group are at, or slightly above, the upper limit of that seen in control animals. The ovarian luteoma instances seen in the current study are slightly high in the control group and well above the normal range at 2500 ppm compared to the historic control animals; however, the luteoma incidences are not outside the upper range for all granulosa cell derived tumors according to the historic data. The statistical significance of the increased incidences in this study depend on a test for trend; the differences are not statistically significant according to the Fisher's exact test performed by the reviewer. The study authors concluded that the increased incidences of mammary and ovarian neoplasms in high-dose female mice compared to the control group were not treatment-related. This conclusion is questionable because the incidences of spontaneous tumors in normal control populations of this strain of mice vary considerably, and the best control is the usually thought to be the one that was performed during the current study. Under the conditions of this study, a positive oncogenic response was seen in high-dose female mice compared to the control group.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200 (§83-2b)] in mice. There were no serious deficiencies found in the study.

C. STUDY DEFICIENCIES

No major deficiencies were noted in the original study (MRID 42159501) combined with the supplement (MRID 43349301). In referencing the historic control sources, the authors reversed the dates when the studies were performed, and there was a disagreement between the tables and the text as to the statistical significance of luteoma and mammary carcinoma incidences ($p \leq 0.05$ or 0.01). The difference appeared to be due to different statistical tests applied, but was confusing. The thymus and parathyroids were not listed in the methods section as organs to be processed microscopically, but both were listed as examined in the section describing the findings for the individual animals

DATA EVALUATION RECORD

014692

DIURON

STUDY TYPE: CHRONIC TOXICITY - DOG [OPPTS: 870.4100 (§83-1b)]
MRID 00091192

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-96D

Primary Reviewer: Donna L. Fefee, D.V.M.	Signature: Robert H. Ross for Donna L. Fefee Date: APR 19 2001
Secondary Reviewers: H.T. Borges, Ph.D., MT(ASCP), D.A.B.T	Signature: HT Borges Date: APR 19 2001
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: APR 19 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L. A. Wilson Date: APR 19 2001

Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

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EPA Reviewer: Yung Yang, Ph.D. Date: 5/10/2001
Toxicology Branch (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, Ph.D. Date: 5/10/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD

This is an updated DER for MRID 00091192 (HED# 001474). The final conclusion and classification of the study have been changed.

STUDY TYPE: Chronic Oral Toxicity Study - Dog [OPPTS 870.4100 (§83-1b)]

DP BARCODE: D272128 SUBMISSION CODE: S591433
P.C. CODE: 035505 TOX. CHEM. NO.: 410

TEST MATERIAL (PURITY): Diuron (80%)

SYNONYMS: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

CITATION: Hodge, H. and W. Downs (1964) Chronic feeding studies of Diuron in dogs. Department of Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, New York. No laboratory or registrant report or study number was provided, July 30, 1964. MRID 00091192. Unpublished.

SPONSOR: E.I. DuPont de Nemours and Company, Inc., Industrial and Biochemical Department, Wilmington, Delaware.

EXECUTIVE SUMMARY: In a 24-month dietary toxicity study (MRID 00091192), groups of three male and three female beagle dogs were given Diuron (80% purity; initial lot number not reported, second quantity coded T 7111 3D) administered in feed at 0, 25, 125, 250, or 2500/1250 ppm (0, 1.8, 9.4, 18.8, or 93.8 mg/kg/day by conversion factor of 0.075). The high-dose group received a diet containing 2500 ppm of the test material for two weeks, then received only the basal diet for a three week reconditioning period, then received a diet containing 1250 ppm of the test material for the remainder of the two-year study.

There were no treatment-related deaths and aside from occasional partial food refusal at the highest dietary concentration, there were no treatment-related clinical signs. Adverse effects of treatment on body weight included an overall day 0-735 body weight losses at the 2500/1250 ppm dietary concentration by both males (18% loss of the pre-treatment weight vs a 15% body weight gain by controls) and females (13% loss of the pretreatment weight vs a 15% body weight gain by controls) and decreased day 0-364 body weight gains by males at the 250 ppm dietary concentration (44% of controls). At the 2500/1250 ppm dietary concentration, normocytic to macrocytic, normochromic anemia was noted in males on days 225-720 and in females at all time points starting at week 2, and the observation of brown pigment (likely hemosiderin) observed in Kupffer cells of all high-dose animals is suggestive of hemolysis. At the highest

dietary concentration, increased numbers of erythroid precursors/1000 bone marrow progenitor cells for male and female dogs (520 vs. 352 for controls), and moderately reduced marrow fat [implying hypercellularity] in histopathological preparations are consistent with attempts at erythrocytic regeneration. At the highest dietary concentration, absolute and relative liver weights were increased in both males (22 and 54% greater than controls) and females (35 and 62% greater than controls), and liver to brain weight ratios were increased in both sexes (25 and 23% for males and females, respectively). There were no other gross or histopathological hepatic changes, and clinical chemistry parameters were not evaluated. Although the increased liver weights may be associated with erythrocyte sequestration, a hepatotoxic treatment-related effect cannot be definitely ruled out.

Under the conditions of this study, the LOAEL for Diuron in male Beagle dogs is 250 ppm (18.8 mg/kg/day), based on decreased body weight gains, and the LOAEL in female Beagle dogs is 1250 ppm (93.8 mg/kg/day), based on a normocytic to macrocytic, normochromic anemia and body weight losses. The NOAEL is 125 ppm (9.4 mg/kg/day) in males and 250 ppm (18.8 mg/kg/day) in females.

This study is classified as **Unacceptable [OPPTS 870.4100 (§83-1b)]** and does not satisfy the Subdivision F guideline requirements. The exact dietary concentrations administered to the animals is unknown, due to the following deficiencies: 1) the test material purity was 80% and it is unknown whether the amount of test material used in diet preparation was adjusted to account for this; 2) stability, homogeneity, and concentration of the test material in food were not determined prior to study initiation; and 3) the physical properties (including stability) of the test substance were not provided.

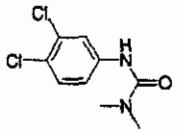
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Diuron

Description: "wettable powder"
Lot/Batch # : Two separate shipments were received. That used during the first year was not reported. The second was coded T 71113D
Purity: a.i. 80%
Stability of compound: Not reported
CAS #: Not reported
Structure:



2. Vehicle and/or positive control

None; the test material was administered in the feed.

3. Test animals

Species: Dog
Strain: Beagle, purebred
Age/weight at study initiation: ages were not provided; males: 8.8-13.6 kg; females: 7.9-13.7 kg.
Source: not provided
Housing: individually, "under comfortable conditions"
Diet: 300 g ground Purina Dog Chow® mixed with 250 g water per dog
Water: municipal tap water, *ad libitum*
Environmental conditions:
Temperature: not reported
Humidity: not reported
Air changes: not reported
Photoperiod: not reported
Acclimation period: six weeks

B. STUDY DESIGN

1. In life dates - start: October 26, 1960; end: November 6, 1962

2 Animal assignment

Animals were assigned to the test groups in Table 1 by an unspecified method.

TABLE 1: Study design				
Test Group	Conc. in Diet (ppm)	mg/kg/day (by conversion factor of 0.075)	Number Assigned	
			Male	Female
Control	0	0	3	3
Low dose	25	1.9	3	4[a]
Low-mid dose	125	9.4	3	3
High-mid dose	250	18.8	3	3
High dose	2500/1250[b]	93.8	3	3

Data taken from Text, pp. 40 and 53, MRID 00091192.
[a] One animal was sacrificed after 8 weeks on study, and an additional animal added during week 11.
[b] The high-dose group received a diet containing 2500 ppm for two weeks, basal diet for a three week reconditioning period, and 1250 ppm for the remainder of the two-year study period.

3. Dose selection rationale

Dose selection was based on a pilot study with two male dogs. One dog received 2.0 mg/kg/day of the test material for one week and then received 8.0 mg/kg/day of the test material for four weeks. The dog did not exhibit any treatment-related changes in clinical signs, body weight, urine glucose and protein, or hematology parameters, and had gross necropsy findings and organ weights within normal limits. A second dog received 20 mg/kg/day of the test material for two weeks, 40 mg/kg/day for two weeks, 80 mg/kg/day for one week, 200 mg/kg/day for nine days, followed by basal diet for five days, then 160 mg/kg/day for three days, the basal diet for five days, 80 mg/kg/day for five weeks and 120 mg/kg/day for eleven days. At the 200 mg/kg/day dose level, the dog vomited and refused food and continued vomiting when the test material was dosed by capsule. The dog vomited and refused food at both the 160 and 120 mg/kg/day doses, but there were no observations of vomiting or inappetence during the final four days of the pilot study. The second dog lost 8% and 7% of its body weight during the 6- and 11-day intervals of dosing at 200 and 120 mg/kg/day, respectively, although most of the weight lost during the later period was attributed to fasting for sacrifice. Hematological analyses done on the sixth day of dosing at the 200 mg/kg/day dose level and two days before termination of the pilot study showed the red blood cell counts, hematocrits and hemoglobin concentrations were decreased compared to baseline values. There were no treatment-related changes in urine glucose or protein; organ weights were within normal limits; and the only abnormal gross and histopathological findings were slight hepatic congestion with minimal hepatic fat. The investigators considered a dose of 1 mg/kg/day to be equivalent to 20 ppm in the diet. The dietary concentrations chosen for the main study were 0, 25, 125, 250, and 2500 ppm based on the results of the pilot study and on the fact that a

high dose of 2500 ppm had been selected for a chronic feeding study with rats. After two weeks of treatment, the animals of the highest dose group were put back on the basal diet for a three week reconditioning period, and then they received 1250 ppm test material in the diet for the remainder of the study.

4. Test diet preparation and analysis

Diets were prepared at weekly intervals by adding appropriate amounts of the test material to 10 kg of ground Purina Dog Chow®. Each dog was offered a food ration that consisted of 300 g of the ground diet mixed with 250 g of drinking water. The storage conditions of the diets were not provided, and homogeneity, stability, and concentration analyses were not conducted. It is unknown whether the actual dosages to the animals were acceptable.

5. Statistics

A t-test was used to compare erythrocyte counts and hemoglobin concentrations of the two highest dose groups to those of controls at selected time points (at one and two years for both groups and at 105 days for the 2500/1250 ppm group only). The same test was also used to compare liver-to-body-weight ratios of the high-dose group and controls. Significance levels of 1 and 5% were used. The values for erythroid precursors per 1000 precursor cells in bone marrow smears were compared by an unspecified statistical method.

C. METHODS

1. Observations

Animals were observed "regularly" for general clinical condition and appetite.

2. Body weight

Animals were weighed weekly throughout the study.

3. Food consumption and compound intake

Food consumption was not determined, and compound intake was not calculated.

4. Blood was collected for hematology analysis from all animals before initiation of treatment and 9 times during the study, on days 16 or 20, 105-106, 223-225, 287-289, 385-386, 475-476, 594-595, 657-658, and 720 or 727-729. An additional female was added to the 25 ppm group on day 78 and for the remainder of the study, blood was collected from this animal on the same days as the other animals on study. Blood collected at the end of the study was subjected to spectral analysis for the presence of pigments. NB: unless stated otherwise, the reviewer will hereafter refer to the collection times by the earliest day of each collection, and data from the replacement animal

will be included with the closest "regular" collection time. The study report did not mention whether animals were fasted prior to blood collection. 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)*		Mean corpusc. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)*
	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		
	(Fibrinogen)		

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical chemistry

Clinical chemistry was limited to plasma spectral analysis for hemoglobin variants.

5. Urinalysis

Urine was collected from all animals prior to initiation of treatment and at monthly intervals during the study. The study report did not mention how the urine was collected or whether the animals were fasted beforehand. The CHECKED (X) parameters were examined quantitatively.

