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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Special Review Submission on Hormonal Effects of Linuron; Caswell 528; EPA I.D. # 035506; Project 7-0134; Record No. 183738

TO: Michael McDavit, Review Manager
Special Review Branch (TS-767C)
and
Robert Taylor, PM #25
Registration Division (TS-767C)

FROM: James N. Rowe, Ph.D.
Section V, Toxicology Branch
Hazard Evaluation Division/HED (TS-769C)

James N. Rowe 1/21/87

THRU: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED (TS-769C)
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch/HED (TS-769C)

1-21/87

1/22/87

ACTION: Review of Dupont submission, "Biochemical and Pathological Effects of Linuron in Selected Tissues of Male and Female Rats"; Caswell 528; EPA I.D. # 03-5506; Project 7-0134; Record No. 183738

CONCLUSIONS/RECOMMENDATIONS:

The biochemical and histopathology data presented in this submission suggest that linuron may affect testosterone metabolism in horse testicular microsomes for a range of dose levels (50-5000 uM = 11-1100 mg) which overlaps the dose levels experienced by rats exposed chronically (0.75-37 mg/kg b.wt). However, the net effect of these enzyme changes and the relevance to the rat in vivo is uncertain. Evidence in young and older rats exposed repeatedly (3-7x) or for 11 or 19 months suggests that Leydig cell incubates are differentially altered in their sensitivity to increasing doses of luteinizing hormone (LH) (ability to stimulate testosterone secretion) with the repeated or chronic exposure to linuron at the intermediate dose levels producing an apparent hyporeactive response to LH as opposed to the hyperactive response of the Leydig cells to LH in the chronic high dose males. While these biochemical effects are worthy of note, they are not definitive in nature, i.e., they do not conclusively show cause-and-effect.

These data are scientifically acceptable. No recommendation is made at this time. The reviewer will request that the Toxicology Branch Peer Review Committee evaluate these data in relationship to the general oncogenicity issue for linuron.

Excerpts of data submitted by Dupont on Linuron were originally attached to this review (38 pages) These pages may be requested by writing FOI (A-101) EPA, Washington DC 20460. Requestors will be asked to sign an Affirmation of Non-multi-national Status. (SEE D17508

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DATA EVALUATION RECORD

STUDY TYPE: Biochemical and histopathological findings under a variety of conditions of linuron administration

CHEMICAL: Linuron [3-(3,4-dichlorophenyl) methoxy-1-methylurea]; CAS 330-55-2

TEST MATERIAL: Linuron (INZ-326); purity 94.5%; material submitted by John C. Summers, Agricultural Products Department, E.I. du Pont de Nemours and Company, Wilmington, Deleware 19898

STUDY I.D.:

1. Title: Biochemical and pathological effects of linuron in selected tissues of male and female rats

2. Laboratory: Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Deleware

3. Study #: Haskell Laboratory Report No. 643-86; MR No. 4580-001

4. Date of report: October 6, 1986

5. Study director: Timothy P. Pastoor, Ph.D.

6. Caswell # 528; Accession # 265422; EPA ID # 035506

CONCLUSIONS:

In conclusion, the biochemical and histopathology data presented in this submission suggest that linuron may affect testosterone metabolism in horse testicular microsomes for a range of dose levels (50-5000 uM = 11-1100 mg) which overlaps the dose levels experienced by rats exposed chronically (0.75-37 mg/kg b.wt). However, the net effect of these enzyme changes and the relevance to the rat *in vivo* is uncertain. Evidence in young and older rats exposed repeatedly (3-7x) or for 11 or 19 months suggests that Leydig cell incubates are differentially altered in their sensitivity to increasing doses of luteinizing hormone (LH) (ability to stimulate testosterone secretion) with the repeated or chronic exposure to linuron at the intermediate dose levels producing an apparent hyporeactive response to LH as opposed to the hyperactive response of the Leydig cells to LH in the chronic high dose males. While these biochemical effects are worthy of note, they are not definitive in nature [illegible] do not conclusively show cause-and-effect. It should be noted that linuron has also produced liver adenomas in male and female mice following chronic administration in the diet. Whether this is related to hormonal alterations is unknown.

This data is considered to be scientifically acceptable.

