



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
RESEARCH AND DEVELOPMENT

SUBJECT Re-evaluation of the Carcinogenicity of Linuron Based on Additional Rat Tumor and Testicular Hormone Data Submitted by the Haskell Laboratory of Dupont

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Wm. Farland

RECEIVED

Conclusions are presented on p. 15 of this document OCT 10 1987

Information Services Branch, PMSI

I. Introduction

This memorandum is in response to your March 4, 1987 memorandum to Dr. Farland requesting the Carcinogen Assessment Group to:

1. Comment on the latest hormone data (from Dupont) for the rat and its relationship to the testicular observed adenomas in the rat.
2. Re-evaluate the prior CAG carcinogenicity position expressed in a CAG document (4/30/84) and memorandum (4/30/85) prepared by Dr. Bernard Haberman.
3. Address the issue of why the mouse liver adenoma was chosen for quantitation rather than the rat testicular adenoma response. Also, respond to the quantitative assessment prepared by Bert Litt (12/31/86).

Our understanding is that CAG's re-evaluation will be used to assist the Hazard Evaluation Division (HED) in finalizing the Position Document 2/3 on the herbicide Linuron.

*18 pages w/Attachments: May 5, 1987 review (3 pages)
April 22, 1987 review (5 pages)*

II. Brief Review of the Previously Submitted Carcinogenicity Data and Current Re-evaluation

In a previous Registration Standard from the Toxicology Branch (OPP) (J. Holder to R. Wright, 9/15/82), it was stated that Linuron caused an increase in benign testicular tumors in the interluminal space commonly known as the interstitium in Charles River CD rats *. This tumorigenic response in the rat primarily occurred in the last few months of life. The total incidence of the testicular adenomas were reported by Dupont as:

<u>Dietary Linuron</u> feed conc. :	0 ppm	50 ppm	125 ppm	625 ppm
Number of rats with testicular adenomas/by # of rats examined, <u>Incidence</u> :	4/70 (5.7%)	9/69 (13.0%)	20/70 (28.6%)	37/70 (52.9%)

As noted in the 1982 Registration Standard, this is a significant response, statistically. However, no malignant testicular neoplasms occurred in the controls or in any of the Linuron-dosed rats. The biological meaning will be discussed in the following text of this Linuron re-evaluation.

The CAG has recently conferred with Dr. Al Singer of Dupont's Haskell Laboratory concerning the occurrence and meaning of the benign neoplasms in the testes †. Dr. Singer related the past frequency of these same types of neoplasms (Table 1). The 65% variation around an average incidence of 7.71% suggests a reasonably repeatable negative control incidence in Charles River CD rats for a tumor usually occurring in less than 10% of the animals. The negative control incidence (4/70 = 5.7%) in the 2-year study seems to be quite near the average historical incidence experienced by Haskell Laboratory personnel (7.7%). It is

* In this report from CAG to HED the cells thought to give rise to these adenomas are the interstitial cells, probably the Leydig cells, and will be noted as ISC.

† Dr. Singer, D.V.M., has taken Dr. Raymond Everett's place at Haskell Laboratory in relating Dupont's position on the pathology since Dr. Everett has left the company.

the opinion of the CAG that the 2-year carcinogenicity bioassay study (study no. H-10080) was adequately controlled and is representative of the spontaneous rate of testicular adenomas of the rat interstitium.

The increases in the number of CD rats (with these testicular hyperplasias and adenomas), treated with Linuron in the diet, are likely to be significant at exposures to Linuron greater than 50 ppm. By comparison to the internal negative control incidence of 4/70 or to the historical control incidence of 84/1005, the incidences at 125 and 625 ppm Linuron are significantly increased (Figure 1). The statistical inferences of trend and increase of tumors are summarized in Table 2.

There could be (not known) an inflection point between 50 and 125 ppm in that the benign tumor response goes from "not different than control" to "significantly increased over controls" in that dose-rate range (cf. note Table 2). As previously pointed out in the 1982 Registration Standard, it is notable that this dose-rate level (50 to 125 ppm) of carcinogenicity coincides with other reported pathological effects (including decreased fertility) reported from other bioassays on Linuron which suggests this could be the beginning of the pathological dose-range of activity for Linuron in the rat. However, establishment of a definite threshold in this range for carcinogenicity is, of course, scientifically unprovable in the formal sense. A threshold might be inferred from more* negative low-dose testicular incidence data (<125 ppm), as well as, a rational understanding of the mechanism of causation of these tumors of the ISC of the aging rat testes which points to a limited dose responsiveness of Linuron. We conclude that the present data sets on Linuron do not provide any determination of an actual threshold for tumorigenicity.

* "More" means considerably more low dose-rate ranges tested and done repeatedly in order to gain some notion of frequency and variation of the tumorigenic responses at low doses of Linuron.

TABLE 1. HISTORICAL EXAMPLES OF TUMOR INCIDENCE OF
 TESTICULAR ADENOMAS IN
 CHARLES RIVER CD RATS IN HASKELL LABORATORY CONTROLS (LIFETIME)

Study	Haskell report number	Incidence of testicular adenomas in rats	Percent incidence (%)
1	H-10080	7/70	6 *
2	H-10108	14/82	17
3	H-10314	12/83	14
4	H-10540	4/68	6
5	H-11987	12/98	12
6	H-11266	7/76	9
7	H-13647	5/69	7
8	H-14331	9/73	12
9	H-14721	2/60	3
10	H-14765	8/68	12
11	H-14793	2/69	3
12	H-14851	3/67	4
13	H-15172	0/60	0
14	H-15527	2/62	3

Testicular adenoma average incidence = 7.71%

standard deviation of this set (n = 14) = 5.03

coefficient of variation = 65.2%

* This control was associated with the 2-year Linuron feeding study in which testicular adenomas were increased.

