BB-133
TXR-7525

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

007525

OCT - 3 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Hormonal Studies of Express® (EPA 352-514) in Female Rats (Tox. Chem. No. 419S; Tox. Proj. No. 9-1992).

TO: L. Schnaubelt, Acting Product Manager #23
Registration Division (H7505C)

FROM: Roger Gardner, Toxicologist
Review Section I
Toxicology Branch 1 Roger Gardner 9-21-89
(Insecticide and Rodenticide Support)
Health Effects Division (H7509C)

THRU: Edwin R. Budd, Section Head
Review Section I
Toxicology Branch 1
(Insecticide and Rodenticide Support)
Health Effects Division (H7509C)
Budd 10/2/89

Actions Requested

Review of a special 90-day feeding study in female rats designed to investigate the potential estrogenic effects of Express®.

Recommendations and Conclusions

The estrogenic effects of Express® were observed at a dose level well above the highest dose tested in the chronic feeding study (1000 ppm), and any of the hormonal effects of the herbicide have not been characterized at dose levels that are not excessively toxic.

I. Background

Express® is a new chemical that is proposed for use as a herbicide on wheat and barley. The pesticide is also known as DPX-L5300, and its chemical name is benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester.

1 7/6

On December 14, 1988, the Health Effects Division Peer Review Committee classified Express® into Group C (Possible Human Carcinogen) based on a weight-of-the-evidence analysis as follows:[1]

1. A dose-related increased incidence of malignant tumors (mammary gland adenocarcinomas) in female Sprague-Dawley strain rats was observed.

2. The increased tumor incidence exceeded the historical control range.

3. There is a possible structure-activity relationship to Atrazine which also causes mammary tumors in female rats.

The Committee concluded that a quantitative risk assessment for Express® was inappropriate, and they noted that a final decision depended upon the results of further required testing to evaluate the role of possible Express®-induced hormonal alterations in the oncogenic process.

On May 9, 1989, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) considered the Peer Review Committee's weight-of-evidence analysis and classification of Express®. The Panel concluded that the herbicide should be placed into Category D (inadequate evidence) because its oncogenicity was observed only at dose levels exceeding the Maximum Tolerated Dose (MTD). Because of this experimental condition, the Panel noted that a study of the hormonal effects of Express® would be of little relevance to low dose risk. In addition, the Panel stated that the absence of oncogenicity in male rats and mice of both sexes as well as the lack of positive genetic toxicology also support a Category D classification for Express®.

On June 1, 1989, the Committee considered the Scientific Advisory Panel's conclusions and upheld the classification of Express® as a Group C oncogen.[2] The Committee also concluded that the oncogenic response observed may be associated with a hormonal imbalance that may not occur at doses below an MTD.

A quantitative risk assessment for Express® was not considered appropriate because:

1. the increased incidence of mammary gland tumors was observed in female rats treated at dose levels exceeding the Maximum Tolerated Dose (MTD);

---

1 Dearfield, K. L. Memorandum dated April 7, 1989. Subject: Peer Review of Express®. To: Richard Mountfort, Product Manager #23, Registration Division (H7509C).

2 Gardner, R. Memorandum dated July 14, 1989. Second Peer Review of Express® - Re-evaluation Following the May 9, 1989 Science Advisory Panel Review. To: Richard Mountfort, Product Manager #23, Registration Division (TS-767C).

2. there was no evidence of genetic toxicity shown in several studies;

3. and structural analogs of Express® (other than Atrazine) were not associated with oncogenic responses in rats and mice.

II. Discussion of New Data

In a special subchronic feeding study to evaluate hormonal effects of Express® (MRID No. 41181901; see Data Evaluation Record in the Appendix below), diets containing 0 or 5000 ppm of the herbicide were fed to female SpragueDawley strain rats for 90 days. The test substance significantly reduced body weight gain (40% decrease in treated rats below controls) and food consumption (27% decrease in treated rats below controls). Express® also increased mean uterus- and ovary-to-body weight ratios. Cell proliferation was increased in the uterus of treated rats, and the incidence of prolonged estrus for treated animals was observed during the 90-day feeding period. Serum prolactin from rats sacrificed in estrus was increased almost twofold (not a statistically significant increase), and mammary progesterone receptor number was increased in rats given the diet containing Express®. A two- to three-fold decrease in uterus and mammary estrogen receptor affinity was also observed in the treated rats.

