KB-183
TXR-6F33

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

AUG 25 1988

006833

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO. Richard Mountfort, Product Manager #23
Registration Division (TS-767)

FROM: Roger Gardner, Toxicologist Roger Gardner 8-23-88
Health Effects Division (TS-769)

THRU: Judith Hauswirth, Ph. D., Acting Chief
Insecticides and Rodenticides Branch
Health Effects Division (TS-769)

SUBJECT: Application for Registration of Express® Herbicide (EPA Reg. No. 352-LNO) and Petition for Tolerances in/on Wheat and Barley (Petition No. 7F3540). Tox. Chem. Nos. 419S and 550A; Tox. Proj. No. 7-0828).

Actions Requested

1. Review of reports listed in Appendices II. and III. below.

2. Tolerances on wheat and barley grain (0.05 ppm) and straw (0.1 ppm).

Recommendations and Conclusions

1. Recommendation on the requested tolerances, registration and data gaps are reserved until a Peer Review of the evidence regarding the oncogenic potential of Express® has been completed (see point 7. below).

2a. Technical grade DPX-L5300 is classified into Toxicity Category III for acute inhalation and dermal toxicity and Category IV for acute oral toxicity (see Section I. B. 1. a. below).

2b. Limited irritation and sensitization studies on the technical grade material (see Section I. B. 1. a. below) and available data on the 75% DF formulation (see point 3. below) suggest that Express® is slightly irritating to the eyes and skin (Toxicity Category IV), and the herbicide is not a skin sensitizer.

1 of 15

3. The 75% DF formulation is classified into Toxicity Category III with respect to acute oral and dermal toxicity. No acute inhalation toxicity study is needed because <0.5% of the granules are less than 105 um in diameter, and the formulation is not respirable. The formulation is a moderate eye irritant (Toxicity Category III) and causes no skin irritation or skin sensitization.

4. Toxic effects observed in a 90-day rat feeding study included decreases in food consumption, body weight gain, food efficiency, and absolute weights for the heart, brain, liver, and kidneys. Relative organ weights for the heart, liver, kidneys, testes, and spleen were increased because of the decreased body weights observed. Serum glucose, globulin, and cholesterol concentrations were also decreased, but there were no treatment-related histopathological effects. The LOEL is 1750 ppm (highest dose tested), and the NOEL is 100 ppm.

5. The NOEL is probably >2500 ppm (highest dose tested) in dogs based on results from a 90-day feeding study.

6. A NOEL was established at 25 ppm (0.625 mg/kg/day), and the LEL was 250 ppm in a one-year dog study based on elevated blood levels of bilirubin and aspartate aminotransferase (AST), increased urinary volume and decreased body weight gain in males, and elevated bilirubin, AST, creatinine and globulin levels along with decreased body weight gain in females. The highest dose tested was 1500 ppm.

7. Based on reduced body weight and body weight gain in treated male and female rats, a NOEL was established in the chronic feeding study at 25 ppm (1.25 mg/kg/day). There was also a statistically significantly increased incidence of mammary gland adenocarcinomas observed in treated female rats. The highest dose tested was 1250 ppm.

8. Based on the increased incidence of seminiferous degeneration and oligospermia in mice from a supplementary long-term feeding study, the suggested NOEL was 20 ppm (3 mg/kg/day), and the LEL was 200 ppm (30 mg/kg/day). Under the limited conditions of the study, Express® was not oncogenic (see Section II. C. 2. below).

9a. Maternal toxicity in a rat teratology study at 125 mg/kg and higher included: decreased body weight gain and food consumption, increased liver-to-body weight ratios, and excess salivation in some animals. Fetuses from dams given toxic doses of 500 or 125 mg/kg had reduced body weights. Increased resorptions, fetal deaths, and incomplete ossification were observed at the 500 mg/kg dose (highest dose tested). These results indicate that the NOEL for maternal and developmental toxicity was 20 mg/kg/day, and the LOEL is 125 mg/kg/day.

9b. A developmental toxicity study in rabbits indicated that the NOEL for maternal toxicity was 20 mg/kg/day based on statistically significantly decreased feed consumption and increased incidence of abortions. The LEL for maternal toxicity was 80 mg/kg/day (highest dose tested). The LEL for fetal effects (reduced fetal weight) was also 80 mg/kg/day, and the NOEL is 20 mg/kg/day. There were no fetal malformations or variations associated with administration of the test substance in pregnant rabbits.