Figure 1

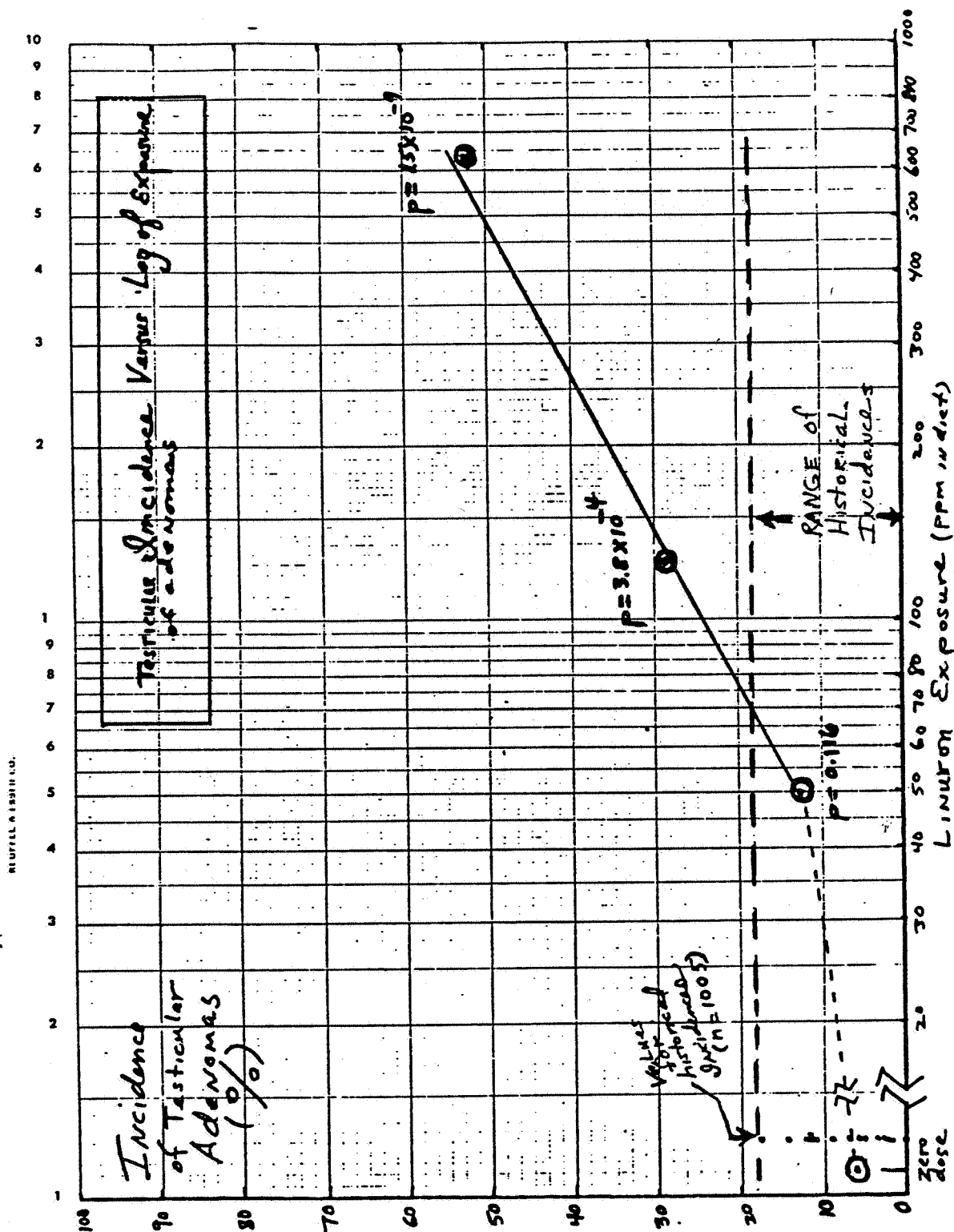


TABLE 2. STATISTICAL EVALUATION OF CHARLES RIVER CD RAT
 TESTICULAR ADENOMA RESPONSE (STUDY NO. H-10080)

1. Probability of no association
 to the set: 4/70, 9/69, 20/70, 37/70
 at 0, 50, 125, 625 ppm dietary Linuron $p = 1.25 \times 10^{-9}$
2. Probability of no-trend
 to the above set: $p = 1.5 \times 10^{-11}$
3. Significance of difference with each dose group response
 and historical or internal controls:

Historical Control Incidence = 84/1005
 Internal control incidence = 4/70

Feed concentration (ppm)	Statistical Significance		Reasonable Interpretation
	Versus historical control	Versus internal control	
0	$p = 0.28$	Not applicable	doses not significantly increased
50	$p = 0.13$	0.12	
<hr/>			
125	$p = 5 \times 10^{-8}$	3.84×10^{-4}	doses statistically increased
625	$p = 5 \times 10^{-10}$	1.5×10^{-9}	

Note: The dashed line refers to an abrupt and dramatic shift in p-values to much lower numbers which could suggest a region of dose-rate of qualitative change in the tumor response.

It should be stressed that a cancerous state* was not caused by Linuron in the rat. It is qualitatively important to recognize that although treated for a lifetime the CD-1 rats did not show evidence of increased malignancies. Rather, a singular response in the testes, which was limited to just adenomas in senescent rats. This response was not life threatening, but was concomittant with decreased body weights and increased testicular weights.