An in vitro experiment with uterus cytosol suggested that Express® and seven of its metabolites may be agonists for the estrogen receptor.

All of these results suggest that Express® may have weakly estrogenic activity in female rats.

APPENDIX

Data Evaluation Record for

MRID No. 41181901 Cook, J. C. March 16, 1989. Ninety-Day Feeding Study with IN L5300-20: Effect on Estrus Cycle. Unpublished report no. 8435-001 (Haskell Research Lab. Report No. 112-89). prepared by Haskell Laboratory for Toxicology and Industrial Medicine. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE.

007525

Reviewed by: Roger Gardner Roger Gardner 8-31-89
Section 1, Toxicology Branch 1
Insecticides and Rodenticides Support (H7509C)
Secondary Reviewer: Sidney Hollenberg 9/1/89

DATA EVALUATION RECORD

STUDY TYPE: Supplement to chronic feeding/oncogenicity (Guideline §83-5)

MRID NUMBER: 402455-11 supplement

TEST MATERIAL: Technical grade INL-5300 with a stated purity of 96.8% was used.

SYNONYMS: Express Herbicide; benzoic acid, 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl]amino]sulfonyl]-, methyl ester

STUDY NUMBER(S): 8435-001 (Haskell Research Lab. Report No. 112-89)

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine

TITLE OF REPORT: Ninety-Day Feeding Study with IN L5300-20: Effect on Estrus Cycle.

AUTHOR(S): Cook, J. C.

REPORT ISSUED: March 16, 1989.

CONCLUSIONS: Diets containing 0 or 5000 ppm Express® were fed to female Sprague-Dawley strain rats for 90 days. The test substance significantly reduced body weight gain (40% decrease in treated rats below controls) and food consumption (27% decrease in treated rats below controls). Express® also increased mean uterus- and ovary-to-body weight ratios. Cell proliferation was increased in the uterus of treated rats, and the incidence of prolonged estrus for treated animals was observed during the 90-day feeding period. Serum prolactin from rats sacrificed in estrus was increased almost 2-fold (not a statistically significant increase), and mammary progesterone receptor number was increased in rats given the diet containing Express®. A two- to three-fold decrease in uterus and mammary estrogen receptor affinity was also observed in the 5000-ppm dose group rats.

An *in vitro* experiment with uterus cytosol suggested that Express® and seven metabolites may be agonists for the estrogen receptor.

These results suggest that Express® may have weakly estrogenic activity in female rats.

Core classification: Supplementary (special study)

007525

§83-5
Supplemental Study

I. PROTOCOL

A. MATERIALS

1. Test species: Female 22-day-old Charles River Crl:CD®BR strain rats were used. Their weights ranged from 37.3 to 62.3 g. The animals were placed on test diets 10 days after receipt at the laboratory.

2. Diet preparation: Basal diet consisted of Purina Lab Chow #5002, and the test substance was added in appropriate concentrations. Test diets were prepared biweekly and stored under refrigeration. Samples of test diets were analyzed for stability, homogeneity and accuracy of test concentration at the beginning of the study, and on test days 49 and 89 feed samples were analyzed for accuracy of test substance concentration.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups No.	Designation	Dose (ppm) *	Animals per group
1	Control	0	20
2	Treated	5,000	20

* Diets were fed to animals for 90 days.

2. Observations schedule

Type of observation	Number of animals per group	Frequency
Mortality	All	At least once daily
Signs of toxicity	All	At least once daily *
Body weight	All	Once each week
Food consumption	All	For all weighing intervals during the study.**
Estrous cycle	All	Monitored daily from study days 27 or 28 to termination.

* Each rat was individually handled at least once each week during the study. Changes in appearance and behavior were noted.