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METHODS:

A photocopy of the materials and methods from the report (pages 20-25) is attached. The studies performed in this report are of an experimental nature and are not described in the 1982 EPA Guidelines for pesticide toxicity assessment. Therefore, these studies will be evaluated in terms of their scientific relevance to the issue of linuron's potential oncogenicity and will not be given a Core grading.

RESULTS:

1. Leydig cell steroidogenic enzyme analyses

[^{14}C]-Labeled substrates (50 uM) for various testicular steroidogenic enzymes (aromatase, 17-20 desmolase, 3-B-hydroxysteroid dehydrogenase/isomerase, 17-hydroxylase and 17-ketosteroid reductase) were incubated in vitro with horse testicular microsomes in the presence of incubation concentrations of 0, 0.5, 5, 50, 500 and 5000 uM of linuron, des-methyl linuron, hydroxy-linuron, nor-demethylated linuron and des-methyl-hydroxy linuron (see attached Table 1 from p. 42 of submitted study).

Linuron produced statistically significant effects on enzyme activity at 500 and 5000 uM for aromatase and desmolase (decreased activity) and reductase (increased activity). Hydroxylase was statistically significantly lower at the high dose of 5000 uM. Nonstatistically significant changes for all the enzymes at lower doses (primarily 50 uM) are consistently suggested by the data: aromatase (14%/50 uM), desmolase (16%/50uM), isomerase (15-20%/all concentrations), hydroxylase (around 10% at all doses except high dose) and reductase (20%/50 uM).

Examination of the enzyme activity of the other metabolites of linuron indicates a variable response. Des-methyl linuron significantly decreased the conversion of labeled testosterone to estradiol 17-B by aromatase only at the high dose of 5000 uM with no other apparent effects on the steroidogenic enzyme activity. Hydroxy-linuron had no statistically significant effects on the enzymatic conversions of labeled substrates. In contrast to linuron, nor-linuron appeared to significantly alter enzymatic activities only at the high dose for all enzymes except isomerase where statistically significant effects (increases or decrease were observed at 50, 500 and 5000 uM concentrations). Finally, des-methyl-hydroxy-linuron generally produced alterations in enzymatic activity for aromatase, desmolase (decreased), and isomerase (increased) at the 500 and 5000 uM concentrations but only at the high dose for hydroxylase and reductase

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2. Testosterone metabolic clearance rate

A summary of the testosterone clearance data is presented below (taken from p. 106 of du Pont report):

Group	Infusion rate(ug/h)	Testosterone conc. (ug/dL) at various sampling times(min) 0	30	60	90	120	150	180	60-180	MCR[b] (ml/h)
control[†]	3	71.4	137	341.0	337	372	200.0	233	297(75.3)[a]	
		41.5	257	251.0	401	*	306.0	256	304(69.6)	
		99.0	365	249.0	298	361	316.0	308	306(40.1)	
		51.6	238	304.0	290	*	*	*	297(9.9)	
		30.5	261	297.0	325	370	353.0	288	327(35.2)	
mean value:		81.4	251.6	288.4	330.2	367.7	293.8	271.2	306(±12.3)[c]	980
testos.	3	184.0	704	491.0	679	303	170.0	375	404(193.0)	
		73.5	256	335.0	353	376	523.0	467	411(80.7)	
		71.5	365	279.0	223	231	294.0	292	264(34.2)	
		16.0	161	256.0	253	324	322.0	399	311(60.0)	
		16.0	32	59.5	*	*	73.8	277	120(92.9)	
		25.4	175	299.0	337	350	267.0	298	296(49.8)	
mean value:		64.4	282.2	274.9	369.0	316.8	330.0	343	301(±107)[c]	997
control	6	73.5	1150	823.0	1060	970	1040.0	1230	1025(147.7)	
		210.0	567	584.0	553	705	649.0	684	635(64.9)	
		173.0	564	562.0	829	742	779.0	855	753(115.6)	
		40.8	427	564.0	522	373	481.0	447	477(73.0)	
		55.5	643	716.0	1090	944	1370.0	2450	1314(677.9)	
		*	335	330.0	236	370	413.0	717	413(181.2)	
mean value:		110.6	614.3	596.5	715.0	684.0	788.7	1063.8	769(±344)[c]	780
testos.	6	26.1	467	576.0	643	652	618.0	622	622(29.5)	
		49.9	408	623.0	656	825	660.0	*	691(90.9)	
		41.0	512	626.0	436	528	637.0	557	557(81.6)	
		39.6	415	569.0	570	612	931.0	614	659(153.5)	
		33.9	441	584.0	559	598	578.0	633	590(27.6)	
		53.8	588	343.0	341	606	737.0	510	507(171.1)	
mean value:		40.7	471.8	501.8	534.2	636.8	693.5	587.2	605(±67.4)[c]	992