Linuron did apparently disturb rat fertility at 125 ppm and 625 ppm. This effect in young rats (at 15 weeks of age) was tested in a standard three generations study. Also scored were decreased pup survival and weights. Decreased fertility could be caused by Linuron effects in male or female rats since the standard protocol does not permit further interpretation. Older rats, an age in which the testicular tumors arose, have never been tested for reproductive competency although this may be difficult to do since rats older than 1 year naturally become sexually inactive.

Fertility not only decreased with increasing dose but also with generation, being lowest (52.6%) in the F3A generation at 625 ppm Linuron. Since reproductive effects are observed, it can not be determined whether the rat male, or the rat female, or both are responsible. It cannot be deduced that male hormone disturbances were necessary linked to the lowered fertility. Therefore, it cannot be stated that any such fertility decrement could be functionally linked to the male rat testicular adenoma response although it cannot be ruled out either that such an interaction might occur. CAG takes the position that the oncogenic response in rat tests is not explained in the present data base by the concomitant reproductive disturbance caused by Linuron.

A tumorigenic response was also observed in Linuron-treated CD-1 mice. That is, hepatocellular adenomas were increased, but with no apparent trend with dose, or with organ locus compared to the rat response. The benign liver tumor

* Stedman's Medical Dictionary definition: Cancer - A general term frequently used to indicate any of various types of malignant neoplasms, most of which invade surrounding tissues, may metastasize to several sites, and are likely to recur after attempted removal and to cause death of the patient unless treated; any carcinoma or sarcoma.

† Adenomas have been defined by Dupont pathologists as the increased number of ISC to the point where the testicular interstitia are largely filled.

incidences in CD-1 mice were:

Tumorigenicity in Linuron Treated CD-1 Mice

(all tumors scored in this Table were hepatocellular adenomas)

Conc:	0 ppm	50 ppm	150 ppm	1500 ppm
Males:	9/79 (11.4%)	<u>18/80 (22.5%)*</u>	10/80 (12.5%)	16/78 (20.5%)
Females	5/79 (6.3%)	6/79 (7.6%)	8/76 (10.5%)	<u>20/80 (25%) †</u>

* p = 0.048 † p = 0.001 [null hypothesis tested against negative control]

No liver carcinomas were observed which were compound related. The actual occurrence of hepatocellular carcinomas was low (2% to 3%) and constant throughout the dose groups thereby demonstrating a lack of malignancy induction by Linuron in the mouse. Due to the heterodisperse nature of the response with dose (low dose, males; high dose, females), the benign nature of the tumor, and the low level of incidence of the liver tumors, the mouse tumor response is considered a minimal tumorigenic response in the mouse which offers little implication as to whether a carcinogenic response would take place if man were exposed to Linuron.

III. Effect of Linuron Fed to Aged Male Rats

The Dupont company has recently submitted an experiment on Linuron under the Data Call-In program of OPP (reviewed by Dr. J. Rowe, OPP, 1/27/87). The experiment exposed older rats in the last year of life (before 24 month sacrifice) to either (1) no linuron (12 months) or (2) no linuron (6 months) then linuron 6 months, or (3) just linuron (12 months). The basic question being asked by these experiments is: does the older rat respond to Linuron with tumors?

Rats in the first group did not show any spontaneous occurrence of testicular adenomas while the second and third group did manifest these tumors in the rat testes. The tumorigenic results are given in Table 3.

These tumor results indicate that these ICS can be induced rather late in life. In a non-parametric sense (all-or-nothing), we agree with Dr. Rowe, OPP, (1/27/87) in that life-time exposure to Linuron is not requisite for any [i.e. any at all] tumors. However, when one compares the same dose (625 ppm, diet), in the prior 2-year oncology study (H-10080), one observes the intensification of the adenoma response (Table 3). This intensification indicates a graded response, and not, an all-or-nothing tumor response. This intensification of the adenoma response in the two-year study is compound-related and demonstrates the first year treatment with Linuron caused more tumors than when Linuron was administered only in the last year for 12 months.

However, even with increased Linuron exposure time periods (Table 3), no malignant tumors were observed thereby showing the testicular adenomas were the terminal state. Moreover, these testicular tumors were not progressing to the cancerous state but rather were limited; going through first hyperplasia of the ISC to finally some crowding of the lumen, which has been defined (by Dupont) as the ISC adenomas so described in this report. These adenomas remained as such (in the terminal state) no matter how long Linuron was administered -- 6, 12, or 24 months.

TABLE 3. DATA CALL-IN ON LINURON

EFFECTS IN AGING ON THE INCIDENCE OF TESTICULAR ISC ADENOMAS

Report date: 9/24/86

Study Number: H-39486

Dr. T. P. Pastoor, Director, Haskell Laboratory

<u>Group</u>	<u>Linuron Exposure (ppm)</u>	<u>Exposure Period</u>	<u>Interstitial Cell Testicular Response</u>	
			<u>Hyperplasia</u>	<u>Adenomas</u>
1	0 + 0	none	8/25 (32%)	0/25 (0%)
2	0 + 625 ppm	18 to 24 months	8/25 (32%)	2/25 (4%)
3	625 ppm + 625 ppm	12 to 24 months	15/25 (60%)	6/25 (24%)
Previous two-year study (H-10080)	625 ppm + 625 ppm plus previous year of 625 ppm	0 to 24 months	6/69 (9%)	37/70 (53%)

IV. Hormonal Effects Described With the Testicular Adenoma Response of the Interstitial Cells

Dupont has recently submitted a data package on Linuron involving studies to evaluate (1) testosterone enzyme changes (2) testosterone clearance rate changes, and receptor-cell (i.e., Leydig cells) sensitivity changes to the effector (testosterone). This report (EPA #03-5506) has been recently reviewed by Dr. J. Rowe (OPP) on 1/27/87 and by Dr. Bob Sonawane (REAG/OHEA) on 5/5/87. The latter review on Linuron hormonal effects is attached in the Addendum to this report for your perusal. We have taken both of these reviews into account in our integration of the hormone tests and the putative effects the hormones have on the interstitial cell hyperplasia and adenoma formation following Linuron administration.

