9-12-91

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

008612

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Linuron - Investigation of a mechanism for Leydig cell tumorigenesis by Linuron in rats

ToxChem No.: 528

Accession (MRID) No. 416301-01
HED Project No.: 1-1238

FROM: Susan L. Makris, M.S. Susan L Makris 9/12/91
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THRU: James N. Rowe, Ph.D., Section Head James N. Rowe 9/16/91
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and

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

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

Summary and Conclusions:

The test material, Linuron, was administered daily at 200 mg/kg by oral gavage to growing and adult Crl:CD®BR rats (approximately 32-33 and 93 days of age, respectively) for 14 days. Flutamide was used as a positive control material. Clinical observations, body weight measurements, and food consumption values were recorded. Following sacrifice, the testis, epididymides, accessory sex organs, and levator ani muscle were weighed; serum testosterone, estradiol, and luteinizing hormone (LH) levels were analyzed. Organ weight data were also evaluated for the P1 and F1 rats of a previously-completed multigeneration study with Linuron (MRID No. 414634-01) and serum hormone data were analyzed for the P1 rats. An in vitro analysis, in which the ability of Linuron and its

metabolites to compete for binding to the androgen receptor, was conducted.

1) The results of this study, particularly the increase in testicular weights, the inhibition of accessory sex organ weights for growing and (to a lesser extent) adult male rats, the increase in serum LH levels for P1 and F1 Linuron-treated rats, and the ability of Linuron to compete for the androgen receptor in vitro, indicate that Linuron may be a weak androgen receptor antagonist. This would support the hypothesis that rats exposed to Linuron could develop interstitial hyperplasia and subsequent adenomas (Leydig cell tumors) of the testicular tissue via a mechanism of sustained hypersecretion of luteinizing hormone (LH) induced by the antiandrogenic potential of Linuron.

2) The hypothesis proposed by the study author, that a threshold mechanism exists for the induction of neoplasms via exposure to Linuron, i.e., that exposure to levels of Linuron that do not cause hypersecretion of LH should pose no additional risk for the development of Leydig cell tumors, was not proven by the results of this study, and therefore cannot be used to support risk assessment decisions.

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.

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Primary Review by: Susan L. Makris, M.S. Susan L Makris 9/12/91
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Secondary Review by: James N. Rowe, Ph.D. James N Rowe 9/16/91
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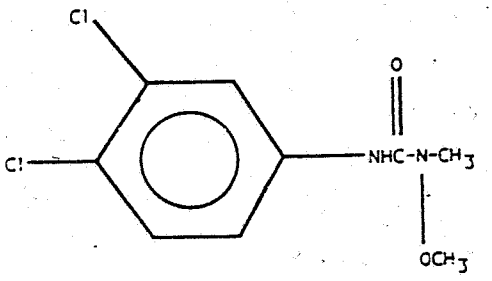
DATA EVALUATION REPORT

STUDY TYPE: Additional information to a multigeneration reproduction study (Guideline 83-4)

EPA ACCESSION NUMBER: MRID No. 416301-01
Caswell No. 528
HED Project No. 1-1238

TEST MATERIAL: IN Z326



SYNONYMS: Linuron
N'-(3,4-dichlorophenyl)-N-methoxy-N-methylurea

STUDY NUMBER: Haskell Laboratory Report (HLR) No. 494-90
Medical Research Project (MRP) No. 8553-001

SPONSOR: E.I. du Pont de Nemours and Company, Inc.
Agricultural Products Department
Wilmington, Delaware 19898

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

TITLE OF REPORT: Investigation of a Mechanism for Leydig Cell Tumorigenesis by Linuron in Rats

AUTHOR: Jon C. Cook, Ph.D.

REPORT ISSUED: September 10, 1990

CONCLUSION: The test material, Linuron, was administered daily at 200 mg/kg by oral gavage to growing and adult rats (approximately 32-33 and 93 days of age, respectively) for 14 days. Flutamide was used as a positive control material. Clinical observations, body weight measurements, and food consumption values were recorded. Following sacrifice, the testis, epididymides, accessory sex organs, and levator ani muscle were weighed; serum testosterone, estradiol, and luteinizing hormone (LH) levels were analyzed. Organ weight data were also evaluated

for the P1 and F1 rats of a previously-completed multigeneration study with Linuron (MRID No. 414634-01) and serum hormone data were analyzed for the P1 rats. An in vitro analysis, in which the ability of Linuron and its metabolites to compete for binding to the androgen receptor, was conducted.

1) The results of this study, particularly the increase in testicular weights, the inhibition of accessory sex organ weights for growing and (to a lesser extent) adult male rats, the increase in serum LH levels for P1 and F1 Linuron-treated rats, and the ability of Linuron to compete for the androgen receptor in vitro, indicate that Linuron may be a weak androgen receptor antagonist. This would support the hypothesis that rats exposed to Linuron could develop interstitial hyperplasia and subsequent adenomas (Leydig cell tumors) of the testicular tissue via a mechanism of sustained hypersecretion of luteinizing hormone (LH) induced by the antiandrogenic potential of Linuron.

2) The hypothesis proposed by the study author, that a threshold mechanism exists for the induction of neoplasms via exposure to Linuron, i.e., that exposure to levels of Linuron that do not cause hypersecretion of LH should pose no additional risk for the development of Leydig cell tumors, was not proven by the results of this study, and therefore cannot be used to support risk assessment decisions.