** Beginning on study day 81, 3 rats from each group were sacrificed each day until test day 95. The investigators noted that an effort was made to sacrifice 9 animals from each group during estrus and 8 per group during diestrus in order to observe hormonal changes during the estrous cycle.

2. Observations schedule (continued)

Type of observation	Number of animals per group	Frequency
Blood samples	17	At necropsy
Cell proliferation	3	At day 80, osmotic minipumps loaded with [^{3}H]-thymidine were implanted (on day of estrus). These animals were sacrificed 7 days later.
Hormone and hormone receptors	17	At 90 days **

** Beginning on study day 81, 3 rats from each group were sacrificed each day until test day 95. The investigators noted that an effort was made to sacrifice 9 animals from each group during estrus and 8 per group during diestrus in order to observe hormonal changes during the estrous cycle.

C. METHODS

1. Observation of estrous cycle: Vaginal washes were conducted with sterile saline, and samples were placed on ring slides and evaluated under a phase contrast microscope. The report described the criteria for observation and interpretation of observations as follows:

> The rat estrous cycle is 4-5 days in length and can be divided into four stages; proestrus, estrus, metestrus, and diestrus. Proestrus occurs for approximately 12 hours and is characterized by a vaginal smear containing epithelial cells. Estrus also has a 12 hour duration and is characterized by a vaginal smear containing cornified cells. Metestrus occurs for approximately 21 hours and is characterized by a vaginal smear containing cornified cells and leukocytes. Diestrus has a duration of approximately 57 hours and is characterized by a vaginal smear containing primarily leukocytes with some epithelial cells.
>
> The individual estrous cycle length was evaluated by counting the number of days that followed the day judged to be estrus and includes the following day judged to be estrus. A prolonged estrus was defined by consecutive day(s) following the day judged to be estrus where the vaginal smear contained primarily cornified cells. In the evaluation of each rat's cycle, the days were identified which had only cornified cells present in the vaginal smear. After identifying these days, the other cells types were evaluated to see if a 4- to 5-day cycle could be identified. For instance, adjustments in the day judged to be estrus were made based upon the cell types that proceeded (sic) and followed the

1. Observation of estrous cycle (continued)

days containing cornified cells. Such an adjustment reflected whether the individual rat had a predominantly four- or five-day cycle. The evaluation of the estrous cycle by vaginal smears required interpretation and, hence, had some element of subjectivity, but these were the general guidelines used by the investigator. The total days of monitoring, mean days of individual monitoring, mean cycle length, total and mean number of cycles, number of cycles with prolonged estrus, and number of rats experiencing a prolonged estrus were determined.

2. Necropsy: Three animals from each group were selected at study day 80 for cellular proliferation studies (see "Observation Schedule" above and "Observation of cell proliferation" below).

The remaining 17 animals in each group were sacrificed as described in the observation schedule listed above. The report also noted that after sacrifice by decapitation, blood was collected from the trunk of these animals for hormone measurements. The body, uterus, ovaries, and mammary glands were also weighed at the time of sacrifice, the number of corpora lutea and follicles were counted, and the liver, uterus, and ovaries were frozen at -85° C for use in future hormone receptor assays (mammary gland and uterus only; see "Hormone receptor observations" below).

a. Observation of cell proliferation: The report noted that on study day 80 three animals from each group were selected for this portion of the experiment. These animals had an osmotic minipump loaded with 1 mCi [^{3}H]-thymidine implanted, and monitoring of their estrous cycles was continued. Seven days after implantation of the minipumps, the animals were sacrificed and the inguinal mammary glands, uterus, ovaries and liver were fixed for further examination. Slides of these tissues were prepared for autoradiography by dipping in Kodak NTB-2 emulsion, storage at -70° C for four weeks. and development in Kodak D-19 developer. The developed slides were stained with hematoxylin and eosin for examination.