The testosterone enzymes measured (aromatase, 17,20-desmolase, 3-ketohydroxysteroid dehydrogenase/isomerase, 17-hydroxylase and 17-ketosteroid reductase, showed some changes with Linuron in vitro. We find there are difficulties with these enzyme changes (some up, some down, some unchanged) in that no consistent pattern emerged that would change the testosterone level in such a way as to necessarily produce the hypergonadotropism observed. Moreover, Dupont did not provide a consistent rationale of the metabolic changes, as measured. Furthermore, the enzymatic alterations were done in the presence of horse microsomes which are not necessarily relevant to human (species at risk) or rat (surrogate test species) microsome-mediated enzyme changes since microsome content varies considerably among species.

Testosterone clearance was measured in male rats which were pretreated with Linuron (8x, 200 mg/ky/day) castrated and then probed with testosterone (exogenous infusion) at the rate of 3-6 mg testosterone per hour. Blood samples were withdrawn at 0, 30, 60, 90, 120, 150, 180 minutes after the start of infusion of the androgen.

Such an experiment measures the steady-state disappearance of testosterone from the blood. This is not a clearance rate from the rat which would require a quantitative balance study of testosterone and testosterone metabolites. The assay was quite variable in measuring testosterone in rat blood. No differences were seen between control and Linuron-treated rats, but this result is in doubt due to the imprecise measurement of testosterone in blood and the incomplete protocol which should have measured the total corporal disposition of testosterone, including appearance of metabolites in the excretia. We draw no useable inference from this "clearance" study and therefore cannot determine if Linuron does, or does not, cause a build-up of corporal testosterone because of effected elimination mechanisms.

Leydig cells of the testicular interstitium were directly tested for LH (lutenizing hormone) effectivity, i.e., how much testosterone was released when a known amount of LH interacted with the recipient Leydig cells. Both young and old rats were used to harvest the ISC for testing for testosterone release following LH treatment in vitro.

Results show cells tended to respond with advanced age or number of Linuron treatments with greater output (at the 625 ppm dose), i.e. greater testosterone/LH. This was observed from treated vs. control for old rats ISC, but not for treated vs. control for young rats. One reason the relative output could increase is because of low responsiveness of old controls thereby making treated/control a high ratio (because of decreased denominator). This did not occur in young rats where the control and Linuron-treated rats tracked together in [LH] responsiveness, i. e., Linuron seemed to make no difference.

Dupont states that the old rat controls lack of response (testosterone/LH) needs to be repeated. We agree, but we might expect the old rat response to

LH to decrease naturally due to senescence. The data show that Linuron may abrogate that natural old-age decline and allow the cells to respond in a hyperactive mode without the proper modulation at the proper time in the life of the animal. Hence, testosterone output in old animals [a time in which the increased ISC are taking place] occurs because of elevated androgen occurring in an anachronistic manner. We disagree with the summary statements in OPP review (J. Rowe, 1/27/87) which relegate these results to "noteworthy" but "undefinitive". The submitted data suggest, in our opinion, a testosterone-related Linuron effect in the testes in old rats which should be investigated further, but that indicate androgenic effects related to Linuron do occur in the aging rat.

The higher control functions of the hypothalamus/pituitary hormonal axis of androgen level maintenance were not investigated by Dupont. Hence, the basic control mechanism for testosterone modulation control was not investigated in the rat with respect to chronic Linuron exposure. One experiment which seems appropriate is to measure LH levels (by radioimmunoassay) in the blood of Linuron-treated rats. We view this omission of experiments on androgenesis control to be a data gap relevant, possibly, to the observed testicular adenomas.

It may be summarized that Linuron possibly alters some testosterone-related enzymes, e.g., desmolase decrease, reductase increase, and Leydig cell responsiveness to lutenizing hormone resulting in the anachronistic and excessive production of testosterone in old rats, a possible mechanism for increased number of ISC leading to ISC adenomas. The CAG views the decrease in testosterone output in the in vitro LH effectivity test at the mid-dose, while at the highdose an increase, a problematic result which will have to be reconciled since both these Linuron doses cause [by whatever mechanism] increased testicular adenomas.

V. Re-evaluation of the possibility that Linuron is a Human Carcinogen

We consider in turn factors for, and factors against, Linuron being a human carcinogen. These factors are presented separately in TABLES 4 and 5. The weight of the evidence indicates that the tumorigenic effects are seen in old rats which may be testosterone-related. These tumorigenic effects are manifest as hyperplasia and adenomas. These adenomas were defined (by Dupont) as hyperplasia to the extent that the cells filled the interstices spaces among the lumen of the rat testes. As such, no malignant state was produced by Linuron which falls short of the normal definition of cancer (cf. footnote pg. 7).

The cancer guidelines takes the position that "benign only" is limited evidence "in most cases". We find this case may be an exception in that "limited" evidence indicates some fraction of the whole, i. e., at least some support for carcinogenicity. Results for increased tumorigenicity were what was actually observed -not carcinogenicity. It follows, then, that a limited amount of carcinogenicity was not produced in bioassay within a substantial portion of the animals' lifetimes.