CORE Classification: Supplementary

I. PROTOCOL

A. MATERIALS:

1. Test compounds: Linuron and its metabolites:

Materials	Haskell No.	Other Identification	CAS No.	Purity
Linuron: N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl-urea	16,569	IN Z326-118; N.B. 7673-8; N.B. 5103-155	330-55-2	96.2%
Metabolite: N-(3,4-dichlorophenyl)-N'-methoxy-urea	17,402	INZ-513; N.B. 5882-105; N.B. AG0079-94	Unknown	Estimated 95%
Metabolite: 3,4-dichloro-benzenamine	17,403	IN 17239-S1; N.B. AG0079-96; Aldrich Chem. Co. #D5,560-1 [95-76-1]	95-76-1	98%
Metabolite: (3,4-dichlorophenyl)-urea	17,404	IN R915-6; N.B. 8416-81; N.B. AG0079-93	Unknown	Estimated 95%
Metabolite: N-(3,4-dichlorophenyl)-N'-methyl-urea	17,405	IN 15654-12; N.B. 5882-103; N.B. AG0079-93	Unknown	Estimated 100%

Descriptions - not provided; Supplier - Sponsor. Structures for Linuron and its metabolites are provided in Attachment 1.

Vehicle: Methocel® (methyl cellulose); Supplier - Fisher Scientific Co., Fair Lawn, New Jersey.

2. Positive control material: Flutamide (α,α,α-trifluoro-2-methyl-4'-nitro-m-propionotoluidide): Description - not provided; Lot No. - SCH 13521; Purity - estimated to be 100%; Supplier - Dr. R. Neri, Schering USA (Bloomfield, New Jersey).

   Vehicle: Arachis (peanut) oil and benzyl alcohol; Supplier - Sigma Chemical Co., St. Louis, Missouri.

3. Other materials: A full description is provided in Attachment 2 (extracted from Materials and Methods, report HLR No. 494-90, page 18).

4. Test animals: Species: Rat; Strain: Crl:CD®BR; Sex: Male; Age at first dose: 32-33 days for studies on growing animals and 93 days for studies on adult animals; Source: Charles River Laboratories, Raleigh, North Carolina; Acclimation and quarrantine: 6 days.

5. Additional test animals: Blood and/or organ weight data from P1 and F1 animals of the multigeneration study HLR No. 20-90 (MRID No. 414634-01) were analyzed.

B. STUDY DESIGN:

A copy of the methodology presented in report No. HLR 494-90 is appended as Attachment 3. This study was designed to identify a mechanism for dose-related Leydig cell tumorigenesis observed in male Crl:CD^{R}(SD)BR rats on a previous two-year feeding study with Linuron. The hypothesis that Linuron possesses antiandrogenic activity was investigated.

1. Group Assignment and Dosage Levels:

Animals were assigned to the following test groups:

Linuron				
Group No.	Test Group	No. of Animals		Dose Level (Oral Gavage)
		Growing	Adult	
1	Control[a]	10	10	5.0 ml/kg/day
2	Pair-fed Control[a]	10	10	5.0 ml/kg/day
3	Linuron	10	10	200 mg/kg/day[b]

a Control vehicle = Methocel^{R}.
b Dose volume = 5.0 ml/kg.

Flutamide (Positive Control)				
Group No.	Test Group	No. of Animals		Dose Level (Subcutaneous Injection)
		Growing	Adult	
1	Control[a]	10	10	0.5 ml/kg/day
2	Flutamide	10	10	10 mg/kg/day[b]

a Control vehicle = 9:1 (v/v) arachis oil/benzyl alcohol mixture.
b Dose volume = 0.5 ml/kg.

In addition, blood and tissue samples were harvested from the following animals assigned to the multigeneration study No. HLR 20-90:

Multigeneration Study No. HLR 20-90[a]			
Group No.[b]	Dose Group (ppm)	No. of Animals (Male[c])	
		P1	F1[d]
1	0	30	30
3	12.5	30	29
5	100	30	29
7	625	30	30

a MRID No. 414634-01.
b Original group numbers were changed for purposes of this study.
c Blood was drawn from P1 females but was not analyzed.
d Reductions in numbers of animals were due to unscheduled deaths.

2. Dose Solution Formulation and Administration:

Linuron was mixed with the vehicle Methocel®, and Flutamide was mixed with a vehicle consisting of 9:1 (v/v) arachis oil/benzyl alcohol to formulate the test material and positive control dosing solutions, respectively. The Linuron solution was administered by oral gavage, and the Flutamide solution was administered by subcutaneous injection. Actual dose volumes administered were based on individual body weight and adjusted daily. All dosing solutions were mixed on a magnetic stir plate during dosing. Rats were treated daily for 14 consecutive days and sacrificed the morning after the last dose.

The report presented no data on stability, homogeneity, or concentration analysis of dosing solutions. Although frequency of mixing is not specified, the report stated that all dosing solutions were stored at 4°C.

3. Husbandry:

Animals were assigned individual unique animal numbers and housed in stainless-steel wire-mesh cages. Purina® Certified Rodent Chow® #5002 meal and tap water were provided ad libitum. Animal rooms were maintained under acceptable environmental conditions.

4. Observations:

Body Weight and Food Consumption: All rats were weighed daily during the 14 days of dosing. Food consumption was measured daily throughout dosing for animals administered Linuron; rats pair-fed to the Linuron group received a daily allotment of food equal to the mean individual daily food consumption of the Linuron-treated rats from the previous day.

Clinical Observations and Mortality: Cage-site observations to detect dead or moribund animals and to identify abnormal behavior and appearance were conducted at least once daily during dosing.

