CASWELL FILE 528

008533

8/28/91

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

AUG 28 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Linuron - Supplemental histopathology data to evaluate eye lesions observed in multigeneration reproduction study No. HLR 20-90 (MR project No. 8511-001), MRID No. 414634-01, under Guideline 83-4

ToxChem No.: 528

Accession (MRID) No. 418647-01
HED Project No.: 1-1279

FROM: Susan L. Makris, M.S. Susan L. Makris 8/8/91
Review Section III
Toxicology Branch II
Health Effects Division (H7509C)

TO: Mr. Walter Waldrop/ Ms. Carol Peterson (PM-71)
Special Review Branch
Special Review and Reregistration Division (H7508W)

THRU: James N. Rowe, Ph.D., Section Head James N. Rowe 8/8/91
Review Section III
Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief M van Gemert 8/12/91
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: E.I. du Pont de Nemours and Company
Elkton Road. P.O. Box 50
Newark, Delaware 19714

Action Requested: Review the histopathology study (supplemental to a 2-generation reproduction study) conducted on the chemical Linuron.

Summary and Conclusion:

Linuron was administered in the diet to Crl:CD^{R}BR^{R} rats at levels of 12.5, 100, and 625 ppm over two generations. Scheduled histopathological evaluation of tissues revealed mineralization of the cornea and degeneration of the lens in high-dose (625 ppm) F1 parental animals. In order to clarify the findings, five additional sections, including the conjunctiva, cornea, lens, iris, and sclera, were cut from the eyes of all adult F1 rats and examined histopathologically.

1. Statistically and/or biologically significant treatment-related increases

were noted for the number of high-dose F1 males with any type of corneal or conjunctival change, including unilateral or bilateral degenerative basophilia of the cornea and unilateral conjunctival inflammation/basophilia. The incidence of any corneal/conjunctival change was increased, but not significantly so, for high-dose females as compared to control; however, this study did not provide enough additional information to rule out a treatment-related effect in the high-dose females. Corneal mineralization noted previously as a treatment-related effect in the high-dose F1 males was not observed at histopathological evaluation of additional corneal sections of ocular tissue.

2. The incidence of lens degeneration (cataracts) was increased, but not significantly, for F1 male and female high-dose animals as compared to control. Lenticular changes were predominantly unilateral and graded "minimal" to "mild", indicating the possibility of a treatment-related effect. Evaluation of additional sections of lens tissue did not clarify or resolve this issue.

There is no change to toxicity levels determined for the 2-generation study in rats with Linuron (Reproductive NOEL > 625 ppm; Systemic NOEL = 12.5 ppm; Systemic LOEL = 100 ppm). These were based upon a decrement in F0 and F1 parental body weight gain values.

CORE CLASSIFICATION: Supplementary

This study was conducted to supply additional data and clarification of findings for a 2-generation reproduction study [Haskell Laboratory Report No. 20-90 (MR No. 8511-001), MRID No. 414634-01] which was previously determined to meet the guideline requirements (83-4) for a multigeneration study in rats.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/SACB
TOX ONELINERS

TOXCHEM NO. 528: Linuron

FILE LAST PRINTED: 5/28/91

CITATION	MATERIAL	ACCESSION/ MRID. NO.	RESULTS	TOX CAT	CORE GRADE/ DOCUMENT #
83-4 Reproduction; 2-generation Species: rat Haskell Lab MR 8511-001; 3/29/90	Linuron Tech., 96.2% a.i.	414634-01	No effect on fertility or reproductive performance at dietary concentrations up to 625 ppm (31.25 mg/kg/day). F1 adult males in the 625 ppm group had testicular and epididymal abnormalities and ocular effects consisting of mineralization of the cornea and lens degeneration. Reprod. NOEL > 625 ppm. Sys. NOEL = 12.5 ppm. Sys. LOEL = 100 ppm, based on body weight decrement.		Guideline 008113
83-4 (Supplemental) Additional histopathology for 2-generation repro. Species:rat Haskell Lab MR 8511-001; 3/29/90	Linuron Tech., 96.2% a.i.	418647-01	Additional histopathology was performed on the conjunctiva, cornea, lens, iris, and sclera of all F1 adult rats from the 2-generation reproduction study (MRID No. 414634-01). Results did not clarify ocular findings noted at initial tissue evaluation, nor was there any change to previously determined levels of toxicity (Reprod. NOEL > 625 ppm; Sys. NOEL = 12.5 ppm; Sys. LOEL = 100 ppm).		Supplementary