Responses of adenomas were observed in two species of test animals, but none have been reported in man (lack of epidemiological support). Since there is some variation in correlating animals to man both in tumor site (could have different organ locus) and intensity (could have different degrees of malignancy/metastasis), we judge the limited evidence category (rather than inadequate) to be satisfactory. We note, however, to treat Linuron as if it were a human carcinogen would go beyond the extent of the above inference. We connote within this tumorigenic response a "possibility" of carcinogenicity which is not ruled out by the data. As such, Linuron is considered Group C, but with low carcinogenic potential.

This categorization of carcinogenicity generally agrees with the prior evaluation by CAG with the addition of the recent data. It is felt that quantitation

of the carcinogenic potency is not appropriate based on such weak qualitative weight-of-evidence evidence for carcinogenicity.

VI. CONCLUSIONS (in order presented in § I, Introduction)

1. The testicular hormone data is suggestive of pathological testosterone/LH related effects in the rat testes, especially since reproduction studies indicate reduced fertility, pup survivability and pup weight. However, since these findings cannot be unequivocally traced to the male rat, the testicular adenomas cannot be explained on the basis of, or be necessarily related to, the observed reproductive effects. We suggest more studies are needed: 1) complete pathology on rat males and females which would reveal reproductive-organ lesions or disorders and, 2) testing older males for reproductive competency, perhaps at 12 - 14 months, since the adenomas occurred late in the life of the rat and therefore took some time to develop.
2. CAG has re-evaluated the cancer data on Linuron to be best represented by Category C - a possible human carcinogen. We note also that interpretation of this data in such a way as to assume human carcinogenicity - either for quantitative (q_1^*) or qualitative purposes - would not be a reasonable interpretation of the data since cancer was not obtained in either the rat or the mouse bioassays. Only benign tumors (adenomas) were observed in the rat testes and mouse liver with only the former bioassay demonstrating dose-relatedness to Linuron. CAG views the carcinogenicity of Linuron to be minimal and is possible to the extent it is not ruled by the actual data and to the extent that the human response, if any, could vary from the animal response toward the pernicious direction, i.e. some malignancy.
3. Because of the lack of any cancer (malignancy) related to Linuron exposure in the bioassays, and of the heterodisperse nature of the tumor response in the mouse, and of the possible hormone (testosterone/LH) mediation of the rat testicular adenomas, it is reasoned that cancer potency estimation is not useful. As such, we recommend against usage of a q_1^* value since we evaluate the potential hazard risk from cancer (malignancy) to be minimal.

TABLE 4. FACTORS IN THE CONSIDERATION OF LINURON BEING A
HUMAN CARCINOGEN

Effect	Comments
1. Caused tumors (adenomas) in Charles River CD rat testes.	1. Dose related trend; only mid (125 ppm) and high dose (625 ppm) significantly greater than controls. No carcinomas at all. Seems to be biologically linked to testosterone disturbance in old rats.
2. Caused hepatocellular adenomas in CD-1 mice.	2. Not dose related with high dose females and low dose males only; significant biological meaning unclear.
3. Scored by more than one Dupont pathologist.	3. Validation - with only "some" difference of opinion of what constitutes by definition testicular hyperplasia or adenomas.
4. More than one tissue responding.	4. Repeat tumorigenic effect, but in different tissues.
5. Two species responding with tumors.	5. Benign tumors replicate in mouse and rat; only rat response is dose-related to Linuron.
6. The Guidelines state that in mouse cases a "benign only" response is limited evidence for carcinogenicity and is a possible human carcinogen.	6. This is not <u>ipso facto</u> evidence of carcinogenicity but rather a consensus opinion to cover most cases where only benign tumors result.
7. Some reproductive problems in the same dose-rate range: lowered fertility, pup weights, and pup survivability. Possible indication of beginning range of pathological effects of Linuron.	7. Effect might be traced back to male test effects but the relationship is unclear since reproductive or postnatal effects could be maternal in origin, or from both parents.

TABLE 5. FACTORS FOR CONSIDERATION OF LINURON NOT BEING A
HUMAN CARCINOGEN

<u>Effect</u>	<u>Comments</u>
1. No malignant state in test animals (mice and rats).	1. Does not satisfy basic definition of cancer (cf. footnote, pg. 7).
2. Benign tumors only - No malignant tumors mixed into the response.	2. Shows definite lack of tumor progression; limited pathological response.
3. Lack of dose-response in male or female mice.	3. Decreases strength of any suspected causality with doses of Linuron in the mouse.
4. Dose response in male rats, but may be androgen related hyperplasia in old rats.	4. Leydig cell responsiveness to testosterone increased in old rats; certain testosterone enzymes may be altered. Cardinal effect could be physiological disturbance based on androgen imbalance in old rats.
5. Lack of genotoxicity supportive of commonly understand mechanisms of tumor initiation.	5. Mostly negative or inadequate data; OPP has evaluated Linuron to be negative in genotoxicity.
6. No life-threatening tumors in mice and rats.	6. Some weight loss that was Linuron-related, but no early deaths that were compound related.
7. No reproductive problems assayed in male rats.	7. Linuron caused hyperplasia and adenomas in the testes late in life, the prelude to which did not compromise male reproductive competence.