Terminal Sacrifice: The Linuron- and Flutamide-treated rats were sacrificed by chloroform anesthesia and exsanguination. Blood was collected from the inferior vena cava, and serum was subsequently prepared and stored at -85°C until analyzed for levels of serum hormone. In addition, following an identical method of sacrifice, blood was collected from P1 males and females and F1 males from the multigeneration study (HLR No. 20-90), and serum was analyzed from the P1 and F1 males only.

For all Linuron- and Flutamide-treated animals, and for the P1 males of the multigeneration study, the following organs were collected and weighed: testes, epididymides, accessory sex organ unit (prostate, seminal vesicles, and coagulating glands), ventral prostate, dorsal lateral prostate, seminal vesicles with fluid removed, coagulating glands, and levator ani muscle. Organ-to-terminal-body weight values were calculated.

Serum Hormone Measurement: Serum was analyzed for levels of testosterone, estradiol, and LH by radioimmunoassay.

Androgen Receptor Competition: DHT, Flutamide, Linuron, and four metabolites of Linuron (Haskell Nos. 17,402, 17,403, 17,404, and 17,405) were evaluated for their ability to compete in vitro for binding to the androgen receptor.

The androgen receptor assay utilized a dextran-coated charcoal (DCC) adsorption procedure. A buffered cytosol was prepared from the ventral prostrate of Crl:CD^{R}BR rats which had been castrated one day earlier in order to clear endogenous androgens; this cytosol was incubated with 7.3 nM [^{3}H]-testosterone in the absence or presence of increasing concentration of the unlabeled ligand dissolved in ethanol. Cytosol was incubated with ligand for 18 hours at 4°C and then treated with a DCC solution. Aliqots of DCC-treated cytosol were counted in a liquid scintillation counter.

From the data collected, the concentration of the ligand which produced 50% displacement (IC_{50}) of [^{3}H]-testosterone from the androgen receptor was calculated using the EZ-FIT program.

5. Statistical Analysis: (The following description was extracted from report No. HLR 494-90, pages 24-25.) Body weight, body weight gain, food consumption, organ weight, and serum hormone measurement data were analyzed by a one-way analysis of variance. When the test for differences among test group means (F test) was significant, pairwise comparisons between the test and control groups were made with the Least Significant Difference and/or Dunnett's test. The Bartlett's test for homogeneity of variances was performed on the organ weight and serum hormone measurement data and, when significant ($p < 0.005$), was followed by

nonparametric procedures. Incidence of clinical observations was evaluated by the Fisher's Exact test with a Bonferroni correction. Except for Bartlett's test, all other significance was judged at $p < 0.05$.

5. Compliance: The following signed and dated statements were provided:

Statement of No Data Confidentiality Claim
Good Laboratory Practice Compliance Statement
Flagging of Studies or Potential Adverse Effects (none claimed)

II. RESULTS

A. Growing Rats:

Clinical observations - There were no compound-related clinical signs observed in either the Flutamide- or Linuron-treated growing male animals.

Body weight, body weight change, and food consumption (Table 1) - Statistically significant decreases in mean body weight, body weight change, and food consumption values were noted for growing male rats treated with Linuron and in the pair-fed control group as compared to the vehicle control group. Body weight and body weight change values for Linuron-treated growing male rats were not significantly different from pair-fed control values, suggesting that significant effects observed were due to detrimental nutritional status, perhaps a secondary effect of treatment. Body weight and body weight change values for rats treated with Flutamide were similar to concurrent control values.

Table 1. Body Weight, Body Weight Change, and Food Consumption in Growing Male Rats

		Linuron			Flutamide (Positive Control)	
Parameter	N=10	Control	Pair-Fed Control	Linuron (200mg/kg)	Control	Flutamide (10mg/kg)
Initial Body Weight (g)	Mean	108.8	109.1	106.8	122.3	122.3
	S.E.	4.2	5.1	4.8	1.2	1.2
	% A		100	98		100
	% B			98		
Final Body Weight (g)	Mean	242.5	191.7*	177.6*	254.0	250.9
	S.E.	6.0	3.0	6.0	4.5	3.5
	% A		93	73		99
	% B			93		
Body Weight Change (g) (Days 1-14)	Mean	117.9	68.0*	64.1*	118.4	116.7
	S.E.	7.4	6.6	8.3	3.7	2.5
	% A		58	54		99
	% B			94		
Daily Food Consumption (g)	Mean	22.3	17.1*	16.7*	-	-
	S.E.	0.7	0.4	0.7	-	-
	% A		77	75		
	% B			98		

% A = Percentage of control value.
% B = Percentage of pair-fed control value.
* Statistically significant difference from control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 36 and 46.

Organ weight data - Significant decreases in the mean absolute and relative organ weight values (Table 2), as compared to the vehicle control values, were noted for nearly all male reproductive organs in Linuron-treated growing male rats. The exceptions were for the relative testes weight, which was significantly increased as compared to control, and for the coagulating gland and relative epididymides weights which were similar to control. In addition, significance, as compared to the pair-fed control values, was also achieved for the absolute and relative epididymides, accessory sex organ unit,

Table 2. Organ Weights and Organ-to-Terminal-Body Weight Ratios in Growing Male Rats (g)