3

Reviewed by: Susan L. Makris, M.S. Susan L Makris 8/8/91
Toxicologist, Review Section III, Toxicology Branch II - HFAS/HED (H7509C)
Secondary review by: James N. Rowe, Ph.D. James N. Rowe 8/8/91
Review Section III, Toxicology Branch II - HFAS/HED (H/509C)

DATA EVALUATION REPORT

STUDY TYPE: Supplemental histopathology data to evaluate eye lesions observed in multigeneration reproduction study No. HLR 20-90 (Guideline 83-4)

EPA ACCESSION NUMBERS: MRID No. 418647-01
Caswell No. 528
HED Project No. 1-1279

TEST MATERIAL: IN Z326-118

SYNONYMS: Linuron, Lorox, (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea)

STUDY NUMBER: Haskell Laboratory Report (HLR) No. 20-90
Medical Research (MR) Project No. 8511-001

SPONSOR: E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714

TESTING FACILITY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714

TITLE OF REPORT: Reproductive and Fertility Effects with IN Z326-118
MultiGeneration Reproduction Study in Rats
Revised Supplementary Appendix I

AUTHOR: Linda S. Mullin, M.A., D.A.B.T.

DATE REPORT ISSUED: April 3, 1991

CONCLUSION: Linuron was administered in the diet to Crl:$CD^{R}BR^{R}$ rats at levels of 12.5, 100, and 625 ppm over two generations. Scheduled histopathological evaluation of tissues revealed mineralization of the cornea and degeneration of the lens in high-dose (625 ppm) F1 parental animals. In order to clarify the findings, five additional sections, including the conjunctiva, cornea, lens, iris, and sclera, were cut from the eyes of all adult F1 rats and examined histopathologically.

1. Statistically and/or biologically significant treatment-related increases were noted for the number of high-dose F1 males with any type of corneal or conjunctival change, including unilateral or bilateral degenerative basophilia of the cornea and unilateral conjunctival inflammation/basophilia. The incidence of any corneal/conjunctival change was increased, but not significantly so, for high-dose females as compared to control; however, this study did not provide enough additional information to rule out a treatment-related effect in the high-dose females. Corneal mineralization noted previously as a treatment-related effect in the high-dose F1 males was not observed at histopathological evaluation of additional corneal sections of ocular tissue.

2. The incidence of lens degeneration (cataracts) was increased, but not significantly, for F1 male and female high-dose animals as compared to control. Lenticular changes were predominantly unilateral and graded "minimal" to "mild", indicating the possibility of a treatment-related

effect. Evaluation of additional sections of lens tissue did not clarify or resolve this issue.

There is no change to toxicity levels determined for the 2-generation study in rats with Linuron (Reproductive NOEL > 625 ppm; Systemic NOEL = 12.5 ppm; Systemic LOEL = 100 ppm). These were based upon a decrement in F0 and F1 parental body weight gain values.

CORE CLASSIFICATION: Supplementary

This study was conducted to supply additional data and clarification of findings for a 2-generation reproduction study [Haskall Laboratory Report No. 20-90 (MR No. 8511-001), MRID No. 414634-01] which was previously determined to meet the guideline requirements (83-4) for a multigeneration study in rats.

MATERIALS:

Test compound: Linuron Technical (IN Z326-118), 96.2% a.i., Batch No. 16,569.