	Appearance*	X	Glucose*
	Volume*		Ketones*
	Specific gravity or osmolality*		Bilirubin*
	pH*		Red blood cells*
	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

* Required for chronic studies based on Subdivision F Guidelines

6. Ophthalmological examinations were not done.

7. Neurotoxicity screening was not done.

8. Sacrifice and pathology

All animals were subjected to gross pathological examination and the CHECKED (X) tissues were collected and subjected to histological examination. The (XX) organs, in

addition, were weighed. The study report did not mention the method of euthanasia or whether the animals were fasted prior to sacrifice.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	XX	Aorta*	XX	Brain (cerebrum, cerebellum, medulla/pons)*+
	Salivary glands*	X	Heart*+		
	Esophagus*	X	Bone marrow*	X	Brain (site unspecified)
X	Stomach*		Lymph nodes*		Periph. nerve*
	Duodenum*	XX	Spleen*+		Spinal cord (3 levels)*
	Jejunum*		Thymus		Pituitary*
	Ileum*				Eyes (retina, optic n.)*
X	Small intestine (site not specified)		**UROGENITAL**		
		XX	Kidneys*+		**GLANDULAR**
	Cecum*	X	Urinary bladder*	X	Adrenal gland*+
	Colon*	XX	Testes*+		Lacrimal gland
	Rectum*		Epididymides*+		Mammary gland (females)*
X	Large intestine (site not specified)		Prostate*		Parathyroids*++
			Seminal vesicle*		Thyroids*++
XX	Liver*+	X	Ovaries*+		
	Gall bladder*	X	Uterus*+		**OTHER**
X	Pancreas*				Bone
				X	Skeletal muscle
	RESPIRATORY				Skin*
	Trachea*				All gross lesions and masses*
XX	Lung*+++				
	Nose*				
	Pharynx*				
	Larynx*				

*Required for chronic studies based on Subdivision F Guidelines
+Organ weight required in chronic studies.
++Organ weight required only for non-rodent studies.
+++Should be weighed if the test substance is administered by the inhalation route.

9. Quantitative analyses

Samples of urine and feces were collected from dogs of all groups six weeks prior to the end of the study, and samples of blood, liver, kidney, spleen, fat, and muscle were taken from dogs of all groups at necropsy. It was not clear from the study report whether samples were taken from every dog or even both sexes.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

During the initial two weeks of treatment, animals receiving 2500 ppm of the test material exhibited food refusal. All dogs in the high-dose group were returned to the basal diet for a 3-week reconditioning period. During week five of the study, treatment of the high-dose group was resumed at a dietary concentration of 1250 ppm.

During the remainder of the study, occasional occurrences of partial food refusal were noted at this dietary concentration, but neither the number of animals or the number of incidences of food refusal were provided in the study report.

2. Mortality

There were no treatment-related deaths during the study. An additional female was added to the 25 ppm group during week 11 as a replacement for an animal sacrificed moribund with heart disease during week 8. A second animal from the 1250 ppm group was sacrificed moribund during week 76 due to a strangulated inguinal hernia and was not replaced.

B. BODY WEIGHT AND WEIGHT GAIN

Selected body weight data are given in Tables 2 and 3. There were no treatment-related effects on absolute body weights and body weight gains of the male 25 and 125 ppm groups. Males of the 1250 ppm group had body weight losses during the first year of the study with a day 0-364 body weight loss of 2.33 kg (20% of the pre-treatment weight) compared to a 1.30 kg body weight gain for controls. Although the high-dose males did have a slight body weight gain for the day 364-735 interval, the overall day 0-735 body weight change was a 2.13 kg loss (18% of the pre-treatment weight) compared to a 1.27 kg body weight gain for controls. Males of the 250 ppm group had a decreased body weight gain for the day 0-364 interval (44% of that of controls). For females, there were no treatment-related effects on absolute body weights or body weight gains in the 25, 125, and 250 ppm groups. High-dose females had slightly decreased body weight gains compared to controls during the first six months of the study and had a 0.47 kg body weight loss during the day 182-364 interval, compared to a 0.70 kg body weight gain by controls. This resulted in body weight losses of 0.27, 1.2, and 1.47 kg during the day 0-364, 364-735, and 0-735 intervals, compared to respective body weight gains of 1.43, 0.13, and 1.57 kg by controls for these same intervals. Interpretation of the body weight data is complicated by a number of different factors: 1) it is unknown whether the dogs were all the same age; 2) the animals were fed fixed, identical portions rather than *ad libitum*; and 3) food consumption was not recorded. However, the significant body weight losses of the high-dose males and females and decreased body weight gains of the 250 ppm males likely represents an adverse effect of treatment.

TABLE 2: Absolute body weights and body weight changes (kg) of male dogs receiving Diuron in feed for 531-735 days[a]

Study day	Dietary concentration (ppm)					
	0	25	125	250[b]	250[c]	2500/1250[d]
Absolute body weights						
0	10.27	10.03	9.97	10.20	10.30	11.60
28	10.27	10.40	10.20	10.47	10.40	10.67
63	10.27	10.63	10.63	10.57	10.30	9.90
91	10.47	10.80	10.87	10.70	10.40	9.87
182	10.77	10.93	11.43	10.70	10.40	9.63
364	11.57	10.93	11.63	10.77	10.25	9.27 (80)[e]
518	11.80	11.27	11.60	10.80	10.10	9.20 (79)
735	11.53	11.13	11.93	n/a	10.70	9.47 (82)
Body weight changes						
0-28	0.00	0.37	0.23	0.27	0.10	-0.93
28-63	0.00	0.23	0.43	0.10	-0.10	-0.77
63-91	0.20	0.17	0.23	0.13	0.10	-0.03
91-182	0.30	0.13	0.57	0.00	0.00	-0.23
182-364	0.80	0.00	0.20	0.07	-0.15	-0.37
0-364	1.30	0.90	1.67	0.57 (44)	-0.05	-2.33
364-735	-0.30	0.20	0.30	n/a	0.45	0.20
0-735	1.27	1.10	1.97	n/a	0.40 (31)	-2.13

Data from Appendix i, pp. 163-222, MRID 00091192.
[a] Calculated by reviewer from individual data.
[b] n = 3, includes the animal sacrificed during week 76.
[c] n = 2, includes only animals that survived to study termination.
[d] The high-dose group received 2500 ppm test material for two weeks, basal diet for a three week reconditioning period, and 1250 ppm of the test material for the remainder of the two-year study period.
[e] Numbers in parentheses are percent of controls, calculated by reviewer.

TABLE 3: Absolute body weights and body weight changes (kg) of female dogs receiving Diuron in feed for 664-735 days[a]

Study day	Dietary concentration (ppm)					
	0	25[b]	25[c]	125	250	2500/1250[d]
Absolute body weights						
0	10.23	10.87	10.75	8.43	9.87	11.40
28	10.87	11.00	11.30	9.00	10.10	11.30
63	11.00	10.93	11.30	9.67	10.23	11.43
91	10.93	11.10	11.55	9.90	10.53	11.50
182	10.97	11.10	11.90	10.27	10.80	11.60
364	11.67	11.07	12.10	10.83	10.73	11.13
518	12.20	11.67	12.60	11.33	10.53	10.40 (85)[e]
735	11.80	n/a	12.80	11.53	11.13	9.93 (84)
Body weight changes						
0-28	0.64	0.13	0.55	0.57	0.23	-0.10
28-63	0.13	-0.07	0.00	0.67	0.13	0.13
63-91	-0.07	0.17	0.25	0.23	0.30	0.07
91-182	0.04	0.00	0.35	0.37	0.27	0.10
182-364	0.70	-0.03	0.20	0.57	-0.07	-0.47
0-364	1.43	0.20	1.35	2.40	0.87	-0.27
364-735	0.13	n/a	0.70	0.70	0.40	-1.20
0-735	1.57	n/a	2.05	3.10	1.27	-1.47

Data taken from Appendix i, pp. 163-222, MRID 00091192.
[a] Calculated by reviewer from individual data and not subjected to statistical analysis.
[b] n = 3, and includes the replacement animal was added during week 11.
[c] n = 2, and includes only animals on study for the entire 735 days.
[d] The high-dose group received a diet containing 2500 ppm for two weeks, basal diet for a three week reconditioning period, and diet containing 1250 ppm of the test material for the remainder of the two-year study period.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Not reported.

D. BLOOD WORK

1. Hematology

Hematology data are given in Table 4. There were no treatment-related effects on hematology parameters at the 25 and 125 ppm dietary groups. At the 250 ppm dietary concentration, hematocrits (HCT), erythrocyte (RBC) counts, and hemoglobin (HGB) concentrations of both sexes exhibited decreasing trends (non-statistical) starting at day 105 and continuing over the course of the study; however, all three parameters remained within normal limits (estimated by the reviewer as pre-test means ± two standard deviations). The study author stated that for 250 ppm males, the decrease in the RBC count was statistically significant on day 720. Males at the 2500/1250 ppm dietary concentration had more pronounced decreases in hematology parameters and some occasionally fell below estimated reference limits (RBC counts on days 225 and

385, HCTs on days 594 and 720, and HGB on day 594). In addition, the calculated MCV of the male high-dose group was increased on days 105, 225, and 385. Females at the 2500/1250 ppm dietary concentration had marked decreases in hematology parameters, with HCTs, RBC counts, and HGBs being below estimated reference limits at all time points (including day 14, not shown in Table 4), but the calculated MCV of this group was only increased on days 385 and 593. The study author stated that the RBC counts and HGB concentrations of males and females at the highest dietary concentration were statistically decreased at 105 days, 1 year, and 2 years.

TABLE 4: Hematology parameters of dogs given Diuron in feed for 531-735 days.[a]

Parameter/Day	Males (ppm)					Females (ppm)				
	0	25	125	250	2500/1250	0	25	125	250	2500/1250
HCT (%)/ 0	57	52	53	54	55	49	49	53	53	47
105	59	52	52	51	48	50	51	56	48	41[b]
225	59	51	50	50	45	51	50	55	47	42[b]
385	58	53	52	49	43	50	53	53	46	40[b]
594	58	52	52	49	42[b]	52	47	53	47	40[b]
720	56	52	52	49	42[b]	52	52	51	46	39[b]
RBC ($10^6/\mu L$)										
0	7.7	7.1	6.9	6.9	7.2	6.5	6.5	6.7	6.2	6.1
105	7.6	7.0	6.8	6.6	5.3*	6.8	6.7	7.1	5.6	4.8*[c]
225	7.4	6.6	6.1	6.3	5.0[c]	7.0	6.4	6.7	5.3	5.0[c]
385	7.3	6.8	6.5	5.8	4.6*[c]	6.4	7.1	6.9	5.3	4.4*[c]
594	7.4	6.8	6.3	5.9	5.1	6.9	5.9	6.9	5.7	4.1[c]
720	6.9	6.9	6.4	5.7*	5.1*	6.3	6.7	6.4	5.5	4.5*[c]
HGB (g/dL)/0	19.7	17.9	17.8	18.3	19.2	17.2	17.0	18.2	17.0	16.7
105	20.6	17.7	17.8	17.1	15.8	18.7	17.9	18.6	16.1	13.7[d]
225	19.9	17.9	17.8	17.4	14.9	18.3	17.8	18.9	16.3	14.1[d]
385	21.5	19.0	19.4	17.3	15.0	18.0	19.6	19.4	16.4	14.3[d]
594	20.9	18.4	18.8	17.4	14.4[d]	18.7	17.8	18.9	16.2	13.2[d]
720	20.0	18.5	18.5	17.3	15.3	18.9	19.0	17.5	16.5	13.7[d]
MCV[e] (fL)/0	74	73	77	78	76	75	75	79	85	77
105	78	74	76	77	91[f]	74	76	79	86	85
225	80	77	82	79	90[f]	73	78	82	89[f]	84
385	79	78	80	84	93[f]	78	75	77	87	91[f]
594	78	76	83	83	82	75	80	77	82	98[f]
720	81	75	81	86	82	83	78	80	84	87

Data taken from Table 16 and Appendix ii, pp. 76 and 223-265, respectively, MRID 00091192.
[a] N = 3, with the exception of the male 250 ppm group on days 597 and 720 when n = 2.
[b] Value is less than the lower end of the estimated reference range of 43-61%.
[c] Value is less than the lower end of the estimated reference range of 5.1-8.4 x 10^6 RBC/μL
[d] Value is less than the lower end of the estimated reference range of 14.7-21.0 g/dL.
[e] Calculated by reviewer as MCV = (HCT x 10)/RBC.
[f] Exceeds the upper end of the estimated reference range of 66-88 fL.
*Reported by study author as $p \leq 0.05$, not flagged in Table 16

2. Clinical chemistry

Spectral analyses were conducted on blood samples collected in week 103 of the study. Oxyhemoglobin spectra were normal for all dogs. There was no indication of

abnormal pigment in the blood of control animals, all animals from the 25 ppm group, or all females and one male from the 125 ppm group. Findings were "somewhat abnormal" for the remaining two males from the 125 ppm group, all animals from the 250 ppm group, and all males and one female from the 2500/1250 ppm group, and the findings for the remaining two females from the 2500/1250 ppm group were considered "abnormal." The general summary of the report stated that the abnormal pigment was "probably a sulfhemoglobin; not methemoglobin."