[a] mean (S.D.); [b] MCR = metabolic clearance rate (calculated by dividing the infusion rate of testosterone by the mean plateau concentration); [c] mean plateau concentration for 60-180 minutes; * report stated testosterone could not be measured in these samples; [†] each value represents an individual animal.

Male rats were administered linuron (200 mg/Kg b.wt.) for eight days, castrated and then infused with testosterone at 3 and 6 ug/hour(see summary table above and attached figure 1 from p. 70 of du Pont report). Based on a rough mean plateau concentration of 306(±12.3)(controls) and 301(±107) (linuron-treated) in the 3 ug/hour testosterone-infused animals at 60-180 minutes, no difference in the MCR (metabolic clearance rate) was apparent (i.e., 980 vs 997, respectively). At the higher infusion rate of 6 ug/hour testosterone, there was a higher mean plateau concentration in the controls as opposed to the linuron-treated rats (769± 344 vs 605 ± 67.4) which gave a higher MCR for the compound-treated animals

(780 vs 992, respectively). However, as pointed out by the authors, there was a wide divergence in the individual animal testosterone concentrations measured resulting in a large standard deviation for the 6 ug/hour controls. Thus, linuron (200 mg/kg b.wt.) administered in the diet for eight consecutive days does not appear to affect the metabolic clearance of infused testosterone in young adult male Crl:CD® BR rats castrated following compound treatment.

3. Leydig cell response to LH (luteinizing hormone) stimulation

Two trials were performed to evaluate the responsiveness of rat Leydig cells incubated in vitro in the presence of luteinizing hormone (LH) from "young" (<3 month old), "old" (approximately one year) or F_{2b} rats (11 months old = Trial 1; 19 months old = Trial 2) derived from the multigenerational reproduction study. In Trial 1, "young" and "old" rats were dosed for three days at 200 mg linuron/kg b.wt./day and in Trial 2, for seven days at the same dosage. Also, in Trial 1 only F_{2b} control and high dose (625 ppm) group testes were evaluated as compared to Trial 2 where testes from all dose levels (0, 25, 125 and 625 ppm) of the F_{2b} male rats were investigated. The data are presented in Figures 2A-C (Trial 1) and 3A-C (Trial 2) photocopied from the report.

In Trial 1 (Figures 2A-C), there appeared to be a difference in the response of the "young" versus the "old" rats to LH. There was no apparent difference in the response of the "young" control or treated animals to LH in secreting testosterone. In the "old" rats, however, the controls appeared to be more responsive than the treated animals, both in terms of maximum response and relative potency of the response. In contrast, the chronic F_{2b} treated Leydig cells were significantly more responsive to hormonal stimulation by LH than the chronic control Leydig cells with a roughly four-fold greater maximal response.

In Trial 2 (Figures 3A-C), there was a consistent shift to the right of the dose-response curve, i.e., a decrease in the responsiveness of the Leydig cell incubates to LH for "young" treated rats as compared to the "young" controls. This decreased responsiveness of linuron-treated animals was even more evident in the "old" treated rat testes as compared to the controls where, as noted in Trial 1, the maximum response was significantly less (three-fold less) and the relative potency was significantly less. In the chronic F_{2b} rats (19 months old), the control and low dose (25 ppm) rat testes had a moderate response to LH, the intermediate dose (125 ppm) was minimally responsive but the high dose group (625 ppm) was significantly greater with a roughly three-fold greater testosterone secretion at the highest dose level of LH as compared to the control Leydig cell preparation. The two trials are reasonably consistent with a preliminary conclusion that there are dose- and time-related effects of linuron upon the sensitivity of rat Leydig cells to stimulation by LH.