		Linuron						Flutamide (Positive Control)			
		Control		Pair-Fed Control		Linuron (200 mg/kg)		Control		Flutamide (10 mg/kg)	
Organ	N=10	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Testes	Mean	2.143	0.887	1.983	1.036*	1.837*	1.038*	2.251	0.888	2.317	0.924
	S.E.	0.036	0.019	0.040	0.022	0.076	0.042	0.036	0.018	0.039	0.016
	% A			92	117	86	117			103	104
	% B					93	100				
Epididymides	Mean	0.291	0.120	0.262	0.137*	0.194*#	0.108#	0.294	0.116	0.228*	0.091*
	S.E	0.014	0.005	0.008	0.004	0.014	0.006	0.010	0.004	0.012	0.004
	% A			90	114	67	90			78	78
	% B					74	79				
Accessory Sex Organ Unit	Mean	0.616	0.254	0.426*	0.221	0.233*#	0.129*#	0.636	0.251	0.342*	0.136*
	S.E.	0.034	0.013	0.030	0.014	0.029	0.012	0.038	0.015	0.025	0.009
	% A			69	87	38	51			54	54
	% B					55	58				
Prostate	Mean	0.294	0.117	0.218	0.114	0.152*#	0.084*#	0.339	0.133	0.205*	0.081*
	S.S	0.014	0.009	0.007	0.003	0.018	0.007	0.019	0.007	0.014	0.005
	% A			74	97	52	72			60	61
	% B					70	72				
Ventral Prostate	Mean	0.147	0.061	0.114*	0.059	0.073*#	0.040*#	0.178	0.070	0.113*	0.045*
	S.E.	0.006	0.003	0.008	0.004	0.010	0.004	0.014	0.005	0.006	0.002
	% A			78	97	50	66			63	64
	% B					64	68				
Dorsal Lateral Prostate	Mean	0.147	0.061	0.104*	0.054	0.080*	0.044*	0.161	0.063	0.091*	0.036*
	S.E.	0.011	0.004	0.008	0.004	0.008	0.00?	0.010	0.004	0.009	0.003
	% A			71	89	54	72			56	57
	% B					77	81				
Seminal Vesicles	Mean	0.130	0.053	0.085*	0.044	0.044*#	0.024*#	0.115	0.046	0.058*	0.023*
	S.E	0.013	0.005	0.00?	0.00?	0.008	0.004	0.008	0.004	0.008	0.003
	% A			65	83	34	45			50	50
	% B					52	54				
Coagulating Glands	Mean	0.039	0.016	0.028	0.015	0.032	0.018	0.035	0.014	0.019*	0.007*
	S.E.	0.004	0.002	0.003	0.001	0.019	0.012	0.005	0.002	0.002	0.001
	% A			72	94	82	112			54	50
	% B					114	120				
Levator Ani Muscle	Mean	0.137	0.057	0.083*	0.043*	0.069*	0.038*	0.121	0.048	0.090*	0.036*
	S.E.	0.005	0.002	0.010	0.005	0.008	0.004	0.007	0.003	0.004	0.002
	% A			60	75	50	67			74	75
	% B					83	88				

% A = Percentage of control value.
% B = Percentage of pair-fed control value.
* Statistically significant difference from control value, $p < 0.05$.
\# Statistically significant difference from pair-fed control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 38-39 and 48-49.

prostrate, ventral prostrate, and seminal vesicle organ weight values of the Linuron-treated rats. The significant change in both absolute and relative organ weight values in Linuron-treated rats, occurring when compared to both negative and pair-fed controls, indicates a direct reproductive organ-specific toxicity resulting from Linuron administration.

The pair fed control group demonstrated significant decreases, as compared to the vehicle control values, in the mean absolute accessory sex organ unit, ventral prostate, dorsal lateral prostate, seminal vesicles, and levator ani organ weight values, as well as in the mean relative testes and levator ani organ weight values. Significant increases were noted for the mean relative testes and epididymides weight values.

Statistically significant decreases were also noted, as compared to the vehicle control values, in absolute and relative organ weight values for the rats treated with Flutamide; the exception being the absolute and relative testes weight values which, although increased, were not statistically significant when compared to control.

Serum hormone levels (Table 3) - Significant increases in serum levels of testosterone, estradiol, and LH, as compared to concurrent control values, occurred for growing male rats treated with Flutamide. Serum levels of these three hormones were similar between the vehicle control, pair-fed control, and Linuron-treated male rats.

Table 3. Serum Hormone Levels in Growing Male Rats

		Linuron			Flutamide (Positive Control)	
Parameter	N=10	Control	Pair-Fed Control	Linuron (200mg/kg)	Control	Flutamide (10mg/kg)
Testosterone (ng/ml)	Mean	0.86	0.64	0.72	0.96	2.92*
	S.E.	0.20	0.17	0.28	0.18	0.63
	% A		74	84		304
	% B			112		
Estradiol (pg/ml)	Mean	8.2	7.0	8.0	7.5	9.2*
	S.E.	0.5	0.5	0.7	0.4	0.5
	% A		85	98		123
	% B			114		
LH (ng/ml)	Mean	0.66	0.72	0.54	0.57	1.73*
	S.E.	0.05	0.12	0.04	0.04	0.28
	% A		109	82		304
	% B			75		

% A = Percentage of control value.
% B = Percentage of pair-fed control value.
* Statistically significant difference from control value, $p < 0.05$.
Data were extracted from report HLP No. 494-90, pages 40 and 50.

B. Adult Rats:

Clinical observations - No treatment-related clinical signs were noted for adult male rats treated with Flutamide. Treatment-related clinical signs observed in Linuron-treated rats were primarily comprised of weakened condition and discharge and/or stains in the perioral, perinasal, and/or periocular regions (Table 4). Significant incidences of rats with any discharge or stain, with eye discharge, and with weakness were noted for Linuron-treated adult males rats as compared to both vehicle and pair-fed control incidences.