Test animals: Species: rat
Strain: Crl:$CD^{R}BR^{R}$
Age at mating: F0 - 19 weeks, F1 -15 weeks
Source (F0): Charles River Laboratories
Kingston, New York

METHODS:

This study was conducted to further clarify ocular findings noted at histopathological evaluation of the F1 parental animals. These findings were predominantly comprised of mineralization of the cornea and degeneration of the lens. Evaluation of the F0 rats did not reveal ocular lesions.

Linuron was administered in the diet throughout the course of the study at the following dose levels for both generations:

Group No.	Dose (ppm)	Animals/Group	
		Male	Female
1	0	30	30
2	12.5	30	30
3	100	30	30
4	625	30	30

Study Design: F0 animals were mated to produce one litter; F1 animals were selected as parents for the F1 generation. Upon sacrifice, control and high-dose tissues, and any grossly abnormal tissues from the low- and mid-dose groups, were prepared for histopathological evaluation.

Preparation of additional specimens: Five additional sections were cut from each eye from all adult F1 rats for both sexes and all dose groups. These included the conjunctive, cornea, lens, iris, and sclera. The distance between sections was approximately 100 microns in order to get a better sample of the entire eye.

Histopathological evaluation: The same pathologist (Edwin F. Stula, D.V.M., Ph.D.) read the specimens from both studies. In the evaluation of lens tissues on the main study, findings were not assigned a severity grading; however, all observations of lens degeneration were graded when additional tissues were read.

Statistical analyses: Fisher's Exact Test was used to analyze histopathology incidence data.

Compliance

- Statement of No Data Confidentiality
- GLP Compliance Statement
- Flagging Statement
- Quality Assurance Statement

RESULTS:

Results of histopathological evaluation of ocular tissues (Tables 1A and 1B) revealed the following:

1. A statistically significant increase was noted in the number of high-dose F1 males with any type of corneal or conjunctival change. There was a statistically significant increase in bilateral, and biologically significant increase in unilateral, degeneration/basophilia of the cornea for the high-dose males. Unilateral conjunctival inflammation/basophilia was biologically increased for the high-dose males. These changes were judged to be treatment-related. The severity of these lesions was generally minimal.

2. There were no significant differences in the incidences of uni- or bilateral lens degeneration (cataracts) between control and treated F1 rats.

Table 1A. Incidences of Microscopic Ocular Lesions in F1 Rats

Observation	Males				Females			
	0 ppm	12.5 ppm	100 ppm	625 ppm	0 ppm	12.5 ppm	100 ppm	625 ppm
No. in group	30	30	30	30	30	30	30	30
No. examined	30	28	29	30	30	30	30	29
Summary counts								
Cornea/conjunctiva: any change	4	4	2	14*	2	2	1	5
Lens: any change	1	0	1	3	0	0	1	3
Conjunctiva								
Bulbar, subepith., inflam./basophilia, focal								
Unilateral	2	1	1	6	1	1	1	1
Bilateral	0	1	0	0	0	0	0	0
Cornea								
Atrophy, focal								
Unilateral	1	0	0	3	0	0	0	1
Bilateral	0	0	0	2	0	0	0	0
Inflammation, focal, unilateral	0	0	0	1	0	0	0	0
Vascularization, focal, bilateral	0	0	0	1	0	0	0	0
Epith.- strom. junct.: degen./basophilia, focal								
Unilateral	2	3	1	7	1	1	0	3
Bilateral	0	0	1	5*	1	1	0	1
Iris: synechia, unilateral	0	1	0	2	0	0	0	0
Lens								
Iris and retina missing, hemosiderin, unilateral	0	0	0	1	0	0	0	0
Degeneration (cataract)								
Unilateral	1	0	1	2	0	0	1	3
Bilateral	0	0	0	1	0	0	0	0
Fibrosis, adhered to cornea, unilateral	0	0	0	1	0	0	0	0
Fibrosis, focal	0	0	0	1	0	0	0	0
Iris: synechia, unilateral	0	0	0	1	0	0	0	0
Retina								
Atrophy, focal, unilateral	0	1	2	0	0	3	2	2
Folds/rosettes	3	3	1	2	2	0	0	2
Sclera								
Osseous metaplasia, focal, unilateral	0	0	0	1	0	0	0	0

* Statistically significantly different from control value, $p \leq 0.05$, by Fisher's Exact Test.
Note: Data were extracted from report No. HLR 20-90 Addendum, pages 12 and 13.