E. URINALYSIS

There were no treatment-related effects on urine protein or glucose. There were occasional increases in the urine protein of individual animals, but the changes were not consistent within groups or over time, and a dose-response pattern was not present.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

Selected organ weight data are given in Table 4. Organ weights from one control female were excluded from comparison due to her liver being grossly and histopathologically abnormal. At the highest dietary concentration, absolute and relative liver weights were increased in both males and females. The study author stated that the increases in the relative liver weights of both sexes were statistically significant. Estimated liver to brain weight ratios were also increased in both sexes.

TABLE 5: Organ weight data from dogs given Diuron in feed for up to 735 days.[a]

Parameter	Males (ppm)					Females (ppm)				
	0	25	125	250[b]	2500/1250	0[c]	25[d]	125	250	2500/1250
Body weight (kg)	11.5	11.1	11.9	10.7	9.3 (81)[e]	11.8	11.9	11.3	11.1	9.9 (85)
Liver weight (g)	360	319	372	340	439 (122)	347	345	362	369	467 (135)
Brain weight (g)	73	73	73	76	71	73	76	78	69	80
Liver/body wt. (g/kg)	31.5	29.1	31.7	31.8	48.8* (154)	29.3	29.8	32.4	34.9	47.4* (162)
Liver/brain wt.[f] (g/g)	4.93	4.37	5.09	4.47	6.18 (125%)	4.75	4.54	4.64	5.35	5.84 (123%)

Data taken from text, p. 83 and Table 19 and Appendix iii, pp. 84 and 266-271, respectively, MRID 00091192.
[a] n = 3, except where otherwise noted.
[b] n = 2; one animal was sacrificed early.
[c] n = 2, one animal with a grossly abnormal liver is excluded from all means.
[d] Include replacement animal that was sacrificed after 664 days of treatment
[e] Numbers in parentheses equal percent of controls, calculated by reviewer.
[f] Estimated by reviewer.
*$p \leq 0.05$

2. Gross pathology

Gross necropsy findings were not summarized in the study report. The most common abnormal findings included depressions on the capsular surface of the liver, scarring on the capsular surface of the kidneys, and nodules on the capsular surface of the liver

and kidneys. There were no dose-response patterns noted, however.

3. Microscopic pathology

The average erythroid precursors/1000 bone marrow progenitor cells for male and female dogs combined were 352, 414, 391, 338, and 520 for the control, 25, 125, 250, and 2500/1250 ppm groups, respectively, with the highest dietary concentration being statistically significant ($p<0.05$). In addition, marrow fat was moderately reduced in histopathological preparations of bone marrow from animals in the 2500/1250 ppm group. Brown pigment (likely hemosiderin) was observed in the Kupffer cells of all high-dose animals, compared to observations in single animals in other treatment groups. Extramedullary hematopoiesis was noted in the spleen of one high-dose male, and "probable hematopoiesis" was noted in the spleens of two high-dose females. Histopathological findings which were not related to treatment included inflammation of the kidney or renal pelvis in eleven animals, inflammation of the liver in six animals, and vacuolar nephrosis and/or tubular pigment in four animals, as well as an acute systemic vasculitis in one male from the 250 ppm group and a mixed mammary tumor and a probable parathyroid adenoma in two control group females.

G. QUANTITATIVE ANALYSES

Quantitative analysis results are given in Table 6. In general, the tissue levels of Diuron were roughly proportional to the dietary concentrations. The reviewer agrees with the study author's conclusion that "no storage occurs."

TABLE 6: Diuron content in urine, feces, and various tissues (ppm) of dogs receiving Diuron in feed for up to 735 days (ppm)

Sample	Dietary concentration (ppm)				
	0	25	125	250	2500/1250
Muscle	NDR[a]	0.25	0.41	0.90	5.1
Fat	NDR	0.54	2.7	5.7	33
Liver	NDR	3.7	9.8	16	56
Kidney	NDR	0.83	4.3	5.4	33
Spleen	NDR	0.36	1.1	1.9	9.7
Blood	NDR	0.37	1.3	2.2	14
Urine	NDR	6.3	41	42	307
Feces	NDR	7.9	41	68	308

Data taken from Table 20, p 88, MRID 00091192.
[a] No detectable residue; <0.1 ppm.

III. DISCUSSION

A. DISCUSSION

There were no treatment-related deaths during the study, and the only treatment-related clinical sign was occasional partial food refusal at the highest dietary concentration. Over the course of the study, 2500/1250 ppm males had an overall body weight loss of 2.13 kg (18% of the pre-treatment weight of this group), compared to a 1.27 kg body weight gain for controls, and 2500/1250 ppm females had an overall body weight loss of 1.47 kg (13% of the pre-treatment weight of this group), compared to a body weight gain of 1.57 kg by controls. The day 0-364 body weight gain of 250 ppm males was only 41% of controls.

Males at the 2500/1250 ppm dietary concentration had pronounced decreases in hematology parameters, with RBC counts decreased below estimated reference limits on days 225 and 385, HCTs decreased below estimated reference limits on days 594 and 720, and HGB concentration decreased below estimated reference limits on day 594. The calculated MCV of the male high-dose group was increased on days 105, 225, and 385. HCTs, RBC counts, and HGB concentrations of females at the 2500/1250 ppm dietary concentration were below estimated reference limits at all time points, and the calculated MCV of this group was increased on days 385 and 593. Evidence of a regenerative response in high-dose males and females included increased erythroid precursors per 1000 progenitor cells in bone marrow smears from the 2500/1250 ppm group; moderate reductions of marrow fat in histopathological preparations from animals in the 2500/1250 ppm group; as well as extramedullary hematopoiesis in one 2500/1250 ppm male and probably two 2500/1250 ppm females.

At the highest dietary concentration, absolute and relative liver weights were significantly increased in both males and females, as well as liver to brain weight ratios. However, there were no corresponding gross or histopathological hepatic changes to account for the increased liver weights. The brown pigment (assumed to be hemosiderin) observed in Kupffer cells of all high-dose animals is consistent with hemolysis, and the increased liver weights may have been associated with erythrocyte sequestration although an adverse effect of treatment cannot be definitively ruled.

The significance of the unidentified pigment in the blood, which may or may not have been sulfhemoglobin, is unknown but considered adverse.

Under the conditions of this study, the LOAEL for Diuron in male Beagle dogs is 250 ppm (18.8 mg/kg/day), based on decreased body weight gains, and the LOAEL in female Beagle dogs is 1250 ppm (93.8 mg/kg/day), based on a normocytic to macrocytic, normochromic anemia and body weight losses. The NOAEL is 125 ppm (9.4 mg/kg/day) in males and 250 ppm (18.8 mg/kg/day) in females.

This study is classified as **Unacceptable [OPPTS 870.4100 (§83-1b)]** and does not satisfy the Subdivision F guideline requirements.

B. STUDY DEFICIENCIES

Numerous deficiencies were noted in the conduct of this study.

1. The storage conditions of the test material were not described, and stability, homogeneity, and concentration of the test material in diet were not determined prior to study initiation. Two separate "quantities" of the test material were used during the study, however, the test substance lot or batch number for the quantity used during the first year of the study was not provided. In addition, the physical properties (including stability) of the test substance were not provided. The purity of the test substance was 80% and since the method of diet preparation was not described it is unknown whether the quantity of test material was adjusted. Finally, the identity of impurities in the test material was not provided.

2. The guideline recommends that dosing begin when the dogs are no older than 9 months of age, and ideally with dogs 4-6 months of age. It is unknown whether this was the case, since the ages of the dogs at study initiation were not provided. The guideline also requires at least 8 animals (4 male, 4 female) per group, and mentions that there should be enough animals alive at termination for statistical evaluation. The study used three animals/sex/group which is too few for meaningful statistical evaluation. It is also unknown whether females were nulliparous and nonpregnant. Necropsy results for one animal mentioned the absence of ovaries and uterus, suggesting that she may have been previously spayed.

4. The report did not specify how often the animals were observed for morbidity, mortality, and clinical signs, and it is unknown whether detailed weekly clinical examinations were made outside the home cage.

5. Food consumption was not measured and the animals were not fed *ad libitum*. These two deficiencies along with not knowing how old the animals were at study initiation make meaningful interpretation of the body weight data problematic.

6. Hematology parameters did not include platelet counts, a measure of clotting potential, MCV (calculated by reviewer), MCH, and MCHC. Clinical chemistry parameters were not evaluated at all. Urinalysis determinations did not include appearance, volume, specific gravity, pH, or blood/blood cells.

7. Ophthalmological examinations and neurotoxicity screening were not done.

8. At necropsy, the following organs were not weighed: heart, epididymides, ovaries, uterus, adrenal glands, thyroid, and parathyroid. The following tissues were not collected and subjected to histopathological examination: salivary glands, esophagus, gall bladder, trachea, nose, pharynx, larynx, lymph nodes, epididymides, prostate, seminal vesicles, peripheral nerve, spinal cord, pituitary, female mammary gland, parathyroid, thyroid, and skin.

9. The method of randomization was not provided.

10. Test result data were not presented as group means and standard deviations. In addition, where individual animal data were provided, it was not presented in an easily understood tabular form, making it difficult and confusing to interpret. The guideline requires that all observed results should be evaluated by an appropriate method, and this was not done. Rather, the study authors chose and analyzed selected results that were outside of normal limits.

11. Clinical signs were not reported.

12. The study authors did not calculate the achieved dose as a time-weighted average, as the guideline requires if the test substance is administered in feed.

13. Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were not provided.

14. Although not considered treatment-related, inflammation of the renal pelvis in 11/30 animals is of concern because it indicates that the health of the animals may have been compromised. Had there been any treatment-related renal effects, interpretation of the data would have been difficult or impossible.

014692

EXECUTIVE SUMMARY

DIURON

STUDY TYPE: SUBCHRONIC ORAL TOXICITY - RAT (NONGUIDELINE) MRID NO. 40886502

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97B

Primary Reviewer:
Carol S. Forsyth, Ph.D., D.A.B.T.

Signature: Carol S. Forsyth
Date: APR 10 2001

Secondary Reviewers:
Sylvia S. Talmage, Ph.D., D.A.B.T.

Signature: Sylvia S. Talmage
Date: APR 10 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: APR 10 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle. LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Yung Yang, Ph.D. Date: 5/10/2001
Toxicology Branch
EPA Work Assignment Manager: Joycelyn Stewart, Ph.D. Date: 5/10/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD- Amended
Original DER- TXR #008176

STUDY TYPE: Subchronic Oral Toxicigy - Rat; Nonguideline

OPP Number: none
DP BARCODE: D272128
PC CODE: 035505

OPPTS Number: none
SUBMISSION CODE: S591433
TOX CHEM NO: 410

TEST MATERIAL: Diuron (98.8%)

CHEMICAL NAME: N′-(3,4-dichlorophenyl)-N,N-dimethyl urea

CITATION: Schmidt, W.M. and Karbe, E. (1988). Diuron: Toxicology study with Wistar rats paying special attention to effects on the blood (administration in the diet for six months). Institute of Toxicology, Bayer AG, Wuppertal, Friedrich-Ebert-Strasse 217-333, West Germany and Institute of Experimental Pathology, Hanover Medical University, West Germany. Study No. Bayer AG T 7018927, Du Pont Report No. D/Tox 18. January 29, 1988. MRID 40886502. Unpublished.