Table 4. Selected Incidences of Clinical Signs from Linuron-Treated Adult Male Rats[a]

Observation	Control	Pair-fed Control	Linuron (200 mg/kg)
Discharge and/or stains (perioral, perinasal, and/or periocular)[b]	0	1	9*#
Discharge, eye	0	0	5*#
Weak	0	0	9*#

a Number of animals with observation noted at least once during the study.
b Incidence calculated by reviewer.
* Statistically significant difference from control value, $p < 0.05$.
# Statistically significant difference from pair-fed control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 52 and 100-101.

Body weight, body weight change, and food consumption (Table 5) - Significant decreases in final body weight and body weight change values were reported for adult male pair-fed control, Linuron-treated, and Flutamide-treated rats as compared to appropriate vehicle control values. In addition, 1) mean food consumption values for the pair-fed control and Linuron-treated groups were significantly less than vehicle control values (food consumption was not measured for Flutamide-treated rats), and 2) the final body weight and body weight change values for Linuron-treated rats were significantly decreased as compared to values of the pair-fed control group, indicating a possible treatment-related toxic effect which, unlike that which was observed in the growing rats, was not secondary to the reduced food intake.

Table 5. Body Weight, Body Weight Change, and Food Consumption in Adult Male Rats

Parameter	N=10	Linuron			Flutamide (Positive Control)	
		Control	Pair-Fed Control	Linuron (200mg/kg)	Control	Flutamide (10mg/kg)
Initial Body Weight (g)	Mean	407.0	403.4	403.1	398.9	392.4
	S.E.	1.8	4.2	3.0	3.0	5.2
	% A		99	99		98
	% B			100		
Final Body Weight (g)	Mean	483.9	416.1*	397.5*#	502.3	468.8*
	S.E.	3.5	4.3	3.8	8.3	7.6
	% A		86	82		93
	% B			96		
Body Weight Change (g) (Days 1-14)	Mean	65.5	5.9*	-10.2*#	93.3	60.9*
	S.E.	3.1	2.9	4.5	6.0	3.3
	% A		9	-15		65
	% B			-173		
Daily Food Consumption (g)	Mean	27.1	19.0*	18.4*	-	-
	S.E.	0.6	0.1	0.6	-	-
	% A		70	68		
	% B			97		

% A = Percentage of control value.
% B = Percentage of pair-fed control value.
* Statistically significant difference from control value, $p < 0.05$.
\# Statistically significant difference from pair-fed control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 41 and 51.

Organ weight data (Table 6) - Decreases in absolute organ weights were noted in Linuron-treated adult rats. These decreases were statistically significant as compared to negative control values for the epididymides, seminal vesicles, coagulating glands, and levator ani muscle and as compared to both negative and pair-fed control values for the accessory sex organ unit, prostate, and ventral prostate. In addition, the relative accessory sex organ unit, prostate, and ventral prostate weight values were significantly decreased for Linuron-treated adult male rats when compared to both controls. As with the growing rats, the significant change in both absolute and relative organ weight values in Linuron-treated rats, occurring when compared to both negative and pair-fed controls, indicates a direct reproductive organ-specific toxicity resulting from Linuron administration. In the case of the adult rats, however, the epididymides and seminal vesicles appeared to be less sensitive to treatment-related effects than in the adolescent rats.

In the pair-fed control group, significant decreases were observed in mean absolute organ weight values for the accessory sex organ unit, coagulating glands, and levator ani muscle, as compared to the vehicle control value; however, mean relative organ weight values were similar to control values for each of these organs.

Table 6. Organ Weights and Organ-to-Terminal-Body Weight Ratios in Adult Male Rats (g)

		Linuron						Flutamide (Positive Control)			
		Control		Pair-Fed Control		Linuron (200 mg/kg)		Control		Flutamide (10 mg/kg)	
Organ	N=10	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Testes	Mean	3.121	0.647	3.126	0.751	2.884	0.726	2.193	0.440	2.84	0.445
	S.E.	0.202	0.043	0.079	0.014	0.242	0.062	0.183	0.040	0.178	0.038
	% A			100	116	92	112			95	101
	% B					92	97				
Epididymides	Mean	1.126	0.233	1.078	0.259	0.966*	0.243	1.016	0.203	0.716*	0.153*
	S.E	0.043	0.010	0.040	0.008	0.043	0.011	0.026	0.007	0.034	0.008
	% A			96	111	86	104			70	75
	% B					90	94				
Accessory Sex Organ Unit	Mean	2.956	0.611	2.595*	0.623	2.065*#	0.519*#	2.997	0.598	2.029*	0.434*
	S.E.	0.081	0.016	0.108	0.023	0.076	0.019	0.085	0.019	0.112	0.024
	% A			88	102	70	85			68	72
	% B					80	83				
Prostate	Mean	1.128	0.233	1.010	0.242	0.784*#	0.197*#	1.077	0.215	0.902*	0.193
	S.S	0.057	0.011	0.058	0.013	0.035	0.008	0.042	0.008	0.055	0.012
	% A			89	104	70	84			84	90
	% B					78	81				
Ventral Prostate	Mean	0.623	0.129	0.531	0.127	0.364*#	0.091*#	0.587	0.117	0.469*	0.100
	S.E.	0.034	0.007	0.034	0.008	0.030	0.007	0.033	0.006	0.036	0.008
	% A			85	98	58	70			80	85
	% B					68	72				
Dorsal Lateral Prostate	Mean	0.505	0.104	0.478	0.115	0.420	0.106	0.490	0.098	0.434	0.093
	S.E.	0.029	0.006	0.029	0.007	0.031	0.008	0.046	0.009	0.027	0.006
	% A			95	110	83	102			88	95
	% B					88	92				
Seminal Vesicles	Mean	0.512	0.106	0.482	0.116	0.400*	0.101	0.612	0.122	0.378*	0.080*
	S.E	0.014	0.003	0.022	0.005	0.037	0.010	0.044	0.008	0.016	0.003
	% A			94	110	78	95			62	65
	% B					83	87				
Coagulating Glands	Mean	0.249	0.051	0.198*	0.048	0.158*	0.040	0.228	0.046	0.166*	0.036*
	S.E.	0.008	0.002	0.016	0.004	0.016	0.004	0.014	0.003	0.012	0.003
	% A			80	94	63	78			73	78
	% B					80	83				
Levator Ani Muscle	Mean	0.372	0.077	0.308*	0.074	0.277*	0.070	0.359	0.071	0.285	0.061
	S.E.	0.017	0.004	0.008	0.002	0.021	0.005	0.031	0.006	0.022	0.004
	% A			83	96	74	91			79	86
	% B					90	94				