ADDENDUM



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

May 5, 1987

OFFICE OF
RESEARCH AND DEVELOPMENT

SUBJECT: Assessment of Linuron's Oncogenicity

TO: William H. Farland, Director
Carcinogen Assessment Group (RD-689)

And

James W. Holder, Toxicologist
Carcinogen Assessment Group (RD-689)

FROM: Bob Sonawane, Pharmacologist,
Reproductive Effects Assessment Group (RD-689)

And

Hal Zenick, Reproductive, Toxicologist
Reproductive Effects Assessment Group (RD-689)

With reference to the above, you have requested us to review and comment on the Rowe's review of the Dupont submission "Biochemical and Pathological Effects of Linuron in Selected Tissues of Male and Female Rats" (Memorandum dated January 27, 1987). We have reviewed the above referred submission and our comments are as follows:

We are in general agreement with the reviewer's conclusion that the biochemical and histopathology data presented in the Dupont submission, are not definitive in nature to demonstrate a cause and effect relationship. We agree with the hypothesis that the Linuron treatment may have caused a prolonged hypergonadotrophism leading to hyperplastic changes observed in aged male and female rats. However, the experimental data do not support conclusively that the Linuron-induced Leydig cell hyperplasia and adenomas in male and cystic endometrial hyperplasia and cervical hyperkeratosis in female rats are related to hormonal alterations. The firm did not provide a convincing argument that the high incidence of the dose-related Leydig cell hyperplasia and adenomas would not lead to malignancy in aged rats. Moreover, there were a number of problems associated with the biochemical studies as outlined below which compromise the relevance of the studies to support the hypothesis. Therefore, we recommend that an independent pathologist should review the slides for histopathological lesions observed in order to address the issue of whether adenomas could lead to malignancy.

The biochemical studies were designed to test the hypothesis that the Linuron treatment may have caused a prolonged hypergonadotrophism leading to hyperplastic changes observed in aged male and female rats, respectively. In order to test this hypothesis, three experiments were conducted to demonstrate that Linuron-induced alterations in feedback loop lead to increase in LH production and Leydig cell hyperplasia.

1. Enzymatic Effects:

Linuron and its four metabolites were tested in vitro for their effects on five key steroidogenic enzymes in horse testicular microsomes. A variable response of different steroidogenic enzymes in horse testes microsomes to Linuron and its metabolites was observed. Although, the choice of testicular steroidogenic enzymes and methods of measurements are appropriate, the use of horse testes microsomal preparations is questionable to explain pathological response in rats. Therefore, we agree with the reviewer that the relevance of study is uncertain.

Testosterone Metabolic Clearance Rate: The time course of plasma concentration of testosterone was monitored in control and Linuron-treated male rats (200 mg/kg/day, 8 days) who were castrated and then infused with 3 or 6 ug testosterone/hour. The data presented in Figure 1 and Appendix B (page 27 and 106 of the DuPont submission dated October 6, 1986), indicate enormous variability in testosterone levels, especially in the controls, suggesting an apparent problem with the assay procedure. The effects of Linuron metabolites were not examined, thus, the effects of Linuron or its metabolites on the metabolic clearance rate (MCR) of testosterone was not demonstrated under the experimental conditions described. Therefore, we disagree with the conclusion of the firm that the metabolic clearance rate of testosterone was not affected by eight doses of Linuron at 200 mg/kg/day.

Leydig Cell Response to LH Stimulation

The studies of Leydig cell LH sensitivity in young and old rats show that there are dose- and time-related effects of Linuron upon the sensitivity of rat Leydig cells to stimulation by LH. The Leydig cells of young and old rats, gavaged for seven days with 200 mg Linuron/kg/day, were less responsive to LH stimulation than corresponding control rats; old rats were more affected than young rats. In the chronic studies, 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 three-fold greater testosterone secretion at the highest LH dose level compared to the control Leydig Cell preparation. It seems that Linuron may have altered the sensitivity of rat Leydig cells to stimulation by LH in a dose- and time related manner.

These observations are consistent with the hypothesis that prolonged LH hyperstimulation of Leydig cells of rats may affect the pituitary feedback loop leading to histopathological changes (hyperplasia and adenoma). Although, the data obtained in the present studies indicate endocrine effects of linuron, it seems possible that these effects may have been secondary to the function of adenomatous foci or of frank tumors of Leydig cell origin. Transplantable Leydig cell tumors often produce androgens as well as estrogens even in

hypophysectomized hosts (Jacobs and Huseby, 1968). The most striking hormone changes accompanying testicular tumor formation involves gradual increments in serum estradiol and prolactin titers (Turek and Desjardins, 1979). However, whether testosterone biosynthesis and secretion continue to depend on LH during the advanced stages of tumorigenesis is questionable. Since hyperprolactinemia paralleled with estrogen elevated titers and histopathological lesions induced by Linuron treatment were not demonstrated in the present study, the existence of cause-effect-relationship cannot be ascertained.

Spontaneous Leydig cell tumors occur infrequently in most strains of rats (Thompson et al, 1961; Coleman et al, 1977, Snell, 1965, Crain, 1958). Testicular interstitial cell tumors have been documented in several other species, including dogs (Hayes and Pendergrass, 1976), mice (Samuels et al, 1967), and humans (Mostofi, 1973). In several instances, the development and maintenance of testicular tumors has been shown to be influenced by gonadotropic and/or gonadal hormones (Walsh, 1977). However, this was not clearly demonstrated by the experimental data presented in the submission.