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

EXECUTIVE SUMMARY: In a 6-month oral toxicity study Diuron (98.8% a.i., Lot No. 232114123) was administered to groups of 10 male and 10 female BOR:WISW (SPF Cpb) rats in the diet at concentrations of 0, 4, 10, or 25 ppm (MRID 40886502). Time-weighted average doses were 0, 0.3, 0.7, and 1.6 mg/kg/day, respectively, for males and 0, 0.3, 0.8, and 1.8 mg/kg/day, respectively, for females.

Deaths of one mid-dose male and one mid-dose female after blood collection during week 12 were considered incidental to treatment; all remaining animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal. Body weights, food consumption, differential blood counts, erythrocyte morphology, and organ weights were unaffected by treatment.

Reticulocyte counts in the high-dose females were increased at all intervals with statistical significance ($p \leq 0.05$) attained at weeks 12 and 26 (150-165% of control values). Mean hemoglobin concentrations in the high-dose females were slightly (n.s.) depressed at all intervals as compared to the controls (within 5% of control levels).

Increased incidences of gross lesions on the urinary bladder were observed in all treated groups of males and females. In the control, low-, mid-, and high-dose groups, dilated blood vessels

were observed in 0/10, 3/10, 1/10, and 3/10 males, respectively, and in 1/10, 3/10, 3/10, and 5/10 females, respectively; increased firmness was observed in 0/10, 1/10, 2/10, and 3/10 males, respectively, and in 0/10, 5/10, 2/10, and 3/10 females, respectively; and reduced transparency was observed in 0/10, 1/10, 1/10, and 2/10 females, respectively.

Microscopic examinations and morphometric measurements of the urinary bladder were done in an attempt to characterize the gross findings. Hyperplasia of the epithelium was observed in 1 low-dose male, 1 control female, 2 low-dose females, and 2 high-dose females. Thickening of the epithelium by enlargement of the epithelial cells (hypertrophy) was seen in 2 low-dose males and 1 high-dose male. In females from the control, low-, mid-, and high-dose groups, the thickness of the urinary bladder wall was 437, 492, 448, and 486 μm, respectively; males were not measured. These observations are judged to be equivocal.

Pigment deposition (iron) in the spleen was observed in all treated and control animals, however, the severity and extent were increased in the high-dose groups. In the control, low-, mid-, and high-dose groups, severity ratings were 2.3, 2.3, 2.3, and 3.0, respectively for males, and 2.5, 3.1, 2.9, and 3.7, respectively, for females (based on a scale of increasing severity of 1-5). Morphometric measurements showed the percent area of iron deposits to be 8.4, 10.1, 9.8, and 14.1, respectively, for males, and 14.7, 15.7, 15.1, and 19.4, respectively, for females.

Under the study condition, the NOAEL can not be determined because some findings were judged to be equivocal.

This study is not designed to be a guideline study and is classified as **Acceptable/Nonguideline** as a supplementary subchronic study in rats.

TABLE . Food consumption (g/animal/day) of male and female rats administered Diuron in the diet for 6 months

Week of Test	Treatment Group			
	0 ppm	4 ppm	10 ppm	25 ppm
Males				
Week 2	17.1	17.9	18.6	17.8
Week 5	18.8	19.5	20.5	19.6
Week 10	20.1	20.4	20.7	20.2
Week 15	21.5	19.9	22.9	20.6
Week 20	20.4	19.1	24.4	19.3
Week 26	20.1	18.5	21.5	18.2
Overall (weeks 2-26)	20.2	19.4	21.8	19.7
Females				
Week 2	13.8	13.2	13.6	14.4
Week 5	14.7	14.0	13.6	13.9
Week 10	15.3	15.5	14.6	14.2
Week 15	17.7	15.2	16.2	15.4
Week 20	15.4	14.9	15.2	14.6
Week 26	14.5	13.7	14.5	14.3
Overall (weeks 2-26)	15.8	14.8	15.2	14.7

Data taken from Table 1, p. 16 and tables on pp. 35-36, MRID 40886502.

TABLE . Absolute and relative organ weights				
Endpoint	**Treatment Group**			
	0 ppm	**4 ppm**	**10 ppm**	**25 ppm**
Males				
Final body weight	366	367	407*	378
Liver				
Absolute (mg)	12576	12171	13010	12448
Relative (mg/100 g)	3481	3316	3200	3292
Spleen				
Absolute (mg)	606	620	683	585
Relative (mg/100 g)	166	169	167	154
Females				
Final body weight	232	219	225	232
Liver				
Absolute (mg)	7526	7629	7240	7470
Relative (mg/100 g)	3255	3475	3216	3225
Spleen				
Absolute (mg)	434	423	435	448
Relative (mg/100 g)	186	193	193	193

Data taken from Table 4, p. 22, MRID 40886502.
Significantly different from control: *$p \leq 0.05$.

TABLE . Histopathological findings in male and female rats fed Diuron for 6 months (no. affected)

Endpoint	Treatment Group			
	0 ppm	4 ppm	10 ppm	25 ppm
Males				
Number examined	10	10	10	10
Bladder				
Cellular infiltration	1	2	3	1
Hyperplasia[a]	0	1	0	0
Hypertrophy[b]	0	2	0	1
Spleen				
Pigment deposition	10	10	10	10
Mean severity[c]	2.3	2.3	2.3	3.0
Percent area[d]	8.4	10.1	9.8	14.1
Females				
Number examined	10	10	10	10
Bladder				
Cellular infiltration	0	0	0	1
Hyperplasia[a]	1	1	0	2
Hyperplasia with vascularization	0	1	0	0
Spleen				
Pigment deposition	10	10	10	10
Mean severity[c]	2.5	3.1	2.9	3.7
Percent area[d]	14.7	15.7	15.1	19.4

Data taken from Table 6, p. 24 and table p. 127, MRID 40886502.
[a]Simple hyperplasia of the urinary bladder epithelium; defined as focal in both low-dose females and one high-dose female.
[b]Thickening of the urinary bladder epithelium by enlargement of epithelial cells.
[c]Severity grades: 1 = slight; 2 = slight to moderate; 3 = moderate; 4 = moderate to severe; 5 = severe.
[d]Mean percent area of iron deposits determined by morphometry of Turnbull blue stain tissues.

014692

EXECUTIVE SUMMARY

DIURON

STUDY TYPE: MULTIGENERATION REPRODUCTION - RAT
[OPPTS 870.3800 (§83-4)]
MRID NO. 41957301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97C

Primary Reviewer:
Carol S. Forsyth, Ph.D., D.A.B.T.

Signature: Carol S Forsyth
Date: APR 10 2001

Secondary Reviewers:
Sylvia S. Talmage, Ph.D., D.A.B.T.

Signature: Sylvia S. Talmage
Date: APR 10 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: APR 10 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle. LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Laurence D. Chitlik, D.A.B.T. Date: 4/25/01
Toxicology Branch
EPA Work Assignment Manager: J. Stewart, Ph.D. Date: 5/10/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD- Amended
Original DER- TXR #009786

STUDY TYPE: Multigeneration Reproduction - Rat; OPPTS 870.3800 (§83-4)

OPP Number: 83-4 OPPTS Number: 870.3700
DP BARCODE: D272128 SUBMISSION CODE: S591433
PC CODE: 035505 TOX CHEM NO: 410

TEST MATERIAL: Diuron (97.1%)

CHEMICAL NAME: Urea, N′-(3,4-dichlorophenyl)-N,N-dimethyl-

CITATION: Cook, J.C. (1990). Reproductive and fertility effects with diuron (IN 14740), multigeneration reproduction study in rats. E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE. Study No. HLR 560-90. December 14, 1990. MRID 41957301. Unpublished.

SPONSOR: Du Pont Agricultural Products, E.I. du Pont de Nemours & Co., Inc., Wilmington, DE

EXECUTIVE SUMMARY: In a two-generation reproduction study Diuron (97.1% a.i., Lot No. 8805540) was administered to groups of 30 male and 30 female Crl:CD®BR rats in the diet at concentrations of 0, 10, 250, or 1750 ppm (MRID 41957301). One litter was produced by each generation. Test substance intake for the treated F_0 groups was 0.58, 14.8, and 101 mg/kg/day, respectively, for males and 0.71, 18.5, and 131 mg/kg/day, respectively, for females. Test substance intake for the treated F_1 groups was 0.77, 18.9, and 139 mg/kg/day, respectively, for males and 0.8, 22.1, and 157 mg/kg/day, respectively, for females. F_0 and F_1 parental animals were administered test or control diet for 73 or 105 days, respectively, prior to mating and throughout mating, gestation, and lactation, and until necropsy.

Deaths or premature sacrifices of several F_0 and F_1 parental animals were considered incidental to treatment. No treatment-related clinical signs of toxicity were observed in the adult animals of either generation. Gross necropsy was unremarkable and testes weights were not affected by treatment.

For the low- and mid-dose groups of both generations, occasional significant differences from the control group for body weights, body weight gains, food consumption, and food efficiencies were considered incidental to treatment.

Body weights of the high-dose F_0 males and females were significantly ($p \leq 0.05$) decreased by an average of 7% beginning on day 7. Body weight gains by the high-dose F_0 males were

significantly ($p \leq 0.05$) less than the control group on days 0-14, 21-28, 42-49, 77-84, and 91-98. Premating, post-mating, and overall (entire study) body weight gains by the F_0 males were significantly ($p \leq 0.05$) decreased by 16%, 28%, and 18%, respectively, compared with the controls. Body weight gains by the high-dose F_0 females were significantly ($p \leq 0.05$) less than the control group on days 0-14 and 21-28 with overall premating body weight gains significantly ($p \leq 0.05$) decreased by 28% compared with the controls. Significant ($p \leq 0.05$) reductions in food consumption were observed in the high-dose F_0 males and females on days 0-14, 21-28, 35-49 (females), 42-56 (males), and 0-70. Food efficiencies for the F_0 males and females were significantly ($p \leq 0.05$) reduced at similar intervals to food consumption with overall premating food efficiency reduced by 8.3% and 22.7%, respectively.

Body weights of the high-dose F_1 males and females were significantly ($p \leq 0.05$) decreased by an average of 16% beginning on day 0 of premating. Body weight gains by the high-dose F_1 males were significantly ($p \leq 0.05$) less than the control group on days 0-28, 42-49, 63-70, 91-98, and 147-154. Premating, post-mating, and overall (entire study) body weight gains by the F_1 males were significantly ($p \leq 0.05$) decreased by 15%, 41%, and 17%, respectively, compared with the controls. Body weight gains by the high-dose F_1 females were significantly ($p \leq 0.05$) less than the control group on days 0-14 with overall premating body weight gains significantly ($p \leq 0.05$) decreased by 14% compared with the controls. Significant ($p \leq 0.05$) reductions in food consumption were observed in the high-dose F_0 males and females throughout premating with the exception of days 77-84 for males. Food efficiencies were significantly ($p \leq 0.05$) reduced for the high-dose F_1 males on days 91-98 and for the high-dose F_1 females on days 0-7, 21-28, and 0-70.

Therefore, the systemic toxicity LOAEL is 1750 ppm (approximately 132 mg/kg/day) based on reduced body weight, body weight gain, food consumption, and food efficiency during both generations. The systemic toxicity NOAEL is 250 ppm (approximately 18.6 mg/kg/day).

For the F_0 and F_1 females, reduced body weights and food consumption during gestation were considered a continuation of premating effects.

No treatment-related effects were noted in either generation on fertility indices, gestation length, pup survival, pup clinical observations, and pup anomalies. Pup body weights for sexes combined or separate were significantly ($p \leq 0.05$) reduced in high-dose litters as compared with the controls throughout lactation for the F_1 pups and beginning on lactation day 7 for the F_2 pups.