Only the mammary tissue was examined microscopically for cell proliferation. The report described the procedure used as follows:

The terminal end buds of the mammary gland were evaluated for cell proliferation by the number of cells in S-phase. Cells were deemed to be in S-phase if the number of grains overlying the nucleus exceeded the background discriminator. The background was determined by counting the number of grains in an equivalent area of cytoplasm in a representative region of a slide. The background discriminator was chosen before scoring began, was determined to be five, and was used as the cut-off value for all sections of mammary tissue. Approximately 500 cells in each region were counted

a. Observation of cell proliferation (continued)

where the number of cells in S-phase was expressed as a percentage of the number of cells counted. This percentage is termed the labeling index (LI).

b. Hormone assays: Radioimmunoassay (RIA) techniques were used to determine serum levels of estradiol, progesterone, prolactin, and insulin. Serum was either extracted or extracted and chromatographed before RIA's were done for estradiol and progesterone. Sensitivity, inter- and intra-assay Standard Error Mean % (SEM%) for the assays were described in the report as follows:

Hormone	Sensitivity	SEM% Inter-assay	SEM% Intra-assay
estradiol	6.0 pg/ml	13.0	4.0
progesterone	0.1 ng/ml	15.4	6.4
prolactin	1.6 ng/ml	8.0	2.3
insulin	2.0 uU/ml	9.9	4.8

c. Hormone receptor observations: These methods were described in the report as follows:

For analysis of the estrogen and progesterone receptors, cytosol was prepared in the following manner from the uterus and mammary gland. The uterus was minced with scisors and the mammary gland ground in a morter and pestle cooled with liquid nitrogen. The uterus and mammary tissue were suspended in 0.1 and 1.0 g/ml buffer, respectively. The buffer was composed of the following: 25 mM Hepes, pH 7.4, 1.5 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol, and 20 mM sodium molybdate (HDGM). The tissue was homogenized...All procedures were performed at 4° C unless otherwise stated. The homogenate was centrifuged at 10,000 X g for 15 min. The supernatant was removed and centrifuged at 100,000 X g for 90 min. After the last centrifugation the cytosol was removed using a pipette, without disturbing the microsomal pellet, divided into aliquots, and stored at -85° C. The cytosolic protein concentration was determined using Biorad protein reagent and BSA fraction V as the standard.

Both the estrogen and progesterone assays utilized the dextran-coated charcoal (DCC) adsorption procedure with an assay volume of 0.2 ml cytosol. The protein concentration was targeted at 1 mg/ml for the uterus and 2.5 mg/ml for the mammary tissue. Cytosol was incubated with labeled ligand in the presence (non-specific binding) or absence (total binding) of a 250-fold excess of unlabeled ligand. The unlabeled ligand for the estrogen receptor assay was diethylstilbestrol and the unlabeled ligand for the progesterone receptor assay was R5020. Each ligand was dissolved/diluted in ethanol and added in 5 ul aliquots to

c. Hormone receptor observations (continued)

silanized...test tubes. Cytosol was incubated with ligand for 3 hours at 4° C. Incubations were ended with the addition of 0.2 ml of a DCC solution followed 10 min later by centrifugation (500 X g, 10 min, 4° C) to pellet the DCC. Two-hundred microliter aliquots of DCC-treated cytosol were counted in a liquid scintilation counter. The DCC solution was made by mixing 1 g charcoal (1%) with 0.05 g dextran (0.05%) per 100 ml HEDGM buffer.

A Schatchard plot was used, where the bound/free steroid is plotted versus the bound steroid. The negative reciprocal of the line is the apparent dissociation constant (Kd) and is expressed in nM. The xintercept is a measure of the total receptor number (n) expressed in fmol per mg cytosolic protein...