% A = Percentage of control value.
% B = Percentage of pair-fed control value.
* Statistically significant difference from control value, $p < 0.05$.
\# Statistically significant difference from pair-fed control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 43-44 and 53-54.

In the Flutamide-treated adult male rats, statistically significant decreases were noted for the absolute prostate and ventral prostate organ weights, and for the absolute and relative epididymides, accessory sex organ unit, seminal vesicles, and coagulating gland weights.

Serum hormone levels - Treatment of adult male rats with Flutamide resulted in significant increases in blood serum levels of testosterone and LH as compared to vehicle control values (Table 7). Blood serum levels of estradiol and LH were significantly increased, as compared to pair-fed controls, for adult male rats treated with Linuron; however, the serum hormone levels for the pair-fed controls were lower than the vehicle control values, suggesting a lack of true treatment-related effect.

Table 7. Serum Hormone Levels in Adult Male Rats

		Linuron			Flutamide (Positive Control)	
Parameter	N=10	Control	Pair-Fed Control	Linuron (200mg/kg)	Control	Flutamide (10mg/kg)
Testosterone (ng/ml)	Mean	1.06	1.21	1.69	1.71	15.65*a
	S.E.	0.12	0.45	0.74	0.25	2.76
	% A		114	159		915
	% B			140		
Estradiol (pg/ml)	Mean	7.2	6.9	8.8#	6.8	6.8
	S.E.	0.3	0.5	0.8	0.8	0.6
	% A		96	122		100
	% B			127		
LH (ng/ml)	Mean	0.57	0.48	0.59#	0.74	2.56*
	S.E.	0.03	0.03	0.05	0.08	0.45
	% A		84	104		346
	% B			123		

% A = Percentage of control value.
% B = Percentage of pair-fed control value.
a N=9 due to elimination of outlier value from animal No. 467379.
* Statistically significant difference from control value, $p < 0.05$.
# Statistically significant difference from pair-fed control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 45 and 55.

C. Multigeneration Study Rats (HLR No. 20-90):

Final body weight and organ weight data (Table 8) - Final body weight values for the P1 male animals were significantly depressed at the 100 and 625 ppm (mid- and high-dose, respectively) levels when compared to the concurrent control value. Mean absolute and relative organ weight values for the 12.5 (low-dose) and 100 (mid-dose) ppm groups were similar to control values. At 625 ppm, significant decreases were noted in the absolute epididymides, dorsal lateral prostate, and levator ani muscle values and significant increases were noted in the relative testes, epididymides, and ventral prostate values. With the exception of the increased mean relative testicular weight, the

Table 8. Final Body Weights, Absolute Organ Weights, And Organ-to-Terminal-Body Weight Ratios (g) of P1 Male Rats from a Multigeneration Study with Linuron (HLR No. 20-90)

Organ	N=30	Group I - 0 ppm		Group III - 12.5 ppm		Group V - 100 ppm		Group VII - 625 ppm	
		Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Final Body Weight	Mean	680.5	--	672.5	--	637.2*	--	552.1*	--
	S.E.	13.1		13.8		10.2		8.5	
Testes	Mean	3.60	0.533	3.50	0.525	3.54	0.559	3.58	0.650*
	S.E.	0.05	0.012	0.05	0.011	0.06	0.012	0.05	0.011
Epididymides	Mean	1.51	0.225	1.58	0.238	1.48	0.233	1.38*	0.251*
	S.E.	0.03	0.006	0.05	0.009	0.03	0.004	0.04	0.006
Accessory Sex Organ Unit	Mean	3.62	0.539	3.54	0.532	3.50	0.552	3.34	0.607
	S.E.	0.13	0.022	0.16	0.027	0.09	0.014	0.11	0.019
Prostate	Mean	1.38	0.206	1.37	0.207	1.37	0.216	1.27	0.230
	S.E.	0.06	0.010	0.05	0.009	0.40	0.007	0.05	0.009
Ventral Prostate	Mean	0.72	0.108	0.69	0.105	0.74	0.116	0.72	0.130*
	S.E.	0.04	0.007	0.03	0.006	0.04	0.006	0.04	0.006
Dorsal Lateral Prostate	Mean	0.66	0.098	0.68	0.102	0.64	0.099	0.55*	0.100
	S.E.	0.03	0.004	0.02	0.004	0.03	0.004	0.02	0.004
Seminal Vesicles	Mean	0.70	0.103	0.66	0.099	0.65	0.102	0.64	0.116
	S.E.	0.03	0.004	0.03	0.005	0.02	0.003	0.02	0.004
Coagulating Glands	Mean	0.24	0.036	0.26	0.039	0.22	0.038	0.22	0.039
	S.E	0.02	0.003	0.02	0.003	0.02	0.002	0.01	0.002
Levator Ani Muscle	Mean	0.42	0.062	0.42	0.063	0.40	0.063	0.35*	0.064
	S.E.	0.01	0.002	0.01	0.002	0.01	0.002	0.01	0.002

* Statistically significant difference from control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 56-57.

effects noted in the P1 animals at termination, following approximately 120 days on study (and at a mean daily Linuron intake calculated to be 35.5 mg/kg during premating [reference: HLR No. 20-90, MRID No. 414634-01]) were not comparable with those produced in the adult male rats dosed acutely at 200 mg/kg.