Therefore, the reproductive/offspring toxicity LOAEL is 1750 ppm (approximately 132 mg/kg/day) based on decreased body weights of the F_1 and F_2 pups during lactation. The reproductive toxicity NOAEL is 250 ppm (18.6 mg/kg/day).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a reproductive toxicity study [OPPTS 870.3800 (§83-4)] in rats.

TABLE . Mean body weights (g) of the F_0 adults during the premating period				
Days on Test	Treatment Group			
	0 ppm	10 ppm	250 ppm	1750 ppm
Males				
Day 0	353.0	355.3	350.0	349.2
Day 7	398.9	396.9	397.6	378.0* (95)[a]
Day 21	471.5	476.7	475.4	440.1* (93)
Day 35	530.0	536.0	531.4	491.3* (93)
Day 56	581.6	589.1	587.5	532.6* (92)
Day 70 (end of premating)	605.7	623.1	622.0	563.8* (93)
Day 112 (termination)	656.5	670.5	665.5	598.0* (91)
Females				
Day 0	223.7	215.7	217.7	217.8
Day 7	245.8	246.8	241.9	229.4* (93)
Day 21	273.3	274.7	274.1	258.4* (95)
Day 35	298.7	297.1	291.7	274.8* (92)
Day 56	310.1	313.0	302.1	282.5* (91)
Day 70 (end of premating)	322.5	324.4	315.7	288.7* (90)

Data taken from Tables 2 and 6, pp. 62 and 66, respectively, MRID 41957301.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

TABLE . Mean body weights (g) of the F_1 adults during the premating period				
Days on Test	Treatment Group			
	0 ppm	10 ppm	250 ppm	1750 ppm
Males				
Day 0	60.0	62.4	58.0	48.5* (81)[a]
Day 7	112.0	112.2	105.0	89.7* (80)
Day 21	244.4	243.7	239.5	204.3* (84)
Day 35	371.2	373.8	367.7	318.8* (86)
Day 56	492.4	497.9	484.7	425.2* (86)
Day 70	551.1	557.5	538.8	472.4* (86)
Day 105 (end of premating)	640.9	648.3	623.8	543.8* (85)
Day 161 (termination)	708.9	719.2	684.0	587.5* (83)
Females				
Day 0	55.4	57.3	54.3	46.2* (83)
Day 7	100.7	101.2	94.4* (94)	81.0* (80)
Day 21	187.1	186.6	175.6* (94)	159.6* (85)
Day 35	238.3	235.0	224.9* (94)	205.8* (86)
Day 56	283.0	282.7	270.1	245.7* (87)
Day 70	303.7	304.9	294.7	264.8* (87)
Day 105 (end of premating)	339.7	342.8	321.8	292.0* (86)

Data taken from Tables 3 and 7, pp. 63 and 67, respectively, MRID 41957301.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

TABLE . Mean food consumption (g/rat) of the F_0 adults during the premating period				
Days on Test	Treatment Group			
	0 ppm	10 ppm	250 ppm	1750 ppm
Males				
Days 0-7	29.5	29.0	29.4	24.7* (84)[a]
Days 7-14	31.2	31.1	31.4	27.2* (87)
Days 28-35	30.2	30.6	30.7	28.7
Days 49-56	30.9	30.6	30.5	28.2* (91)
Days 63-70	30.0	30.0	30.4	28.5
Days 0-70	30.3	30.5	30.9	27.9* (92)
Females				
Days 0-7	21.0	21.0	21.1	18.8* (90)
Days 7-14	22.3	21.0	21.8	21.0
Days 28-35	20.8	20.2	20.5	19.6
Days 49-56	21.4	20.5	19.7	20.1
Days 63-70	20.3	20.3	21.0	19.4
Days 0-70	21.2	20.7	21.1	19.9* (94)

Data taken from Tables 14 and 18, pp. 74 and 78, respectively, MRID 41957301.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

TABLE . Mean food consumption (g/rat) of the F_1 adults during the premating period				
Days on Test	Treatment Group			
	0 ppm	10 ppm	250 ppm	1750 ppm
Males				
Days 0-7	16.6	17.0	15.4	13.3* (80)[a]
Days 7-14	22.7	22.5	21.4	19.8* (87)
Days 28-35	30.3	30.8	30.3	27.6* (91)
Days 49-56	31.5	32.3	31.2	28.2* (90)
Days 63-70	32.6	31.8	30.5	27.8* (85)
Days 98-105	30.9	32.1	29.8	27.3* (88)
Days 0-105	29.3	29.7	28.5	25.9* (88)
Females				
Days 0-7	15.7	15.7	14.5	12.5* (80)
Days 7-14	20.1	20.0	18.5* (92)	16.7* (83)
Days 28-35	22.7	21.3	20.8* (92)	19.6* (86)
Days 49-56	22.6	22.0	21.7	19.4* (86)
Days 63-70	22.0	21.6	21.9	19.5* (89)
Days 98-105	22.5	21.3	20.1* (89)	19.0* (84)
Days 0-105	21.8	21.2	20.4* (94)	18.6* (85)

Data taken from Tables 15 and 19, pp. 75 and 79, respectively, MRID 41957301.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

TABLE . Absolute and relative testes weights				
Endpoint	Treatment Group			
	0 ppm	10 ppm	250 ppm	1750 ppm
F_0 Males				
Final body weight	670.4	684.6	678.3	611.4* (91)[a]
Absolute testes wt. (g)	3.775	3.883	3.836	3.820
Relative testes wt. (% of body wt.)	0.5675	0.5703	0.5689	0.6278* (111)
F_1 males				
Final body weight	723.2	734.7	696.0	594.2* (82)
Absolute testes wt. (g)	3.896	4.174* (107)	3.969	3.898
Relative testes wt. (% of body wt.)	0.5435	0.5718	0.5728	0.6611* (122)

Data taken from Tables 44 and 46, pp. 106 and 108, respectively, MRID 41957301.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

EPA Reviewer: Yung G. Yang, Ph.D. Date 5/15/2001
Toxicology Branch (7509C)
EPA Secondary Reviewer: Alberto Protzel, Ph.D. Date 5/15/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD
This revised executive summary is an addendum to HED Tox. No. 10441.

STUDY TYPE: Twenty-one day dermal toxicity-rabbit; OPPTS 870.3200 (rabbit)

DP BARCODE: D191321 SUBMISSION CODE: S440777
P.C. CODE: 035505 TOX. CHEM. NO.: 410

TEST MATERIAL (PURITY): Diuron (96.8%)

SYNONYMS: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

CITATION: MacKenzie, S. (1992) Repeated Dose Dermal Toxicity: 21-Day Study with DPX-14740-166 (Diuron) in Rabbits: Lab Project Number: 9122-001: 484-92. Unpublished study prepared by E.I. du Pont, Haskell Lab. September 21, 1992 MRID no. 42718301 . Unpublished

SPONSOR: Du Pont Agricultural Products

EXECUTIVE SUMMARY:
In a 21-day dermal toxicity study (MRID no. 42718301), diuron (98.6%) was administered dermally at 0 (deionized water), 50, 500 and 1200 mg/kg/day to 5 New Zealand White rabbits/sex/dose for 6 hours/day. Diuron was administered on the backs of rabbits whose hair was clipped. Body weight, good consumption, clinical signs, mortality, clinical chemistry, hematology, gross pathology, histopathology, and organ weights were observed. Erythema and edema were scored using the Draize scoring system.

There were no treatment related effects on clinical signs, body weight, body weight gain, food consumption, hematology, organ weights, or histopathology parameters were noted.

In treatment and control groups, slight to mild erythema was observed by days 10-14 and was attributed to mechanical skin injury from experimental treatment. In the 1200 mg/kg/day group, moderate erythema was noted at 1200 mg/kg/day in 2 of 5 females. Slight or mild edema was noted in one male and one female, respectively, of the 1200 mg/kg/day group.

The NOAEL for 21 day dermal toxicity study is 1200 mg/kg/day (HDT). This study is classified **acceptable-guideline** and satisfies the guideline requirement for a subchronic dermal study (82-2) in rabbits.

EXECUTIVE SUMMARY

DIURON

STUDY TYPE: DEVELOPMENTAL TOXICITY - RABBIT
[OPPTS 870.3700 (§83-3B)]
MRID NO. 40228802

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97A

Primary Reviewer: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Carol S Forsyth Date: APR 10 2001
Secondary Reviewers: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Sylvia S. Talmage Date: APR 10 2001
Robert H. Ross, M.S., Group Leader	Signature: Robert H Ross Date: APR 10 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battelle. LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Laurence D. Chitlik, D.A.B.T. Laurence D. Chitlik Date: 4/25/01
Toxicology Branch 1/HED (7509C)
EPA Work Assignment Manager: J. Stewart, Ph.D. Jacqueline Stewart Date: 5/10/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD- Amended
Original DER- TXR #008339

STUDY TYPE: Developmental Toxicity - Rabbit; OPPTS 870.3700 (§83-3b)

OPP Number: 83-3
DP BARCODE: D272128
PC CODE: 035505

OPPTS Number: 870.3700
SUBMISSION CODE: S591433
TOX CHEM NO: 410

TEST MATERIAL: Diuron (99%)

CHEMICAL NAME: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

CITATION: Dearlove, G.E. (1986). Developmental toxicity study of H-16035 (Diuron) administered by gavage to New Zealand white rabbits. Argus Research Laboratories, Inc., 935 Horsham Rd., Horsham, PA. Study No. HLO 332-86. May 16, 1986. MRID 40228802. Unpublished.

SPONSOR: E.I. du Pont de Nemours & Co., Inc., Agriculture Product Department.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 40228802), 24-25 artificially inseminated New Zealand white rabbits per group were administered 0, 2, 10, or 50 mg/kg/day of Diuron (99% a.i.; Lot No. not given) by gavage on gestation days (GD) 7-19, inclusive. On GD 29, all surviving does were sacrificed and examined grossly. The liver from each doe was weighed. All fetuses were weighed, sexed, and examined for external and visceral malformations/variations. The brain of each fetus was free-hand transverse-sectioned and examined. All fetal carcasses were processed for skeletal examination.

One control animal died on GD 0 due to an anaphylactic shock reaction during insemination and one high-dose doe aborted and was killed on GD 26. These deaths were considered unrelated to treatment. All remaining animals survived to scheduled termination. No treatment-related clinical signs of toxicity were observed in any animal. Maternal liver weights were comparable between the treated and control groups and gross necropsy was unremarkable.

Maternal body weights, body weight gains, and food consumption for the low- and mid-dose groups were similar to the control levels throughout the study. Absolute body weights of the high-dose does were significantly ($p \leq 0.01$) less than the controls on GD 20. Mean body weight gains by the high-dose group were significantly ($p \leq 0.05$ or 0.01) reduced as compared with the controls during the intervals of GD 10-13, 13-16, and 7-20 (weight loss). Weight gain by the high-dose group was significantly ($p \leq 0.05$ or 0.01) greater than the controls during the post-dosing interval. Food consumption by the high-dose group was significantly ($p \leq 0.01$) less than the controls during the GD 13-16, 16-20 and 7-20 intervals.

The maternal toxicity LOAEL is established at 50 mg/kg/day based on decreased body weights and food consumption during the dosing interval. The maternal toxicity NOAEL is 10 mg/kg/day.

At cesarean section, the pregnancy rates, numbers of corpora lutea, implantation sites, resorptions, and live fetuses, and fetal body weights were similar between the treated and control groups. No dose- or treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus.

The developmental toxicity NOAEL is ≥50 mg/kg/day and the developmental toxicity LOAEL is not identified.

This study was previously classified as unacceptable/upgradable by the reviewer based on deficiencies in analytical data of sample analysis. However, the HIARC determined that this study is acceptable because the low nominal level of sample concentration was observed at the low dose only and the NOAEL was established at the mid-dose with the LOAEL at the high-dose. Therefore, the deficiencies in the analytical data did not affect the results of the study (HIARC Report, HED Doc. No. 014596).

This study is classified as **acceptable** and **does** satisfy the guideline requirements for a developmental toxicity study [OPPTS 870.3700 (83-3b)] in rabbits.