4. Clinical, gross and histopathology findings, etc.

a. Food consumption, food efficiency

Mean daily food consumption data for the F_{2b} male and female rats were presented in Tables 10 and 11 of the report for days 0-98 and 519-526 (males) or 533-540 on test. The individual data were not submitted, but based on data tables presented, mean daily diet in males appeared to be similar in all dose groups except for the high dose which was somewhat lower, e.g., control values

at days 519-526 = 28.1g vs 21.8g. All female values were generally similar. On the basis of mean daily food intake at the latter period, the mean daily intake of linuron was calculated to be 0, 0.75, 4.1 and 22 mg linuron/kg b. wt. in males and 0, 1.1, 6.1 and 37 mg linuron/kg b.wt. in females. Based on the limited data, especially for the later time periods, it is not possible to determine the significance of the food efficiency data. For males, at the 519-526 time period, the mean food efficiency (g wt gain/g diet consumed) appeared to be diminished at the mid and high doses (0.014/control, 0.037/low, -0.011/mid, and 0.002). The female data at 533-540 was quite variable, i.e., -0.026/control, 0.020/low, 0.005/mid and 0.023/high dose.

b. Body weights, clinical signs

Mean body weights for F_{1b} and F_{2b} male and female rats are presented in Figures 4 A, B and 5 A, B (pages 73-76 of Dupont report). There was a statistically significant depression in mean body weights for F_{1b} male and female rats at the high dose level during days 469 to 708 ($p<0.05$); and a statistically significant depression in the female body weights for days 540-596 on test. For the F_{2b} male and female rats there was also a statistically significant depression in mean body weights at the high dose level ($p<0.05$) for days 0-533 (no high-dose group male rats remained on test after this day) for males and 0-709 days for females. As with the F_{1b} females, the mid dose group mean body weights of the F_{2b} females were also significantly depressed from day 0-554 on test ($p<0.05$).

Examination of the clinical signs data (F_{1b}, F_{1b} male and female data) did not indicate any unusual findings except for a statistically significant increase in alopecia in the F_{2b} female rats at the mid and high dose level (see below):

Study group	Dose group (ppm) 0	25	125	625
F_{1b} females	7 (225)[a]	8 (469)	11 (225)	11 (225)
F_{2b} males	6 (582)	9 (652)	12 (519)	4 (428)
F_{2b} females	9 (245)	12 (344)	17*(302)	19 (302)

[a] the number preceding the parentheses indicates the number of rats within each group exhibiting the sign; the number within the parentheses indicates the median days-on-test when the sign was first observed; there were 20 rats/group at the study start; * statistically significantly different from controls by the Fisher's Exact Test with a Bonferroni correction ($p \leq 0.017$)

The F_{1b} females and F_{2b} males also appeared to have a slight to moderate increase in alopecia (not statistically significant).

c. Gross and microscopic pathology

There was an apparent increase in the number of small or discolored testes in the high dose F_{1b} or F_{2b} males as compared to the control males (4/10 small and 5/10 discolored testes in high dose F_{1b} vs 5/10 small and 1/10 discolored testes in controls; 4/7 small testes in high dose F_{2b} vs 0/10 in controls). Microscopic examination did not appear to reveal any unusual atrophy of the testes as compared to the control testes.

Two uterine adenocarcinomas (1/9 in F_{1b} and 1/20 in F_{2b}) and one squamous cell carcinoma of the cervix (1/20 in F_{2b}) were reported in the high dose group females. These tumors were stated in the pathology report (p. 82) to be "... uncommon in aged Crl:CD®:BR rats at Haskell. Of 400 recent control females assigned to 2-year tests, no uterine adenocarcinomas or cervical squamous cell carcinomas were observed, although both tumors were seen in various treated-group rats on most of these studies. The incidences of these tumors in this multigeneration test, however, were not statistically significant (alpha = 0.05, Fisher exact test) when compared to the study controls. In addition, no uterine adenocarcinomas or cervical squamous cell carcinomas were found in any of the rats on the previous 2-year study ..."