Serum hormone levels - Significant increases in estradiol and LH serum hormone levels were noted for both the P1 and F1 Linuron-treated males at the 625 ppm dose level as compared to the control values (Table 9). Mean hormone levels determined for the males of both generations appeared to be similar to concurrent vehicle control values at the 12.5 and 100 ppm dose levels.

Table 9. Serum Hormone Levels of P1 and F1 Adult Male Rats from a Multigeneration Study (HLR No. 20-90)

Parameter		Group I 0 ppm	Group III 12.5 ppm	Group V 100 ppm	Group VII 625 ppm
		P1 Males			
Testosterone (ng/ml)	Mean	1.38	1.06	1.35	1.60
	S.E.	0.27	0.14	0.30	0.27
	% A		77	98	116
	N	30	30	30	29
Estradiol (pg/ml)	Mean	2.9	2.7	3.2	4.5*
	S.E.	0.4	0.4	0.4	0.7
	% A		93	110	155
	N	30	30	30	29
LH (ng/ml)	Mean	0.53	0.64	0.64	0.93*
	S.E.	0.03	0.05	0.06	0.08
	% A		121	121	175
	N	30	29	30	28
		F1 Males			
Testosterone (ng/ml)	Mean	2.22	2.20	1.61	2.12
	S.E.	0.35	0.38	0.25	0.44
	% A		99	72	95
	N	30	29	29	30
Estradiol (pg/ml)	Mean	7.3	8.0	7.3	8.4*
	S.E.	0.3	0.3	0.3	0.3
	% A		110	100	115
	N	25	29	29	29
LH (ng/ml)	Mean	0.60	0.58	0.59	1.01*
	S.E.	0.40	0.02	0.02	0.10
	% A		97	98	168
	N	27	28	28	30

% A = Percentage of control value.
* Statistically significant difference from control value, $p < 0.05$.
Data were extracted from report HLR No. 494-90, pages 58-59.

In vitro competition for the androgen receptor from ventral prostate cytosol - The concentration of ligand which produced a 50% displacement (IC_{50}) of [H^3]-testosterone from the androgen receptor in rat ventral prostate cytosol is presented in Table 10. Dihydrotestosterone (DHT) and Flutamide were used as positive control

materials; IC_{50} results determined experimentally in this study varied from results reported in published scientific literature (1.7 nM for DHT and 35,000 nM for Flutamide). In addition, the internal variability of test results (high standard errors with small N values) indicates the possibility of erroneous test results, which cannot be confirmed from the reported data. Based upon the results as presented, Linuron and three of its metabolites (17,402, 17,403, and 17,405) were able to compete with [^{3}H]-testosterone for binding to the androgen receptor, although not to the extent demonstrated by DHT or Flutamide. The metabolites of Linuron all had a lower affinity for the androgen receptor when compared to Linuron.

Table 10. IC_{50} Values (nM) for Displacement of [^{3}H]-Testosterone Binding to the Cytosolic Androgen Receptor in Rat Ventral Prostate

Test Substance	Mean	S.E.	N
DHT	1.4	0.2	2
Flutamide	18,000	3,500	2
Linuron	64,000	11,000	3
17,402	120,000	12,000	2
17,403	110,000	-	1
17,404	a	-	1
17,405	260,000	130,000	2

a IC_{50} value could not be determined; the compound did not displace [^{3}H]-testosterone.

Data were extracted from report HLR No. 494-90, page 60.

D. DISCUSSION:

1. The use of Flutamide as a positive control material: Flutamide was selected as a positive control material for this study based upon previously published studies which identified it as an androgen antagonist. Flutamide, which is structurally related to Linuron (Attachment 2), has been shown to block the formation of the testosterone-androgen receptor complex, preventing the hypothalamus and adenohypophysis from recognizing the presence of testosterone, and causing hypersecretion of luteinizing hormone (LH). In addition, in a study conducted by the manufacturer (Shering Canada, Inc.) dietary administration of Flutamide to Sprague-Dawley rats for one year resulted in Leydig cell hyperplasia, which progressed to Leydig cell adenoma by study termination. Similar Leydig cell lesions in male Sprague-Dawley rats were observed in a two-year Linuron feeding study conducted by Haskell Laboratory (report HLR No. 100-80, MRID No. 241897).

   Flutamide administration did not result in any observable

clinical/systemic toxic effects in growing male rats, although in adult male rats a significant decrease in body weight gain was noted. In addition, while mean absolute and relative testicular weight values were increased as compared to a concurrent vehicle control group, all mean absolute and relative accessory sex organ weight values were decreased (generally by a significant margin). In adult animals treated with Flutamide, the effects were very similar, but not as pronounced: the mean relative testes weight value was increased slightly but not significantly, while the mean relative accessory organ weight values were decreased somewhat, occasionally at a significant level.