Table 1B. Incidences of Selected Microscopic Ocular Lesions in F1 Rats - Results of Main Study

Observation	Males				Females			
	0 ppm	12.5 ppm	100 ppm	625 ppm	0 ppm	12.5 ppm	100 ppm	625 ppm
No. in group	30	30	30	30	30	30	30	30
No. examined	30	28	29	30	30	30	30	29
Cornea								
Mineralization, focal, unilateral	1	0	0	4	0	0	0	1
vacuolation, epithelium	0	0	0	1	0	0	0	0
Lens								
Hemosiderin, loss of lens, possible trauma	0	0	0	1	0	0	0	0
Degeneration								
Unilateral	0	0	0	2	0	0	0	2
Bilateral	0	0	0	1	0	0	0	0
Outer nuclear membrane: atrophy, focal, unilateral	0	0	0	0	0	0	0	2

Note: Data were extracted from report No. HLR 20-90, pages 122 and 125.

Deficiencies/Errors:

1. The report did not address the lack of data for several animals. Tissues from Group 2 male Nos. 448454 and 448455 and Group 3 male No. 448504, which were listed as scheduled deaths, and Group 4 female No. 448645, which was sacrificed in extremis, were not included in the results of histopathological evaluation.

2. Histopathological findings from the main two-generation reproduction study and the addendum study, which address some of the same tissues from the same animals, were not combined in any meaningful manner. Differences and correlations should have been addressed.

3. An unexplained discrepancy in histopathological findings in the lens was identified for two Group 4 F1 male rats. In the main study, No. 448509 was noted to have bilateral lens degeneration (cataract) and No. 448538 was noted to have unilateral lens degeneration. Results of the additional histopathological evaluations indicated that the lens findings were unilateral in No. 448509 and bilateral in No. 449538.

DISCUSSION:

Correlation to the main study:

1. In the results of histopathological evaluation of ocular tissue in the main study, it was noted that focal mineralization of the cornea was observed in 1/30 control F1 males and 4/30 high-dose F1 males. This was one of the findings that was judged to be potentially treatment-related and was the basis upon which additional histopathological evaluations were performed. However, in this expanded study, there was no incidence of corneal mineralization in any group, including in the same animals for which the finding was previously reported. This discrepancy was not addressed by the investigators.

2. Lens degeneration (cataracts) noted in the main study (1/30 bilateral and 2/30 unilateral in the high-dose males, 2/30 unilateral in the high-dose females) was noted in the same individual animals in the expanded histopathological ocular evaluation study. Additional observations of unilateral lens degeneration were reported for one control male, one mid-dose male, one mid-dose female, and one high-dose female; no additional observations of bilateral lens degeneration were noted.

Correlation to findings in previous studies:

1. The corneal and lens findings noted in this study were not previously reported in a 2-year dietary rat study (HLR No. 100-80, MRID No. 241897) which was dosed at 625 ppm. In addition, in two multigeneration rat reproduction studies (HLR No. 436-84, MRID No. 255829 and HLR No. 413-85, MRID No. 259393) previously submitted to the Agency, both of which were dosed at 625 ppm, ocular toxicity was not cited as an effect of treatment.

2. Similar findings were not noted in other species (previously conducted chronic beagle and mouse studies, respectively: HLR No. 181-88, MRID No. 409526-01 and HLR No. 758-82, no MRID No. on file), thereby suggesting that the ocular effects may be species-specific in nature.

Other issues relating to interpretation of study results:

1. The investigators stated that although they previously considered that the eye lesions noted in the two-generation study indicated a compound-related effect on both male and female F1 rats at 625 ppm, the additional histopathological evaluations performed indicate that the effect of treatment with Linuron is only observed in the males. In argument against this hypothesis, it appears that although the incidences of corneal and lenticular findings in the female rats were not statistically significant for treated groups as compared to control, there is an increase in the findings noted for the high-dose females. The additional information provided by this study is not conclusive enough to completely disregard the possible treatment-related effects in the high-dose females.