Additional selected histopathology findings are presented on the following pages. Interstitial cell adenomas and hyperplasia were primarily observed in linuron-treated males of both generations as compared to the controls. Combining the data from both the F_{1b} and F_{2b} groups further supports this finding for the mid and high dose levels [i.e., for adenomas, control = 1/19 (5%) vs mid = 6/25 (24%) and high = 2/16 (12%); for hyperplasia, control = 2/19 (11%) vs mid = 7/25 (28%) and high = 3/16 (19%)]. In most instances, the adenomas are associated with hyperplasia. The lower incidence in the high dose group is probably due to the more limited number of animals available for histopathological examination. Thirteen of the male rat testes were utilized in the testosterone assay and many of the rats died several months prior to final sacrifice. The death of the animals prior to final sacrifice may have obscured the testicular changes which might have been expressed in that tissue if the rats had survived longer. These observations are consistent with the findings in the 2-year chronic rat study where a statistically significant, dose-related increase in testicular adenomas were observed.

For the F_{1b} and F_{2b} female groups, the combined data indicated a dose-related increase in cervical endometrial hyperplasia (control = 0/28, low = 6/30, mid = 9/29, 13/29) and a increase in cervical cystic hyperkeratosis of the high dose group as compared to the controls (control = 0/28, low = 1/30, mid = 1/29, high = 7/29). As with the male findings, these changes are consistent with the findings in the females of the 2-year chronic study where uterine endometrial hyperplasia was observed in the high dose group (625 ppm). The dose-related increase in cystic endometrial hyperplasia suggests that the NOEL for this effect is possibly below 25 ppm although the limitations of the study, i.e., in terms of study design and animal numbers, make this uncertain.

DISCUSSION/CONCLUSIONS:

The authors of this submission have suggested that the mechanism of action for the oncogenic response of rats fed linuron is mediated through an effect on the hypothalamic-pituitary/testes feedback loop for luteinizing hormone (LH), i.e., linuron-induced alterations in the feedback loop that increase LH production may lead to Leydig cell hyperplasia and adenomas. They have further proposed that there are four mechanisms which could account for an increase in LH including:

1) androgen resistance: linuron blocks hypothalamic recognition of testosterone thereby preventing regulation of LH secretion

2) LH sensitivity: linuron alters Leydig cell sensitivity to LH thereby reducing testosterone secretion and increasing LH secretion

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SELECTED HISTOPATHOLOGY SUMMARY FOR F1B AND F2B MALE AND FEMALE RATS*
(approximately 2-years old derived from 3-generation reproduction study)

	<----------Dose (ppm)---------->			
F1B male rats	0	25	125	625
Testes:				
adenoma (interstitial cell)	1/10	0/10	2/10	2/10
hyperplasia (interstitial cell)	2/10	0/10	5/10	4/10
adenoma plus hyperplasia	1/10	0/10	2/10	1/10
Adrenal cortex: hyperplasia	3/10	4/10	5/10	7/10
Pituitary: adenoma	5/10	8/10	6/10	6/10
F2B male rats				
Testes:				
adenoma (interstitial cell)	0/9	0/15	4/15	0/6
hyperplasia (interstitial cell)	0/9	0/15	5/15	1/6
adenoma plus hyperplasia	0/9	0/15	3/15	0/6
Adrenal cortex: hyperplasia	2/9	0/15	3/15	1/6
Pituitary: adenoma	6/9	12/15	12/15	4/6
Combined data: (F1B and F2B)				
Testes:				
adenoma (interstitial cell)	1/19	0/25	6/25	2/16
hyperplasia (interstitial cell)	2/19	0/25	7/25	3/16
Adrenal cortex: hyperplasia	5/19	9/25	8/25	8/16
Pituitary: adenoma	11/19	20/25	18/25	10/16

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SELECTED HISTOPATHOLOGY TABLE*(continued)

	<----------DOSE (ppm)---------->			
F1B female rats	0	25	125	625
Uterus:				
cervix: cystic hyperkeratosis	0/10	0/9	0/9	4/9
cystic endometrial hyperplasia	0/10	2/10	1/9	3/9
Adrenal cortex: hyperplasia	3/10	7/10	3/9	2/9
Pituitary: adenoma	5/10	7/10	6/9	3/9
F2B female rats Adrenal cortex:				
Uterus:				
cervix: cystic hyperkeratosis	0/18	1/20	1/20	3/20
cystic endometrial hyperplasia	0/18	4/20	8/20	10/20
Adrenal cortex: hyperplasia	7/18	6/20	6/20	4/20
Pituitary: adenoma	13/18	12/20	16/20	7/20
Combined data: (F1B and F2B)				
Uterus:				
cervix: cystic hyperkeratosis	0/28	1/30	1/29	7/29
cystic endometrial hyperplasia	0/28	6/30	9/29	13/29
Adrenal cortex: hyperplasia	10/28	13/30	9/29	6/29
Pituitary: adenoma	18/28	19/30	22/29	10/29