TABLE : Cesarean section observations				
Observation	**0 mg/kg/day**	**2 mg/kg/day**	**10 mg/kg/day**	**50 mg/kg/day**
No. Animals Assigned	24 (23)[a]	24	25	25
No. Animals Pregnant	21	22	23	23
Pregnancy Rate (%)	91	92	92	92
Maternal Mortality	0	0	0	0
Delivered Early/Aborted	0	0	0	1
Corpora Lutea/Doe	11.0	10.7	10.0	10.4
Implantation/Doe	7.2	7.1	7.6	7.7
Preimplantaion Loss (%)[b]	34.5	33.6	24.0	26.0
Postimplantation Loss (%)[b]	6.9	4.2	7.9	2.3
Total Live Fetuses	141	149	161	166
Live Fetuses/Litter	6.7	6.8	7.0	7.5
Mean Fetal Weight (g)	46.11	45.89	46.13	45.17
Total Dead Fetuses	1	0	0	0
Does With All Resorptions	0	0	0	0
Mean Resorptions/Dam	0.5	0.4	0.6	0.2

Data taken from Table 7, p. 42, MRID 40228802.
[a]One animal died during insemination and was not replaced.
[b]Calculated by reviewer from group means.

TABLE : Summary of fetal external and visceral malformations/variations [no. fetuses (no. litters) affected]				
Observation	**0 mg/kg/day**	**2 mg/kg/day**	**10 mg/kg/day**	**50 mg/kg/day**
Multiple external malformations	0 (0)	0 (0)	1 (1)	0 (0)
Umbilical hernia (observed externally and viscerally)	0 (0)	0 (0)	1 (1)	0 (0)
Kinked tail	1 (1)	0 (0)	0 (0)	0 (0)
Subcorneal hemorrhage	2 (2)	1 (1)	1 (1)	3 (3)
Agenesis of intermediate lobe of lung	1 (1)	1 (1)	0 (0)	1 (1)

Data taken from Table 8, pp. 43, MRID 40228802.

TABLE : Summary of fetal skeletal malformations/variations [no. fetuses (no. litters) affected]				
Observation	**0 mg/kg/day**	**2 mg/kg/day**	**10 mg/kg/day**	**50 mg/kg/day**
Skull - irregularly shaped fontanelle	0 (0)	0 (0)	0 (0)	4 (2)
Skull - hole in parietal(s)	0 (0)	0 (0)	3 (3)	0 (0)
Hyoid - ala(e) angulated	2 (2)	5 (4)	6 (5)	1 (1)
Ribs - thickened areas	3 (3)	0 (0)	6 (6)	2 (2)
Xiphoid - unossified	7 (4)	5 (3)	9 (5)	6 (5)

Data taken from Table 8, pp. 44-46, MRID 40228802.

DATA EVALUATION REPORT

014692

DIURON
(H-16035)

STUDY TYPE: DEVELOPMENTAL TOXICITY – RAT
OPPTS 870.3700 (§83-3a)
MRID 40228801

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-96A

Primary Reviewer: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Carol S. Forsyth Date: APR 0 4 2001
Secondary Reviewers: Kowetha A. Davidson, Ph.D., D.A.B.T.	Signature: K A Davidson Date: APR 0 4 2001
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: APR 0 4 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L. A. Wilson Date: APR 0 4 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

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EPA Reviewer: Laurence D. Chitlik, D.A.B.T. Date: 4/25/01
Toxicology Branch 1/HED (7509C)
EPA Work Assignment Manager: J. Stewart, Ph.D. Date: 5/10/2001
Toxicology Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat [OPPTS 870.3700 (§83-3a)]

DP BARCODE: D272128
P.C. CODE: 035505

SUBMISSION CODE: S591433
TOX. CHEM. NO.: 410

TEST MATERIAL (PURITY): Diuron (99% a.i.)

SYNONYMS: H-16035; 3-(3,4-dichlorophenyl)-1,1-dimethylurea

CITATION: Dearlove, G.E. (1986) Developmental toxicity study of H-16035 (Diuron) administered by gavage to rats. Argus Research Laboratories, Inc., 935 Horsham Road, Horsham, PA. Laboratory Project ID. HLO 410-86. June 16, 1986. MRID 40228801. Unpublished.

SPONSOR: Agricultural Products Department, E.I. du Pont de Nemours and Company, Inc.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 40228801), 25 presumed pregnant Crl:COBS®CD®(SD)BR rats per group were administered H-16035 (99%; Lot No. not given) by gavage in 0.5% aqueous hydroxypropyl methylcellulose at doses of 0, 16, 80, or 400 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, dams were sacrificed, subjected to gross necropsy, and all fetuses were examined externally. Approximately one-half of all fetuses were examined viscerally by the Staples technique; these fetuses were decapitated, and the heads fixed in Bouin's solution for subsequent free-hand sectioning. The remaining one-half of the fetuses were eviscerated and all carcasses were processed for skeletal examination.

All dams survived to terminal sacrifice. One high-dose animal appeared thin on GD 13-18 as a result of marked weight loss. No other treatment-related clinical signs of toxicity were observed in any group. Body weights, body weight gains, and food consumption by the low-dose group were similar to the controls throughout the study. No treatment-related lesions were observed in any dam at necropsy.

Absolute body weights of the mid- and high-dose groups were significantly ($p \leq 0.01$) less than the controls during the dosing interval and ranged from 92-94% and 84-88%, respectively, of the control levels. Body weight gains by the mid- and high-dose dams were significantly ($p \leq 0.05$ or 0.01) less than that of the controls during the dosing period with the exception of GD 12-16. The most pronounced effect on body weight gain occurred immediately after the initiation of dosing (GD 6-9) when the mid- and high-dose groups had a net weight loss compared to a gain by the

controls. The high-dose group also had a weight loss for GD 9-12. Weight change during the entire dosing interval was 37% of the control level for the mid-dose group and a weight loss of 12.2 g by the high-dose group. Food consumption by the mid- and high-dose groups was significantly (73 and 47%, respectively, of controls; $p \leq 0.01$) less than the controls during the dosing interval. Weight gain and food consumption by the mid- and high-dose dams during the post-dosing period was significantly ($p \leq 0.01$) greater than the controls.

Therefore, the maternal toxicity LOAEL is tentatively established at 80 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal toxicity NOAEL is tentatively 16 mg/kg/day.

No differences were observed between the treated and control groups for pregnancy rate, number of corpora lutea, number of implantation sites, number of fetuses/litter, or fetal sex ratios. No dead fetuses or late resorptions were observed. Two high-dose dams had total litter resorption and the number of early resorptions/dam in the high-dose group (3.2) was slightly greater than that of the controls (1.2). Mean fetal body weight in the high-dose group was significantly ($p \leq 0.01$; 91% of controls) less than that of the controls.

In the 0, 16, 80, and 400 mg/kg/day groups, the total number of fetuses(litters) examined for external and skeletal malformations/variations was 288(22), 305(23), 297(22), and 279(20), respectively, and for visceral malformations/variations was 138(22), 149(23), 144(22), and 134(20), respectively. No treatment-related external or visceral malformations/variations were observed in any group.

Delayed ossification of the vertebrae and sternebrae was observed in fetuses of the high-dose group. In the 0, 16, 80, and 400 mg/kg/day groups the incidence rates for litters containing fetuses with bifid thoracic vertebral centra was 1/22, 1/23, 2/22, and 7/20 ($p \leq 0.05$), respectively. Incomplete ossification of the sternebrae was observed in fetuses from 3/22, 3/23, 1/22, and 9/20 ($p \leq 0.05$), litters respectively. Unossified thoracic vertebral centra was observed in fetuses from 3/20 ($p \leq 0.05$) high-dose litters but not in fetuses from the other treated or control groups.

Therefore, the developmental toxicity LOAEL is tentatively established at 400 mg/kg/day based on whole litter resorption, reduced fetal body weights, and delayed ossification of the vertebrae and sternebrae. The developmental toxicity NOAEL is tentatively 80 mg/kg/day.

This study is **NOT** classified as **Acceptable** and **does not** satisfy the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats. Test article concentrations in the mid- and high-dose solutions were highly variable and well outside of acceptable ranges. Based upon available analytical data, it appears that target doses may not have been representative of the actual doses to the animals. In addition, the lot number and corresponding analyses were not provided. It is unlikely that this study may be upgraded. (See Deficiencies)

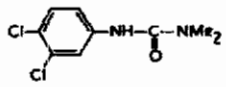
COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, and Data Confidentiality statements were included. A Flagging statement was not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: H-16035

Description: tan powder
Lot No.: not given
Purity: 99% a.i.
Stability of compound: responsibility of sponsor
CAS No.: 330-54-1
Structure:



2. Vehicle and/or positive control

A 0.5% aqueous hydroxypropyl methylcellulose (Lot #52F-0103; Sigma Chemical Company, St. Louis, Missouri) solution was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: rat
Strain: Crl:COBS®CD®(SD)BR
Age and weight at study initiation: 84 days; 234-294 g
Source: Charles River Laboratories, Kingston, NY
Housing: Animals were individually housed in wire-bottomed stainless steel cages suspended above absorbent paper liners.
Diet: Certified Rodent Chow Meal® 5002M (Ralston Purina) was available *ad libitum*.
Water: Reverse osmosis-treated water was available *ad libitum*.
Environmental conditions:
- Temperature: 72±4°F
- Humidity: 50-70%
- Air changes: 10/hour
- Photoperiod: 12 hr light/dark

Acclimation period: 14 days

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of H-16035 when administered by gavage to rats on GD 6-15, inclusive.

1. In life dates

Start: January 07, 1986; end: January 30, 1986

2. Mating

Females were mated to a male of the same strain and source at a ratio of one female to one male for a maximum of four days. Positive evidence of mating was confirmed by the presence of a copulatory plug or of sperm in a vaginal smear. The day on which evidence of mating was observed was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Animals were assigned to a control or treatment group using a computer-generated (weight-ordered) randomization procedure based on GD 0 body weight.

TABLE 1. Animal assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	0	25
Low Dose	16	25
Mid Dose	80	25
High Dose	400	25

Data taken from text table p. 16, MRID 40228801.

4. Dose selection rationale

A dose selection rationale was not given.

5. Dose solution preparation and analysis

Test solutions were prepared twice weekly during the study. All solutions were adjusted for purity of the test article. The method of preparation was not described. Two samples of each preparation were reserved for concentration analyses. Stability was determined by the sponsor prior to initiation of the study; details were not given. Homogeneity was not measured.

Results –
Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the low-dose solution were 97-112% of nominal. In the mid-dose solutions, the concentrations in sample #1 from each preparation were 124, 69, 42, and 86% of nominal; analysis of the second sample from the first three preparations showed concentrations of 114, 116, and 102%, respectively, of nominal. An additional result for analysis of the second sample from the second mid-dose preparation was 54% of nominal, but it is unclear why this sample was analyzed. For the high-dose solutions, results form the third preparation were not given; concentrations in samples from the

first preparation were 100-105% of nominal. Concentrations in the second and fourth high-dose preparations were 154 and 150%, respectively, of nominal in the first sample and 63 and 108%, respectively, of nominal in the second sample.

Homogeneity analysis: not determined

Stability analysis: The sponsor stated that the test article was stable in the vehicle for the duration of use. These data were not provided for review.

Analyses of the dosing solutions showed highly variable concentrations in the mid- and high-dose solutions. Therefore, target doses may not have been representative of the actual doses to the animals. These dosing preparation deficiencies raise issues as to the acceptability of the submitted study.

6. Dosing

All doses were administered in a volume of 5 mL/kg of body weight based on the most recently recorded individual body weight.

C. OBSERVATIONS

1. Maternal observations and evaluations

All animals were checked twice daily for viability. Rats were observed for clinical signs of toxicity "several times" during the pre-dosing interval, several times daily during the dosing interval, and daily during the post-dosing interval. Body weights were measured on GD 0 and 6-20. Food consumption was measured on GD 0, 6, 10, 16, and 20. Dams were sacrificed on GD 20 by carbon dioxide inhalation and examined grossly. The liver was weighed. The number of corpora lutea on each ovary were counted. Gravid uteri were examined for number and location of live and dead fetuses and number and location of early and late resorptions and implantation sites. Uteri that appeared nongravid were placed in an ammonium sulfide solution for detection of very early resorptions. Gravid uterine weights were not obtained.