The combined incidence of Leydig cell hyperplasia and adenomas in F_{1b} and F_{2b} male rats in the control, low-, intermediate- and high-dose groups was 3/18, 0/25, 16/25 and 7/16, respectively. In female F_{1b} and F_{2b} rats, the combined incidence of cystic endometrial hyperplasia and cervical hyperkeratosis in the control, low-, intermediate-, and high-dose groups was 0/28, 7/30, 10/29, and 20/29. In the previously submitted chronic two-year study (Kaplan, 1980), endometrial hyperplasia was observed only in the high-dose group and there were two incidences of adenocarcinomas (one in F_{1b} and one in F_{2b}) and one incidence of cervical squamous cell carcinoma (F_{2b}). The incidence of these rare type tumors was not statistically significant. It seems important to note that these hormone-dependent tumors (as claimed) are induced by Linuron treatment only in aged rats. However, the major question remains as to whether these tumors represent extensive hyperplasia or adenomatous overgrowths or may lead to neoplastic transformation or actually an example of malignant neoplasia. The preliminary review of the descriptions of the individual microscopic observations of gonadal tissues (testes, uterus and ovaries) as presented in the submission are inadequate to make any distinct judgements. Since different morphological cell types are being affected by Linuron treatment, it seems unlikely that a single mechanism triggers this response. This assumption is only cautionary and needs to be carefully evaluated by examining the degree of pleomorphism and hyperchromatism, the frequency of bizarre large cells, and the occurrence of multipolar mitotic figures to qualify these tumors as being malignant.



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

RELEASABLE

APR 22 1987

SUBJECT: Abbreviated Peer Review of Linuron

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

FROM: Esther Rinde, Ph.D. *E. Rinde 4/1/87*
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Robert Taylor
Product Manager #25
Registration Division (TS-767c)

RECEIVED

The Toxicology Branch Peer Review Committee met on March 2, 1987 to discuss and evaluate the weight-of-evidence on Linuron with particular reference to its oncogenic potential.

1. Individuals in Attendance:

- A. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated.)

Theodore M. Farber

Theodore M. Farber

William L. Burnam

Mr L Burnam

Anne Barton/Gary Burin

(over) Dr. G. Burin

Reto Engler

Reto Engler

Louis Kasza

Louis Kasza

Richard Levy

Richard Levy (correction attached)

Judith Hauswirth

Judith W. Hauswirth

Jack Quest

John A. Quest

Esther Rinde

Esther Rinde

1. B. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report.)

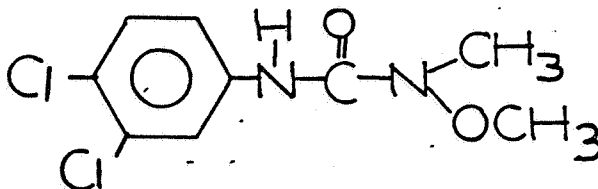
James Rowe

Quang Bui

James Rowe
Quang Bui

2. Background Information:

Linuron, currently in Special Review, was designated by the Carcinogen Assessment Group (CAG) as a class C oncogen and a risk assessment has been performed. Dupont recently submitted biochemical and histopathology data which purportedly indicates that the mechanism of action is mediated through the pituitary-testes (ovary) feed-back mechanism; based on this data, Dupont maintains that linuron should be considered as having a threshold for its oncogenic effect.



LINURON

3. Oncogenicity Studies:

The following 2 studies in the rat and in the mouse were reviewed by CAG ("Review of Rat and Mouse Data from the Dupont Chemical Company for the Carcinogenicity of Linuron" CHEA-C-117, April 30, 1984):

In the chronic rat study, conducted by DuPont, Charles River CD rats were fed linuron for two years at 0, 50, 125 or 625 ppm in the diet. Linuron produced a statistically significant increase in the incidence of interstitial-cell adenoma in the testes of male rats in mid and high-dose groups (controls, 4/68 or 5.9%; low dose, 9/56 or 16.1%; mid-dose, 19/64 or 29.7%; high-dose, 37/66 or 56.1%).

In the mouse study, conducted by Haskell Laboratory, Charles River CD-1 mice were fed linuron for 2 years at 0, 50, 150, or 1500 ppm in the diet. (The equivalent dose rates were reported as 0, 12, 35, and 455 mg/kg/day, respectively.) A statistically significant increase in the incidence of hepatocellular adenomas was observed at the highest dose group in female mice (controls, 5/79 or 6.3%; low dose, 6/79 or 7.6%; mid dose, 8/76 or 10.5%; high dose, 20/80 or 25% ($p=0.001$)), and in the lowest dose group (50 ppm) male mice (controls, 9/79 or 11.4%; low dose 18/80 or 22.5% ($p=0.048$); mid dose, 10/80 or 12.5%; high dose, 16/78 or 20.5%). Hepatocellular carcinomas were not significantly increased at any dose, in either sex.

The following 2 studies were reviewed by TOX Branch:

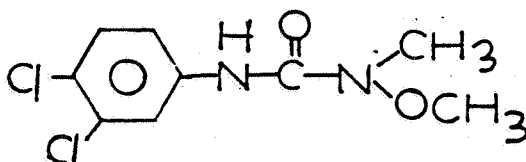
In a multi-generation rat reproduction study, also conducted by Dupont ("Special Review Submission on Hormonal Effects of Linuron" EPA I.D. #035506, Caswell # 528) Crl:CD strain rats were fed linuron at 0, 25, 125, or 625 ppm (estimated dose: 0.75-37 mg/kg body wt.) in the diet. Two uterine adenocarcinomas and one squamous cell carcinoma of the cervix were reported in the high dose group females*, however this incidence was not statistically significant when compared to concurrent controls. (Neither of these tumor types were seen in the chronic rat study, discussed above.) There was an increase in testicular interstitial-cell adenomas and hyperplasia in treated males of both generations, as compared to controls. The incidence of adenomas for the F_{1b} and F_{2b} groups combined was: controls, 1/19 or 5%; low dose, 0/25 or 0%; mid-dose, 6/25 or 24%; high-dose, 2/16 or 12%. In most instances, the adenomas were associated with hyperplasia.