* animals sacrificed by design or unscheduled deaths (found dead/sacrificed in extremis)

3) enzymatic effects: linuron alters the activity of key enyzme(s) in the Leydig cell thereby reducing testosterone secretion

4) metabolic clearance: linuron increases the metabolic clearance rate (MCR) of testosterone

The authors stated that the first hypothesis was not tested since no study design was available to adequately address this hypothesis. The other three hypotheses were evaluated and reported in the present submission.

As noted in the results, linuron primarily affected horse testicular microsomal enzyme activity in vitro (aromatase: decreased activity; reductase: increased activity) at the high dose concentrations of 500 and 5000 uM. However consistent but not statistically significant effects were seen at the lowest dose used of 50 uM (aromatase, desmolase, isomerase, hydroxylase and reductase). Metabolites of linuron produced a variable response on Leydig cell enzymes primarily at the 500 and 5000 uM concentrations. Fifty micromolar, 500 and 5000 uM equals to, respectively, 10.9, 109 and 1090 mg of linuron, and as noted in the study the male rats from the multigeneration study chronically consumed 0, 0.75, 4.1 and 22 mg linuron/kg b.wt. and the females consumed 0, 1.1, 6.1 and 37 linuron mg/kg b.wt. Thus, it is possible that blood levels approaching $\geq$ 50 uM range could have been experienced by the rats (although the degree of gastrointestinal absorption of the linuron from the dietary feed could impact this figure) and alterations in the rate of testosterone metabolism could have occurred. However, the interpolation of enzymatic changes in the horse testes to the rat is uncertain. Furthermore, the effect of the various enzyme changes on testosterone secretion is unclear. Linuron decreased the activity of aromatase, an enzyme which converts testosterone to estradiol-17B, and increased the activity of 17-20 desmolase, an enzyme which converts 17-alpha-Hydroxyprenenolone to Dehydroepiandrosterone. The net effect of this would seem to be to increase not decrease testosterone secretion, but the interaction of the total enyzme changes induced by linuron on net testosterone secretion of the Leydig cells is uncertain.

Testosterone clearance (in vivo) in male rats treated for eight consecutive days with linuron (200 mg/kg) and then castrated was not significantly affected. Thus, in animals exposed to high doses of linuron on a short-term basis there did not appear to be a change in the MCR (metabolic clearance rate) which presumably would have been mediated through the liver. However, no examination of the liver microsomal enzyme activity was performed in order to support this hypothesis. It would have been appropriate to also test the effect of chronic administration of linuron on testosterone clearance.

There appears to be an age and dose-related effect on the sensitivity of Leydig cells of the rat testes to luteinizing hormone (LH) as evidenced by the in vitro secretion pattern of testosterone. Young rats (<3 months) or old rats (one year) treated with linuron for three to seven consecutive days were generally less responsive to LH in testosterone secretion than the respective controls. The intermediate dose level animals (125 ppm) in chronically treated F_{2b} animals (11-19 months treatment) were significantly less responsive than the control or low dose animals with the same dosage regimen. However, there was a significantly greater maximal release of testosterone (three to four-fold) in the F_{2b} high dose animal incubates (either 11 or 19 months of treatment). These finding suggest that linuron is altering the sensitivity of the Leydig cells, i.e., repeated or chronic exposure to linuron at the intermediate dose levels produced

an apparent hyporeactive response to LH as opposed to the hyperactive response to LH in the chronic high dose males.