The ability of IN L5300-20 and its metabolites to compete, in vitro, for binding to the estrogen and progesterone receptors was investigated by incubating uterine cytosol with either 0.5 nM [^{3}H]estradiol or 0.5 nM [^{3}H]R5020 in the presence (non-specific binding) or absence (total binding) of unlabeled ligand. Diethylstibestrol was defined as producing 100% displacement from the estrogen receptor where percentage displacement was calculated by the following:

$$100 \times \frac{\text{(Total binding of [}^3\text{H]estradiol) - (Non-specific binding of [}^3\text{H]estradiol + Test Compound)}}{\text{(Total binding of [}^3\text{H]estradiol) - (Non-specific binding of [}^3\text{H]estradiol + Diethylstilbestrol)}}$$

R5020 was defined as producing 100% displacement from the progesterone receptor where percentage displacement was calculated as follows:

$$100 \times \frac{\text{(Total binding of [}^3\text{H]R5020) - (Non-specific binding of [}^3\text{H]R5020 + Test Compound)}}{\text{(Total binding of [}^3\text{H]R5020) - (Non-specific binding of [}^3\text{H]R5020 + R5020)}}$$

D. Statistical Analyses:

The report described the statistical analyses used in the study as follows:

Body weights, body weight gains, organ weights, corpora lutea and follicle number, and serum hormone measurements 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 treated and control groups were made with the Least Significant Difference and/or Dunnett's test. The incidence of clinical observations

D. Statistical Analyses (continued)

and estrous cycle data were evaluated by the Fisher's Exact test. Significance was judged at alpha = 0.05.

II. REPORTED RESULTS

A. Mortality and Signs of Toxicity: No treatment-related clinical signs or mortalities were observed according to the report.

B. Body Weight and Food Consumption: Results for body weight, body weight gain, food consumption, and food efficiency can be summarized from the report as follows:

Observation *	Dose group 0 ppm	5000 ppm	% Difference
Mean body weight (g)	302.9	224.5 †	-25.9
Mean body weight gain (g)**	194.9	117.7 †	-39.6
Mean daily food consumption (g)**	20.1	14.7	-26.9
Mean food efficiency ** ††	0.116	0.095	-18.1

* At day 84 of the study.
** For study days 0 to 84.
† Statistically significantly different from control, $p < 0.05$.
†† Calculated as the amount of body weight gain/amount of food consumed.

C. Test substance intake: According to the report, dietary analyses indicated that test substance concentrations were 95 to 99% of the nominal concentrations, and homogeneity tests indicated a ±2% variation in concentration. Based on results of these analyses, body weight and food consumption measurements, the daily intake of test substance was calculated to be 390 mg/kg/day during the entire study.

D. Estrous cycle: These results were described in the report as follows;

> ...Although not statistically significant, the individual cycle length was longer in the rats receiving diets containing IN L5300-20 (4.4 vs. 4.2 days in the controls). This finding is consistent with the total (253 vs. 263 for the control) and mean number (12.6 vs 13.2 for the control) of estrous cycles being less in the treated rats. The number of cycles with a prolonged estrus was greater in the treated rats (11.5% vs. 2.7% for the control). The number of rats experiencing a prolonged estrous cycle was also greater in the treated rats (70% vs. 30% for the control). The number of rats experiencing two or more prolonged estrus cycles was more pronounced in the treated group (45% vs. 5% for the control)...

E. Necropsy

1. Organ weight: Group mean organ weights are summarized from the report as follows:

Organ	Absolute weight (g) 0 ppm	Absolute weight (g) 5000 ppm	Relative organ weight 0 ppm	Relative organ weight 5000 ppm
Animals sacrificed in estrus				
Final body weight (g)	307.7	219.0*	---	---
Liver	10.086	9.704	3.274	4.426 *
Uterus	0.449	0.422	0.147	0.193 *
Ovaries, total	0.108	0.098	0.035	0.045 *
Mammary tissue	3.219	1.141	1.013	0.520 *
Animals sacrificed in diestrus				
Final body weight (g)	308.3	228.7*	---	---
Liver	10.631	10.365	3.449	4.525 *
Uterus	0.448	0.327	0.114	0.143 *
Ovaries, total	0.107	0.098	0.035	0.043
Mammary tissue	2.341	1.046	0.760	0.458 *

* Statistically significantly different from controls, $p < 0.05$.

2. Corpora lutea and follicle number: According to the report, there was no difference in the numbers of corpora lutea or ovarian follicles in the control and treated groups for rats sacrificed in estrus or diestrus.