Another study in Crl:CD(SD)BR rats was submitted by DuPont, in response to a data call-in ("Effects of linuron fed to aged male rats" study #394-86, 1986; MR No. 7351-001, Caswell 528). The estimated average age of the rats at the beginning of the study was 12 months. A statistically significant increase in testicular adenoma and hyperplasia was observed in male rats fed dietary linuron (625 ppm) for 12 months. Rats fed normal diet for 6 months, followed by 6 months of dietary linuron, had a non-statistically significant increase in testicular adenomas without elevation in testicular hyperplasia (see J.Rowe memo, 2/27/87 for details).

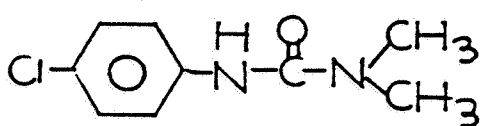
*A high degree (dose-related) of uterine endometrial hyperplasia was also observed in these female rats, which could be related to the testicular hyperplasia in males <L. Kasza>.

4. Ancillary Data for Weight of Evidence Determination:

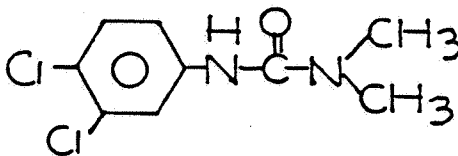
Linuron was negative in the following acceptable studies for mutagenicity: Ames Test, UDS, CHO-HGPRT gene mutation and in vivo bone marrow chromosomal aberration. No metabolism data were available for review. SAR: Monuron, a structural analog, was carcinogenic (kidney and liver) in the male (F344/N) rat; preliminary information suggests that diuron causes bladder tumors in Wistar rats; other analogs (propanil, and dimilin) were negative for oncogenicity in the mouse and rat (propanil was tested in the rat only).



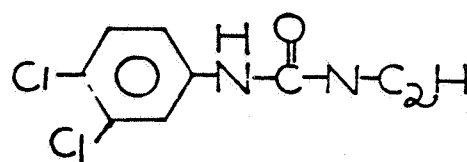
Linuron



Monuron



Diuron



Propanil

Biochemical and histopathological data were presented which suggest that linuron may affect testosterone metabolism in horse testicular microsomes for a range of dose levels (50-5000 μ M = 11-1100 mg).

The Leydig cells of chronically dosed (625 ppm, high dose) male rats exhibited a hyperactive response to luteinizing hormone (LH) manifested by increased testosterone secretion. On the other hand, Leydig cells of rats exposed repeatedly (200 mg/kg per os for 3-7 days) or for 11 or 19 months at intermediate dietary dose levels (125 ppm), apparently were hyporeactive to LH (decreased testosterone secretion).

In vitro secretion patterns of testosterone suggest that linuron effects on Leydig cells of rat testes are age and dose-related. Also, Husby (Cancer Res. (1981) 41:3172-3178) reported that prolonged LH hyperstimulation of Leydig cells in Fischer rats gives rise to hyperplasia and adenoma. (Leydig cell tumors occur spontaneously in older males of this strain).

5. Conclusion

The registrant (DuPont) maintains that these biochemical and pathological data (presented in section 4.) indicate that the mechanism of action for oncogenicity of linuron is mediated through a pituitary-testes (ovaries?) feedback mechanism and therefore linuron should be considered as having a threshold for this effect.

The Peer Review Committee concluded that these data are suggestive (but not definitive) of an hormone-mediated effect for oncogenicity; furthermore, whether or not this might be the only mechanism for oncogenicity could not be determined.

Linuron was classified by the Peer Review Committee as a Group C Carcinogen, in accordance with the 1986 Guidelines*; based on limited evidence in the rat and mouse (there was a statistically significant increase in the incidence of benign tumors only). The Committee also recommended that a quantitative risk assessment should be performed on the testicular tumors in the rat.

6. Additional Data Required for a Definitive WOE Determination

Two impurities found in linuron of potential toxicological concern are 3,3'(-) 4,4'-tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB), analogs of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent carcinogen and acute toxicant. TCAB and TCAOB are contaminants of concern due to their structural similarity to TCDD, and recent preliminary reports of teratogenicity, chloracne, mutagenicity and binding potential to the apparent TCDD receptor site in the liver. In addition, it is known that TCDD and other dioxins strongly resist biodegradation and it is quite likely that TCAB and TCAOB would not be quickly degraded, as well. In order to properly compare the risks of use of two linuron product contaminants under consideration, a determination of the comparative maximal residues on food crops of TCAB and TCAOB has been requested from the Residue Chemistry Branch (RCB/OPP) based on the lb a.i./acre of each crop, that is, where it is possible to make such estimates. In addition, due to concern that these impurities are likely to be found in other pesticides of a similar chemical structure, RCB has also been requested to determine which compounds are known to contain TCDD-related impurities. If these appear significant in concentration, then OPP will evaluate whether or not bioassay data should be generated, possibly through the National Toxicology Testing Program (NTP) <J.Rowe>.

It was recommended that the DERs prepared by Dr. Rowe be sent to CAG for consideration in their weight of evidence determination (CAG has received the DERs and is currently evaluating the OPP conclusions). It was also recommended that CAG should be asked for assistance in the future (e.g., in the preparation of Position Documents for the SAP) since the comprehensive review of linuron was performed by CAG.

*EPA Guidelines for Carcinogen Risk Assessment, 1986 FR51: 33992-34003.