The expected short-term net effects of Flutamide treatment, that of hypersecretion of LH resulting in elevated levels of serum testosterone, were observed in both adolescent and adult Flutamide-treated male rats on this study, and the ability of Flutamide to compete with testosterone for the binding sights of the testosterone receptor was adequately demonstrated.

Thus, based upon the positive control results reported in this study, 1) Flutamide was judged to be an appropriate positive control substance, and 2) the validity of the laboratory assay techniques utilized in this study was demonstrated.

2. Systemic toxicity of Linuron: A dose- and treatment-related decrease in body weight change was demonstrated for the adult P1 and F1 rats of the multigeneration study with Linuron (HLR No. 20-90, MRID No. 414634-01). Decreases in body weight gain were also noted for both growing and adult male rats dosed with 100 mg/kg of Linuron in this study. Comparisons to a pair-fed control group suggested that body weight decrements in the growing animals may have been a secondary effect of Linuron administration, related more closely to reduced food intake; however, comparison of body weight, food consumption, and clinical observation data for the adult rats to vehicle and pair-fed control groups, indicate the probability of an additional primary toxic response to Linuron administration.

3. Effects of Linuron administration on organ weight data: 1) The overall effect of Linuron treatment on growing male rats appeared to be a significant increase in the mean relative testes weight value and significant decreases in the mean relative weight values of most of the accessory reproductive organs studied. These results were similar to those noted for Flutamide-treated growing male rats. 2) In the adult male rats treated with Linuron, the mean testes weight value was again increased, although not significantly, and the mean relative accessory organ unit, prostate, and ventral prostate weight values were significantly decreased as compared to control values. The other accessory reproductive organ relative weight values were similar between control and treated animals. The results, although dissimilar from effects observed in the growing male rats, were comparable to those seen with Flutamide treatment of adult rats and appeared to be mediated by factors related to the age or maturity of the

animals. 3) With the exception of the significantly increased mean relative testicular weight noted in the high-dose group (625 ppm), the effects observed in the adult P1 animals at termination of the first generation, were not comparable with those produced in the adult male rats dosed acutely. The relative accessory reproductive organ weight values in the high-dose group were generally increased, not decreased, as compared to control values, occasionally achieving statistical significance. The disparities could be the result of differences in dosing regimen, since the P1 animals were administered the test material in the diet for approximately 120 days prior to sacrifice, receiving a mean daily Linuron intake of 35.5 mg/kg at the 625 ppm dietary level (calculated from data collected during premating); whereas the adult male rats on this study were administered 200 mg/kg of Linuron daily for 14 days.

4. Serum hormone data: 1) Serum hormone levels in growing male rats were similar to vehicle and pair-fed control values following treatment with Linuron. 2) Although the study author reported that the serum hormone level results for adult male rats were indicative of treatment-related increases in luteinizing hormone (LH) and estradiol, this reviewer regards the results as equivocal, particularly in regard to the LH values. The mean values for the Linuron-treated group were significantly increased as compared to the pair-fed control group only, for which values were noticeably lower than those of the vehicle control group. This would suggest that the significance noted might be an artifact, and not the result of a true biological effect. 3) In the P1 and F1 adult male rats from the multigeneration study, significant increases in estradiol and LH levels were noted in the high-dose groups (625 ppm). These differences appeared to be treatment-related but were not dose dependant. 4) It is interesting to note that estradiol levels were not affected in the positive control (Flutamide-treated) growing and adult male rats, while testosterone levels were increased quite substantially. This difference from results obtained with Linuron-treated animals was not addressed by the report.

5. In vitro competition for the androgen receptor: There are indications that the data generated to demonstrate in vitro competition for the androgen receptor from ventral prostate cytosol may not be valid. Specifically, 1) IC_{50} results for Flutamide varied from results reported in published scientific literature and 2) internal variability of results determined experimentally in this study (as evidenced by high standard errors with low N values) suggest the possibility of erroneous results. However, since there is no confirmation of error within the reported data, the decision was made to accept the data and resulting conclusions as valid. Therefore, the receptor data demonstrated that Linuron, as well as three of the four Linuron metabolites tested, can compete for binding to the androgen receptor, although none have the high affinity demonstrated by Flutamide.

6. Conclusions: 1) The results of this study, particularly the increase in testicular weights, the inhibition of accessory sex organ weights for growing and (to a lesser extent) adult male rats, the increase in serum LH levels for P1 and F1 Linuron-treated rats, and the ability of Linuron to compete for the androgen receptor in vitro, indicate that Linuron may be a weak androgen receptor antagonist. 2) The concept of a threshold level for chemicals that produce neoplasia via a hormonally-controlled mechanism has been proposed, based upon studies of the induction of thyroid follicular cell carcinogenesis. The study author contends that this hypothesis can be applied to Linuron exposure, and that "exposure to levels of Linuron which do not disrupt the hypothalamic-pituitary-thyroid axis (i.e., do not elevate LH) should pose no additional risk for the development of Leydig cell tumors" (report HLR No. 494-90, pages 12 and 33). It is the opinion of this reviewer that this hypothesis has not been confirmed by the results of this study. Therefore, the concept of a threshold level for Linuron-induced neoplasia (Leydig cell tumors) remains a hypothesis and cannot be used to support risk assessment decisions.

Study/Report Deficiencies

1. No signed/dated Quality Assurance Statement was provided.
2. No information was provided regarding the description of the test and positive control materials.
3. It was not specified how often the Linuron and Flutamide solutions were mixed.
4. Stability, homogeneity, and concentration analysis data were not presented (nor discussed) in the report; it is assumed that these tests were not performed.

Page_____ is not included in this copy.

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