2. Fetal evaluations

At necropsy, each live fetus was weighed and examined for external abnormalities. Approximately one-half of all fetuses were examined viscerally by the Staples technique; these fetuses were decapitated, and the heads fixed in Bouin's solution for subsequent free-hand sectioning. The remaining one-half of the fetuses were eviscerated and all carcasses were processed for skeletal examination.

D. DATA ANALYSIS

1. Statistical analysis

Maternal incidence data were analyzed by the Cochran-Armitage test for a linear trend in proportions and the Fisher's exact test. Maternal body weight, food consumption, and liver weight data were analyzed by Bartlett's test of homogeneity of variances. If Bartlett's test was positive, the Mann-Whitney U test was used to compare the treated and control groups; if Bartlett's test was negative, a one-way Analysis of Variance (ANOVA) followed by Dunnett's t-test was used to separate the means. Litter incidence data were analyzed with Jonckheere's test followed by either the Mann-Whitney U test or Fisher's exact test.

2. Historical control data were provided to allow comparison with concurrent control and treated groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

All animals survived to scheduled sacrifice. One high-dose animal appeared thin on GD 13-18. No other treatment-related clinical signs of toxicity were observed in any group. Alopecia was a common finding in rats in the control and treated groups.

2. Body weight

Selected maternal body weights and body weight gains during gestation are given in Table 2. Although dams were weighed daily during the dosing interval, means and statistical comparisons were only given for the days shown below. Absolute body weights of the mid- and high-dose groups were significantly ($p \leq 0.01$) less than that of controls during the dosing interval and ranged from 92-94% and 84-88%, respectively, of the control levels. Body weight gains by the mid- and high-dose dams were significantly ($p \leq 0.05$ or 0.01) less than the controls during the dosing period with the exception of GD 12-16. The most pronounced effect on body weight gain occurred immediately after the initiation of dosing (GD 6-9) when the mid- and high-dose groups had a net weight loss compared to a gain by the controls. The high-dose group also had a weight loss for GD 9-12. Weight change during the entire dosing interval was 37% of the control level for the mid-dose group and a weight loss of 12.2 g by the high-dose group. The high-dose dam that appeared thin clinically had marked weight loss during the dosing interval. Weight gain by the mid- and high-dose dams during the post-dosing period was significantly ($p \leq 0.01$) greater than that of controls. Body weights and body weight gains by the low-dose group were similar to that of controls throughout the study.

TABLE 2: Maternal body weights, body weight gains, and food consumption during gestation				
GD	**0 mg/kg/day**	**16 mg/kg/day**	**80 mg/kg/day**	**400 mg/kg/day**
Absolute body weights (g)				
0	266.4	266.8	268.4	265.2
6	299.6	299.0	297.7	297.6
9	304.1	299.4	286.4** (94)	267.9** (88)[a]
10	308.4	305.4	288.6** (94)	266.2** (86)
12	317.1	314.0	290.4** (92)	265.1** (84)
16	338.7	336.8	312.3** (92)	285.4** (84)
20	395.8	396.6	380.2** (96)	363.3** (92)
Body weight change (g)				
0-6	33.2	32.1	29.3	32.4
6-16 (dosing interval)	39.1	37.9	14.6** (37)	-12.2**
16-20	57.0	59.7	67.9** (119)	78.3** (137)
Food consumption (g/day)				
0-6	21.0	21.3	20.5	21.2
6-16 (dosing interval)	22.0	21.8	16.0** (73)	10.4** (47)
16-20	24.7	26.1*	27.1** (110)	26.9** (109)

Data taken from Tables 3 and 4, pp. 34-36, MRID 40228801.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: **$p \leq 0.01$.

3. Food consumption

Selected food consumption data are given in Table 2. Food consumption by the mid- and high-dose groups was significantly ($p \leq 0.01$) less than that of controls during the dosing interval. The high-dose dam that appeared thin clinically had a marked reduction in food consumption during the dosing interval. Post-dosing food consumption by all treated groups was significantly ($p \leq 0.01$) greater than that of controls. Food consumption by the low-dose group was similar to that of controls throughout the pre-dosing and dosing intervals.

4. Gross pathology and liver weights

No treatment-related gross abnormalities were observed at maternal necropsy. Absolute liver weights were similar between the treated and control groups. However, the liver-to-body weight ratio was significantly ($p \leq 0.01$) increased in high-dose dams to 107% of the control value.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 3. No differences were observed between the treated and control groups for pregnancy rate, number of corpora lutea, number of implantation sites, number of fetuses/litter, or fetal sex ratios. No dead fetuses or late resorptions were observed. Two high-dose dams had total

litter resorptions; thin appearance was noted clinically in one of these dams. In addition, the number of early resorptions/dam was slightly greater in the high-dose group than that of the controls. Fetal body weights in the high-dose group were significantly ($p \leq 0.01$) less than those of the controls.

TABLE 3: Cesarean section observations

Observation	0 mg/kg/day	16 mg/kg/day	80 mg/kg/day	400 mg/kg/day
No. Animals Assigned	25	25	25	25
No. Animals Pregnant	22	23	22	22
Pregnancy Rate (%)	88	92	88	88
Maternal Mortality	0	0	0	0
Delivered Early/Aborted	0	0	0	0
Corpora Lutea/Dam	17.4	18.0	16.4	18.0
Implantation/Dam	14.4	14.6	14.2	15.8
Preimplantaion Loss (%)[a]	17	19	13	12
Postimplantation Loss (%)[a]	9.0	8.9	7.4	11.4[b]
Total Live Fetuses	288	306	297	279
Live Fetuses/Litter	13.1	13.3	13.5	14.0
Mean Fetal Weight (g)	3.36	3.42	3.35	3.07** (91)
Sex Ratio (% Male)	47.9	54.4	57.7	52.1
Total Dead Fetuses	0	0	0	0
Dams With All Resorptions	0	0	0	2
Resorptions/Dam				
Early Resorptions/dam	1.3	1.4	0.7	3.2
Late Resorptions/dam	0.0	0.0	0.0	0.0

Data taken from Table 7, p. 38, MRID 40228801.
[a]Calculated by reviewer from group means.
[b]Excludes litters with total resorptions.
Significantly different from control: **$p \leq 0.01$. Number in parentheses is percent of control; calculated by reviewer.

B. DEVELOPMENTAL TOXICITY

In the 0, 16, 80, and 400 mg/kg/day groups, the total number of fetuses(litters) examined for external and skeletal malformations/variations was 288(22), 305(23), 297(22), and 279(20), respectively, and the number examined for visceral malformations/variations was 138(22), 149(23), 144(22), and 134(20), respectively. The high-dose group had increased litter incidence rates ($p \leq 0.05$) of delayed ossification of the vertebrae and sternebrae, A summary of the fetal findings is given in Table 4.

1. External examination

No treatment-related external malformations were observed in any fetus. One fetus from each of the control and high-dose groups had multiple malformations of the head and face. No external variations were observed.

2. Visceral examination

No visceral malformations or variations were observed in any fetus.

3. Skeletal examination

The control and high-dose fetuses with grossly malformed heads also had multiple malformations in the bones of the skull. The litter incidence rates of delayed ossification of the vertebrae and sternebrae were significantly ($p \leq 0.05$) increased in the high-dose group as compared with the controls (Table 4). The high-dose group also had an increased ($p \leq 0.05$) litter incidence of bifid vertebrae.

TABLE 4: Summary of fetal malformations/variations [No. fetuses (no. litters) affected]

Observation	0 mg/kg/day	16 mg/kg/day	80 mg/kg/day	400 mg/kg/day
Multiple malformations of the head	1 (1)	0 (0)	0 (0)	1 (1)
Vertebrae, bifid thoracic centra	1 (1)	2 (1)	4 (2)	10 (7*)
Vertebrae, not ossified thoracic centra	0 (0)	0 (0)	0 (0)	4 (3*)
Sternebrae, incompletely ossified	3 (3)	5 (3)	1 (1)	16 (9*)
Femur bent	0 (0)	1 (1)	0 (0)	0 (0)
Fibula compressed	0 (0)	0 (0)	0 (0)	1 (1)

Data taken from Table 8, pp. 39-44, MRID 40228801.
Significantly different from control: *$p \leq 0.05$.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that H-16035 resulted in maternal toxicity at doses of 80 and 400 mg/kg/day as evidenced by reduced body weight gain and food consumption. In addition, developmental toxicity was observed at 400 mg/kg/day as decreased fetal body weights and delayed ossification. Based on these results, the NOEL for maternal toxicity was 16 mg/kg/day and the NOEL for developmental toxicity was 80 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Maternal toxicity was evident at the mid and high doses as decreased body weights and food consumption. During the dosing interval, marked reductions in weight gains were noted for the mid-dose dams and weight loss occurred in the high-dose dams. The reduced body weight gains correlated with reduced food consumption for the mid- and

high-dose groups during the dosing interval. Thin appearance of one high-dose dam corresponded with weight loss, reduced food consumption, and complete litter resorption. Increased weight gain by the mid- and high-dose groups during the post-dosing interval suggested slight recovery. However, the greater food consumption by all treated groups during post-dosing attained statistical significance most likely due to a low value by the control group since the effect was not dose-related and food consumption by the low-dose group was not affected during treatment.

Therefore, the maternal toxicity LOAEL is 80 mg/kg/day based on decreased body weights, body weight gains, and food consumption. The maternal toxicity NOAEL is 16 mg/kg/day.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

Complete litter resorption by two high-dose dams and the slightly greater number of early resorptions/dam in the high-dose group were considered treatment-related and may have resulted from maternal toxicity.

b. Altered growth

In the high-dose group, fetal body weights were reduced compared with the controls and delayed ossification was observed in vertebrae and sternebrae. These are indicative of reduced growth by the fetuses and may have been a result of the marked maternal toxicity observed at this dose.

c. Developmental variations

Variations common to the rat fetus were observed equally in the treated and control groups.

d. Malformations

No dose- or treatment-related increased incidence of any malformation was observed.

Therefore, the developmental toxicity LOAEL is 400 mg/kg/day based on whole litter resorption, reduced fetal body weights, and delayed ossification of the vertebrae and sternebrae. The developmental toxicity NOAEL is 80 mg/kg/day.

C. STUDY DEFICIENCIES

Test article concentrations in the mid- and high-dose solutions were highly variable such that target doses may not have been representative of the actual doses to the animals. On 1/10/86, 1/14/86, 1/17/86, 1/21/86, and 1/26/86 analyses revealed dosing concentrations well outside of acceptable levels ranging from 42% to 161% of nominal. These data reflect a serous study flaw compromising interpretation and establishment of NOAEL's and LOAEL's.

D. CORE CLASSIFICATION

This study is **NOT** classified as **Acceptable** and **does not** satisfy the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats. It is doubtful that it can be upgraded.

014692

EXECUTIVE SUMMARY

DIURON

STUDY TYPE: MUTAGENICITY- REVERSE MUTATION ASSAY IN BACTERIA [OPPTS 870.5100 (§84-2)] MRID NOS. 00146608, 40228805

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97

Primary Reviewer:
C. B. Bast, Ph.D., D.A.B.T.

Signature: Cheryl B Bast
Date: APR 10 2001

Secondary Reviewers:
B. L. Whitfield, Ph.D.

Signature: B L Whitfield
Date: APR 10 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: APR 10 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, Managed and Operated by UT-Battell. LLC., for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

Supplement to Tox Document 005039, review for Accession 258877 and Tox Document 008751, review of re-submitted data, mutagenicity- bacterial reverse mutation study. This supplement provides an executive summary to update the original review.