3. Serum hormone levels: Data were presented for both control and treated group rats killed during estrus or diestrus. There were no effects observed on estradiol or progesterone levels or on the estrogen/progesterone ratio in rats killed at either stage of the cycle. There was nearly a two-fold increase in serum prolactin levels reported in treated rats sacrificed during estrus (9.0 ng/ml in control rats compared with 17.2 ng/ml in the treated rats), but the difference was not statistically significant because of large standard deviations for group mean values.

Insulin levels were decreased in rats sacrificed at both estrus (16.0 mU/ml in control rats vs. 10.4 mU/ml in treated rats) and diestrus (16.7 mU/ml in control animals compared with 11.2 mU/ml in treated rats). Only the difference between control and treated insulin levels in rats killed during estrus was statistically significant ($p<0.05$). These results are limited because insufficient blood samples were available for insulin determinations. Samples from only 6 control and 3 treated animals killed during estrus and 3 control and 2 treated animals sacrificed during diestrus were used. Although the investigators described the apparent effect as tenuous, it was a consistent observation in rats sacrificed during either stage of the cycle.

4. Hormone receptor observations: The report noted a two-fold increase in the dissociation constant (Kd) for estrogen receptors in the uterus of treated rats. These results indicate a decreased estrogen-binding afinity for that tissue in treated rats. There was no effect on progesterone receptor afinity or the numbers of estrogen and progesterone receptors in the uterus of treated rats.

Results for the mammary gland were described in the report as follows:

> ...the Kd for the estrogen receptor was 2.9- and 2.5-fold greater in the IN L5300-20-treated rats sacrificed in estrus and diestrus, respectively, when compared to the controls. For the mammary progesterone receptor from rats sacrificed in estrus, the receptor number was approximately 2-fold greater in the IN L5300-20-treated rats while the Kd was unaffected...

The report indicated that Scatchard plots for the progesterone receptor analyses from animals sacrificed at diestrus were nonlinear, and Kd values could not be determined. Efforts were made to determine the reason for nonlinearity of the plots, but none was found. Experimental error was ruled out by testing the reagents involved in the assay and by increasing the concentrations of protein in the cytosol containing the receptors.

5. Cell proliferation observations: The report noted that there was a high background of radioactivity in the uterus (high labeling index) that interfered with quantitative measurements of cell proliferation in that tissue. The report described the qualitative differences between control and treated uterine tissue samples by stating, "The amount and intensity of labeling in the uterus from IN L5300-20-treated rats is greater than the respective control which is consistent with the finding that the mean relative uterine weight is greater in the treated animals." Representative photomicrographs from the control and treated groups were included to support investigators' conclusion that cell proliferation was increased in the uterus of treated rats.

In mammary tissue from treated rats a 2.8-fold decrease below control group values in the labeling index was reported (the difference was not statistically significant). The investigators concluded that the decreased cell proliferation in the mammary glands of treated rats was associated with the significant decreases in body weight.

6. In vitro competition for receptors: The report indicated that there was competition for estrogen receptors in vitro for seven metabolites of IN L5300-20, but no competition was observed for progesterone receptors.

Results of these experiments are summarized in the report as follows:

6. In vitro competition for estrogen and progesterone receptors: The report indicated that there seven metabolites of Express® capable of competing for binding to estrogen receptors. No competition was observed for binding to progesterone receptors. Results of these experiments are summarized as follows:

Test Compound (final incubation concentration)	% Displacement of [^{3}H]estradiol	% Displacement of [^{3}H]R5020
Diethylstilbestrol (0.125 mM)	100	---
R5020 (0.125 mM)	---	100
IN L5300 (1.0 mM)	3	0
IN L5300 acid (1.0 mM)	15*	3
Triazine amine (1.0 mM)	0	4
N-Demethyl triazine amine (1.0 mM)	9*	0
O-Demethyl triazine amine (1.0 mM)	0	1
alpha-Hydroxy triazine amine (1.0 mM)	25*	1
N-Demethyl-6-hydrosymethyl triazine amine (1.0 mM)	6*	0
Metsulfuron methyl (1.0 mM)	76*	0
Hydroxylated metsulfuron methyl (1.0 mM)	2	0
Sulfonamide (1.0 mM)	0	3
Acid sulfonamide (1.0 mM)	0	0
Sulfonamide urea (1.0 mM)	80*	0
Saccharin (1.0 mM)	0	0
Hydroxylated saccharin (1.0 mM)	72*	0

* Statistically significantly different from control, $p < 0.05$.