As pointed out by the authors, Husby (Cancer Research 41:3172- 3178, 1981) reported that prolonged LH hyperstimulation of Fisher rat Leydig cells (a strain of rats in which Leydig cell tumors spontaneously occur with a significant frequency in older males) results in hyperplasia and adenoma formation. As noted in the histopathology section, Leydig cell hyperplasia and adenomas related to linuron administration have been observed in male rats from a two-year chronic rat study as well as male rats taken from a three-generation reproduction study. Furthermore, uterine adenocarcinoma, cervical squamous cell carcinoma, cystic endometrial hyperplasia and cervical cystic hyperkeratosis have been observed in female rats fed linuron in the same two studies; these tissues are hormonally sensitive tissues. These histopathological changes would appear consistent with an endocrine effect of linuron if it is producing an effect on the hypothalamic/hypophysial-testes or -ovarian feedback loop.

The EPA Guidelines for Carcinogen Risk Assessment (1986) state that, "The carcinogenic effects of agents may be influenced by non-physiological responses (such as extensive organ damage, radical disruption of hormonal function (reviewer's emphasis), saturation of metabolic pathways, formation of stones in the urinary tract, saturation of DNA repair with a functional loss of the system) induced in the model systems. Testing regimes inducing these responses should be evaluated for their relevance to the human response to an agent and evidence from such a study, whether positive or negative, must be carefully reviewed." Thus it is appropriate for the EPA to consider the proposal that the oncogenic effect of linuron in rats may be mediated through a secondary hormonal mechanism by possible modification of the Leydig cell LH receptor, particularly in light of the generally negative evidence that linuron is a classical mutagenic compound with direct interaction with cellular DNA .

It is the opinion of the reviewer that while the submitted data is suggestive of an effect upon the pituitary feedback loop, particularly in regard to LH, the data is not definitive enough, at this time, to cause a reevaluation of the Agency's risk assessment. This is based on the following points:

1) interpretation of the hypo- or hypersensitivity of Leydig cells in secreting testosterone upon LH stimulation after subchronic-high dose/chronic intermediate dosages or from chronic high dose animals is uncertain. How does one integrate these findings with the dose-related increases in testicular hyperplasia and adenomas? If the chronic high dose results in an increased secretion of testosterone wouldn't this decrease in LH release from the pituitary make a hyperplastic/adenomatous response less likely. An additional test which might have confirmed LH hyperstimulation would have been to monitor via radioimunoassay the in vivo blood levels of LH of the chronic rats at various time periods to determine if the LH profile is consistent with the in vitro findings, e.g., if the intermediate dose is producing down regulation of the LH receptor in the testes, than a decrease in testosterone secretion might be expected and an increase in blood levels of LH and vice versa at the high dose.

2) extrapolation of the results from rats to other rodent species, in this case the mouse, is not appropriate as noted by Husby (1981) where Leydig cell tumorigenesis appears to relate to estrogenic stimulation and not gonadotropic stimulation. Therefore, its relevance to humans would obviously be unclear. In vitro tests examining the relative response of isolated human and rat testicular Leydig cell responses to LH stimulation including LH receptor binding studies might answer in part this issue.

3) It may that changes in the responsiveness of the testicular interstitial cells are occurring in addition to a direct oncogenic effect of linuron, that is one may not necessarily be the end result of the other

4) If linuron is altering the endocrine system in some subtle fashion in the mammalian organism, or possesses some hormonal-like action of itself, this may predispose the animals to potentially deleterious effects (possibly of oncogenic nature) which can not be determined at this time.

In conclusion, the biochemical and histopathology data presented in this submission suggest that linuron may affect testosterone metabolism in horse testicular microsomes for a range of dose levels (50-5000 uM = 11-1100 mg) which overlaps the dose levels experienced by rats exposed chronically (0.75-37 mg/kg b.wt). However, the net effect of these enzyme changes and the relevance to the rat in vivo is uncertain. Evidence in young and older rats exposed repeatedly (3-7x) or for 11 or 19 months suggests that Leydig cell incubates are differentially altered in their sensitivity to increasing doses of luteinizing hormone (LH) (ability to stimulate testosterone secretion) with the repeated or chronic exposure to linuron at the intermediate dose levels producing an apparent hyporeactive response to LH as opposed to the hyperactive response of the Leydig cells to LH in the chronic high dose males. While these biochemical effects are worthy of note, they are not definitive in nature, i.e., they do not conclusively show cause-and-effect. It should be noted that linuron has also produced liver adenomas in male and female mice following chronic administration in the diet. Whether this is related to hormonal alterations is unknown.

These data are considered to be scientifically acceptable.