EPA Reviewer: Nancy McCarroll Nancy E. McCarroll Date: 04/24/01
Toxicology Branch I
EPA Work Assignment Manager: J. Stewart, Ph.D. Jacqueline Stewart Date: 5/1/2001
Toxicology Branch 1 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity- bacterial reverse mutation test (Ames); OPPTS 870.5100

OPP Number: 84-2
DP BARCODE: D272128
PC CODE: 035505

OPPTS Number: 870.5100
SUBMISSION CODE: S591433
TOX CHEM NO: 410

TEST MATERIAL: Diuron (98.19 %)

CHEMICAL NAME: N`-(3,4-dichlorophenyl)-N,N-dimethyl-urea: 3-(3,4-chlorophenyl)-1,1-dimethylurea)

CITATION: Poet, L.B., Arce, G.T., Sarrif, A.M. (1985). Mutagenicity evaluation in Salmonella typhimurium. Study No. HLR 471-84. E. I. duPont de Nemours and Company, Newark, DE. MRID 00146608. Unpublished.

Memorandum to Walter Waldrop and Carol Peterson from David S. Liem. Review of a resubmitted mutagenicity study using technical Diuron (Guideline 84-2). October 30, 1991. MRID 40228805.

SPONSOR: E. I. duPont de Nemours and Company, Newark, DE.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 00146608), *S. typhimurium* strains TA97, TA98, TA100, and TA1535 were exposed to Diuron (98.19%, lot number T50906) in dimethylsulfoxide at concentrations of 0.5, 1.0, 2.5, 5.0 and 10.0 µg/plate in the absence of mammalian metabolic activation (S9-mix) and 10, 25, 50, 100, and 250 µg/plate in the presence of S-9 mix. Duplicate plates were utilized for each test concentration, and two independent assays were performed. The S9-fraction was obtained from Aroclor 1254 induced Charles River CD rat liver.

Diuron was not cytotoxic at any dose; however, higher levels (≥50 µg/plate without S-9 mix or 500 µg/plate with S-9) were cytotoxic (see MRID 40228805). The solvent (DMSO) and positive controls (N-methyl-N'-nitro-N-nitrosoguanidine, 2-nitrofluorene, 9-aminoacridine, and 2-aminoanthracene) induced the appropriate response in the respective strains. **There was no evidence of a mutagenic effect with or without S9 activation.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5100 (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

EXECUTIVE SUMMARY

014692

DIURON

STUDY TYPE: MUTAGENICITY- FORWARD MUTATION *in vitro*
[OPPTS 870.5300 (§84-2)]
MRID NO. 00146609

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97

Primary Reviewer:
C. B. Bast, Ph.D., D.A.B.T.

Signature: Cheryl B Bast
Date: APR 10 2001

Secondary Reviewers:
B. L. Whitfield, Ph.D.

Signature: B L Whitfield
Date: APR 10 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: APR 10 2001

Disclaimer

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Supplement to Tox Document 005039 review for Accession 258877, mutagenicity- forward mutation study in mammalian cells *in vitro*. This supplement provides an executive summary to update the original review.

EPA Reviewer: Nancy McCarroll, Date 01/24/01
Toxicology Branch
EPA Secondary Reviewer: J. Stewart, Ph.D. Date 05/01/2001
Reregistration Branch 1 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity- forward mutation study in mammalian cells *in vitro*

OPP Number: 84-2
DP BARCODE: D272128
PC CODE: 035505

OPPTS Number: 870.5300
SUBMISSION CODE: S591433
TOX CHEM NO: 410

TEST MATERIAL: Diuron (98.19%)

CHEMICAL NAME: N`-(3,4-dichlorophenyl)-N,N-dimethyl-urea: 3-(3,4-chlorophenyl)-1,1-dimethylurea)

CITATION: Rickard, L.B., Ullman, D,V., Choy, W.N., Fisher, R.L. (1985) . Mutagenicity evaluation of Diuron in the CHO/HGPRT assay. Study No. HLR 282-85. Prepared and submitted by E. I. DuPont de Nemours and Co., Inc., Newark, DE. MRID 00146609. Unpublished.

SPONSOR: E. I. DuPont de Nemours and Co., Inc., Newark, DE

EXECUTIVE SUMMARY: In independently performed mammalian cell gene mutation assays in vitro, triplicate (in the absence of activation) or duplicate (in the presence of activation) cultures of Chinese hamster ovary (CHO) CHO-K1-BH3 cells (MRID 00146609) were exposed to Diuron (Lot No. T50906; Batch No. 04, 98.19% a.i.) in dimethyl sulfoxide at concentrations of 0.01, 0.5, 1.0, 1.125, and 1.250 mM (≈2.3 - 288 μg/mL) in the absence of mammalian metabolic activation (S9-mix), and at 0.05, 0.1, 0.20, 0.25, 0.5, or 0.75 mM (≈11.5 - 173 μg/mL) in the presence of Sprague Dawley S9-mix. The S9-fraction was obtained from Aroclor 1254-induced 8 to 9 week-old male CrL:CD(SD) BR rats.

Diuron was tested up to concentrations limited by cytotoxicity. Cell survival was 8.4-39.3% in the absence of S9-mix at 1.250 mM.. In the presence of S9-mix, no cells survived treatment with 0.75 mM and cell survival was 15% at 0.5 mM. There was no increase in the mutant frequency in cells treated with diuron in either the presence or absence of metabolic activation. The ethyl methane sulfonate (EMS) positive control (without S9) and the dimethylnitrosamine positive control (with S9-mix) induced the expected responses. **There was no evidence of an increased mutant frequency either in the presence or absence of S9 metabolic activation.**

This study is classified as **Acceptable/Guideline**. It does satisfy the requirement for FIFRA Test Guideline [OPPTS 870.5300 (§84-2)] for *in vitro* mutagenicity (mammalian forward gene mutation) data.

EXECUTIVE SUMMARY

014692

DIURON

STUDY TYPE: MUTAGENICITY- *In vivo* CYTOGENETIC ASSAY
[OPPTS 870.5385 (§84-2)]
MRID NO. 00146611

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97

Primary Reviewer: C. B. Bast, Ph.D., D.A.B.T.	Signature: Cheryl B Bast Date: APR 10 2001
Secondary Reviewers: B. L. Whitfield, Ph.D.	Signature: B L Whitfield Date: APR 10 2001
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: APR 10 2001

Disclaimer

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Supplement to Tox Document 005039, review for Accession 258877, mutagenicity- *in vivo* cytogenetic assay. This supplement provides an executive summary to update the original review.

EPA Reviewer: Nancy McCarroll Date: 05/08/01
Toxicology Branch
EPA Secondary Reviewer: J. Stewart, Ph.D. Date: 5/16/01
Reregistration Branch 1 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity- *in vivo* cytogenetic assay

OPP Number: 84-2
DP BARCODE: D272128
PC CODE: 035505

OPPTS Number: 870.5385
SUBMISSION CODE: S591433
TOX CHEM NO: 410

TEST MATERIAL: Diuron (98.19%)

CHEMICAL NAME: N'-(3,4-dichlorophenyl)-N,N-dimethyl-urea: 3-(3,4-chlorophenyl)-1,1-dimethylurea)

CITATION: Sarrif, A. (1985). Assessment of Diuron in the in vivo Cytogenetic Study in Rats. Study No. 36685. E. I. duPont de Nemours and Company, Haskell Laboratory, Newark, DE. MRID 00146611. Unpublished.

SPONSOR: E. I. duPont de Nemours and Company, Newark, DE.

EXECUTIVE SUMMARY: In an *in vivo* cytogenetic assay (MRID 0014661), five Sprague-Dawley rats per sex per harvest time were administered Diuron (98.18%, lot number T50906) by single gavage at doses of 0, 50, 500, or 5000 mg/kg. Bone marrow cells were harvested 6, 24, or 48 hours after test compound administration and 24 hours after administration of the positive control. The vehicle was corn oil (12 mL/kg) and the positive control was a single 20 mg/kg dose of cyclophosphamide.

One high-dose female died 48 hours after dosing. High-dose males and females exhibited body weight loss, mouth, nasal, and ocular discharge, decreased activity or depression, wet, stained perineum, labored respiration, diarrhea, salivation, tremors and moribundity. Mid-dose females exhibited decreased body weight at 48-hours and one mid-dose animal of each sex exhibited diarrhea. Red staining of the neck was noted in one low-dose female. Significantly decreased ($p \leq 0.01$) mitotic indices were seen at 24 and 48 hours for high-dose males; data combined for both sexes were also significantly decreased at these time intervals. There was also a significant ($p<0.05$) increase in the percentage of abnormal cells when the data for both sexes were combined (0.11 versus 0.00 in controls). A significant positive linear trend ($p<0.01$) was also recorded for the combined (by sex) percentage abnormal cells. A total of 4/10 animals in

the high-dose group were affected: single chromatid breaks were seen in two males and one female and a chromatid fragment was seen in one male. **Based on these data, there is evidence that 5000 mg/kg Diuron induced a weak clastogenic effect in the bone marrow cells of rats.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS [870.5385 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

within the range of the historical control [percent abnormal cells/group: 0-2.6% (♂) and 0-2.0% (♀); average number of aberrations/cell: 0-0.023% (♂) and 0-0.060 % (♀)]. **Based on these data, there is no clear evidence that Diuron is clastogenic in the bone marrow cells of rats in this study.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS [870.5385 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

EXECUTIVE SUMMARY

DIURON

STUDY TYPE: MUTAGENICITY- UDS ASSAY in MAMMALIAN CELLS
[OPPTS 870.5550 (§84-2)]
MRID NO. 00146610

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order 01-97

Primary Reviewer:
C. B. Bast, Ph.D., D.A.B.T.

Signature: Cheryl B Bast
Date: APR 10 2001

Secondary Reviewers:
B. L. Whitfield, Ph.D.

Signature: B L Whitfield
Date: APR 10 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: APR 10 2001

Disclaimer

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Supplement to Tox Document 005039, review for Accession 258877, mutagenicity- UDS in mammalian cells in culture. This supplement provides an executive summary to update the original review.

EPA Reviewer: Nancy McCarroll Nancy E. McCarroll Date: 4/26/01
Toxicology Branch
EPA Work Assignment Manager: J. Stewart, Ph.D. Joycelyn Stewart Date: 5/1/2001
Reregistration Branch 1 (7509C)

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity- UDS in mammalian cells in culture; OPPTS 870.5550

OPP Number: 84-2
DP BARCODE: D272128
PC CODE: 035505

OPPTS Number: 870.5550
SUBMISSION CODE: S591433
TOX CHEM NO: 410

TEST MATERIAL: Diuron (98.19%)

CHEMICAL NAME: N'-(3,4-dichlorophenyl)-N,N-dimethyl-urea: 3-(3,4-chlorophenyl)-1,1-dimethylurea)

CITATION: Acre, G.T. and Sarrif, A.M. (1985) . Assessment of Diuron in the in vitro Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes. Study No. HLR 349-85. E.I. duPont de Nemours and Co., Inc., Newark, DE. MRID 00146610. Unpublished.

SPONSOR: E.I. duPont de Nemours and Co., Inc., Newark, DE.

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis assay (MRID 00146610), primary rat hepatocyte cultures were exposed to Diuron (98.19% a.i. in dimethylsulfoxide; Lot No. T50906, Batch No. 04) in Williams' Medium E (WME) at concentrations of 0.001, 0.01, 0.1, 0.33, 1.0, and 20.0 mM (≈0.23 - 4662 μg/mL) for 18 hours.

Diuron was tested up to cytotoxic concentrations as evidenced by marked increases (2.3-fold over control) in lactate dehydrogenase activity at 0.33, 1.0, and 20 mM Diuron. Compound precipitation was also observed at 1.0 and 20.0 mM. The mean net nuclear grain counts were calculated from 100 cells/group. The mean net nuclear grain counts were <1.0 at 0.001, 0.010, and 0.1 mM, indicating no induction of UDS activity and average cytoplasmic grain counts were significantly decreased ($p<0.01$) at 0.33, 1.0, and 20.0 mM due to the observed cytotoxicity. The solvent (DMSO) and positive control (0.1 mM dimethylbenzanthracene) values were appropriate. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts], was induced.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5550 (§84-2)] for other genotoxic mutagenicity data.

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