III. DISCUSSION

A. Investigators' Conclusions:

IN L5300-20 appears to alter the endocrine system of female rats based on the following: 1) increased mean relative uterine weight accompanied with a qualitative increase in cell proliferation; 2) increased mean relative ovarian weight; 3) increased incidence of prolonged estrus; 4) two-fold increase in serum prolactin from rats sacrificed in estrus, although not statistically significant; 5) two- to three-fold decrease in uterus (from rats sacrificed in estrus) and mammary estrogen receptor affinity; and 6) two-fold increase in mammary progesterone receptor number. These findings are all consistent with the demonstration that seven IN L5300-20 metabolites appear to be agonists for the estrogen receptor based on in vitro competition studies.

A. Investigators' Conclusions (continued)

> ...estrogens increase uterine weight and produce a persistent estrus which is consistent with the increased mean uterine weight and increased incidence of prolonged estrus observed in the IN L5300-20-treated rats. Although the 2-fold increase in serum prolactin was not statistically significant, compounds with estrogenic activity...increase serum prolactin levels. The lower affinity estrogen receptor in the uterus and mammary tissue could occur from occupation of the high affinity receptor sites by IN L5300-20 metabolites that appear to be agonists for the estrogen receptor. Estrogens ...elevate the level of cytoplasmic the progesterone receptor which is consistent with the (observed) elevation in mammary progesterone receptor number. Taken together, these data strongly suggest that the seven metabolites of IN L5300-20 possess estrogenic activity..., their action, in vivo, is subtle since there were no differences in follicle or corpora lutea number and a persistant estrus was not observed.

B. Reviewer's discussion: There are points discussed by the investigators regarding the influence of reduced food intake on serum hormone levels in rats. These comments are exerpted from the original report as follows:

> A recent paper has shown that a 30% decrease in body weight due to chronic reduction in food intake, increases the sensitivity of the hypothalamus and/or pituitary to the suppression of luteinizing hormone secretion by estradiol. Whether this enhanced sensitivity lowers the serum levels of estrogen in a chronically underfed rat was not investigated. Hence, in the absence of a pair-fed control, the serum hormone data are difficult to interpret since possible increases in serum hormone levels by IN L5300-20-treatment could be masked by the body weight losses. (from page 31 of the original report)

In view of these comments and results of body weight and food consumption observations from previously conducted 90-day and chronic feeding studies in rats, a concurrent "pair-fed" control group would be useful in the interpretation of the serum hormone results obtained.

Additional comments by the investigators about the effects of estrogenic compounds on many of the parameters used in the IN L5300-20 study (see previous section above) suggest that a concurrent "positive control" group treated with a test substance having estrogenic activity would also be useful to an interpretation of the results of the in vivo experiments.

B. Reviewer's discussion (continued)

The results described in the report suggest that the test substance and its metabolites have weak estrogenic activity in the female rat. As a weak estrogen, the test compound may also be anti-estrogenic by binding to estrogen receptors in the uterus or mammary gland and inhibiting the binding of circulating estradiol to those receptors. Whether IN-L5300-20 has estrogenic or anti-estrogenic activity could be resolved by testing for both types of activity in rat uterotropic assays. Comparisons could be made between the herbicide and a known estrogen such as estradiol and a known anti-estrogen such as clomiphene or tomoxifen.

The weak estrogenic activity exhibited by IN-L5300-20 in the experiments described above is consistent with the hypothesis that the increased incidence of mammary gland tumors in female rats is a result of the hormonal effects of the test compound. Further studies are needed to determine the nature of the hormonal effects with respect to tumor induction in the rat.