2. A suggestion was made by the investigators that the lens degeneration (cataracts) noted in the high-dose animals could be due to nutritional deficiency. The mean F1 high-dose male body weight value (511.4 g) was, in fact, approximately 25% lower than the control value (685.1 g) at study termination, and the mean F1 high-dose female body weight value (269.9 g) was approximately 21% lower than the control value (343.7 g). To support this hypothesis, a reference was provided: Hogan and Zimmerman, Ophthalmic Pathology, 2nd ed., 1962, pp. 663-664. This text described the two known dietary deficiencies which lead to cataracts: amino acid (usually tryptophan) and vitamin (riboflavin) deficiencies. Under experimentally induced deficiency conditions, cataract formation is associated with vascularization and ulceration of the cornea, conjunctivitis, dermatitis with areas of baldness, irregularities in the teeth, and gonadal atrophy. In the main study, a significant treatment-related incidence of testicular atrophy was noted in the high-dose males, and nonsignificant incidences of upper incisor malformations were noted in all dose groups, including control (Table 2). It cannot be determined from the information currently available if the incisor malformations reported are comparable to the "irregularities of the teeth" as described in the literature. Inflammation of the conjunctiva (6 males and 1 female) and corneal vascularization (1 male) were noted in the high-dose animals at evaluation of additional ocular tissue (Table 1A); however, no other observations

common to this nutritional deficiency syndrome were found. The conclusion, regarding correlation of lens degeneration to nutritional deficiency following treatment with Linuron, is somewhat equivocal. Nevertheless, the lens degeneration observed in this study appears to be treatment-related, whether it was a primary response to toxic insult or a secondary response to the significantly reduced food consumption and resulting nutritional deficiency observed in the F1 parental rats.

Table 2. Incidences of Selected Microscopic Lesions in F1 Rats (No. with positive findings/No. examined)

Observation	0 ppm	12.5 ppm	100 ppm	625 ppm
No. in group	30	30	30	30
MALES				
Testes				
Atrophy (all types)	1/30	1/1	2/30	14/30*
Granuloma/fibrosis, intratubular (all types)	0/30	1/1	2/30	8/30*
Hyperplasia, interstitial cell, focal	0/30	1/1	1/30	7/30*
Teeth: upper incisor				
Malformation	3/30	3/9	4/30	3/30
Malformation and inflammation	0/30	3/9	0/30	0/30
FEMALES				
Teeth: upper incisor				
Malformation	3/30	0/7	3/8	0/30
Malformation and inflammation	1/30	0/7	2/8	0/30

* Statistically significantly different from control values, $p \leq 0.05$.
Note: Data extracted from report HLR No. 20-90, pages 122-123 and 126.

3. A letter, dated April 17, 1990 and submitted to Robert J. Taylor, E.P.A. Project Manager, from Marie M. Chubb, Du Pont Registration Specialist, concerning this study stated that "the fact that lesions in the rat were not observed bilaterally, as would be expected for compound-related effects, suggests an equivocal association with linuron administration." In this study, many of the observations were, in fact, unilateral. However, only one unilateral observation was graded as severe (Group 4 F1 male No. 448538) and one was graded as moderate (Group 4 F1 female No. 448640); all other unilateral lenticular findings were graded as minimal or mild. This could be interpreted as an indication that most lesions were in early stages and had the potential to develop bilaterally. The predominance of unilateral lesions is not, therefore, an unequivocal argument repudiating the presence of a treatment-related effect in the lens.

Toxicity levels:

There is no change to toxicity levels determined for the 2-generation study in rats with Linuron (Reproductive NOEL > 625 ppm; Systemic NOEL = 12.5 ppm; Systemic LOEL = 100 ppm) which were based upon a decrement in F0 and F1 parental body weight gain values.