10. In a two-generation reproduction study, no effects were seen on fertility, gestation, or lactation at dietary levels as high as 1000 ppm (highest dose tested). Effects associated with Express® included reduced group mean body weight for the adult females and offspring and reduced spleen weight in the second litter of the final generation. The NOEL was established at 25 ppm, and the LEL was 250 ppm.

11a. No mutagenic activity was observed in Chinese Hamster Ovary cells in vitro; no cytogenetic effects were seen in bone marrow cells from treated rats; no induction of micronuclei were found in normochromatocytes from treated mice; and no unscheduled DNA sysnthesis was induced in primary hepatocytes from treated rats.

11b. A reverse mutation assay in Salmonella typhimurium is unacceptable because the report was not complete (missing page).

12a. Orally administered Express® is readily absorbed by male and female rats. The excretion half-life (time required for excretion of half of the dose) for a low dose was 26 to 33 hours. At high single doses the excretion half-life for male rats was 51 to 54 hours, and that value for female rats was 69 to 96 hours. The major route of excretion in rats was the urine.

12b. Tissue levels of Express® and its metabolites increased with dose, but there was no concentration of radioactivity in any particular organ or tissue.

12c. Major metabolites in the urine and feces included metsulfuron methyl, saccharin, and O-demethyl triazine amine. There was no evidence of glucuronide of sulfate conjugation.

13a. DPX-L5296 (4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine) is a moderately persistent soil metabolite and a possible minor rat metabolite of Express®.

13b. Its acute oral LD_{50} for male and female rats is 410 mg/kg, and its dermal LD_{50} is greater than 2000 mg/kg in rats (highest dose tested). The metabolite did not cause skin irritation in male rabbits, and it caused slight eye irritation (conjunctival reactions) that reversed in 3 days in rabbits. No delayed dermal sensitization reactions were observed in guinea pigs treated with DPX-L5296.

13c. In a four-week oral toxicity study, an LEL was established at 40 mg/kg/day, and the effects associated with the test substance included reduced body weight and weight gain, decreased blood glucose levels, and reduced platelet counts. These effects were also observed in males and females given the highest dose tested (200 mg/kg/day). In addition, the high dose group females exhibited decreased spleen-to-body weight ratios and increased white blood cell counts. High dose group males and females also had decreased potassium, increased SGPT, and an increased incidence of myocardial degeneration (often associated with fibrosis) in the ventricular apex. The 200 mg/kg dose group males also had elevated total serum protein. Based on these results, the suggested NOEL is 8 mg/kg/day.

13d. DPX-L5296 did not increase the frequency of reverse mutations with or without metabolic activation in Salmonella typhimurium or cause structural or numerical chromosome aberrations in human lymphocytes in vitro.

14. Using a NOEL of 25 ppm (0.625 mg/kg/day) established in a one-year dog study and a Safety Factor of 100, a Reference Dose (RfD) of 0.006 mg/kg/day is derived. This value has not been verfied by the ADI Committee.

I. Background

A. General Information

DPX L5300 (chemical name: benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester) is proposed as a herbicide for use on wheat and barley. It is to be formulated for that purpose as a dry flowable (75% active ingredient). The inert ingredients have been cleared for food use.

The formulation, which is called Express® Herbicide, is to be applied at rates of 1/6 to 1/3 oz. active ingredient per acre according to the label, and the application is to be made between the 2-leaf and boot stages of winter or spring wheat and spring barley. The herbicide is to be mixed with water, and the mixture is to be sprayed by air (1 gal/A) or ground equipment (5 gal./A) in the spring.

The proposed tolerances are 0.05 ppm in/on wheat and barley grain and 0.1 ppm in/on wheat and barley straw.

According to a memorandum from the Residue Chemistry Branch (Worthington, October 18, 1985), DPX-L5300 is the major residue, and there are adequate data to indicate that residues will not exceed the proposed temporary tolerances which are the same as the permanent tolerances under consideration herein. Meat, milk, poultry, and egg tolerances were not requested because of a restriction imposed against feeding treated crops or forage to livestock.

B. Summary of Previously Submitted Data

Appendix I. below contains Toxicology "One-Liners" for all the toxicity studies discussed in this section.

1. Technical grade DPX L5300

a. Acute toxicity

The results of acute toxicity studies on technical DPX L5300 are summarized as follows:

006833

Route of administration	Species	Sex	LD_{50} or LC_{50}	Toxicity Category
Oral	Rat	Both	>5,000 mg/kg	IV
Inhalation	Rat	Both	>6.7 mg/L*	III
Dermal	Rabbit	Both	>2,000 mg/kg	III

*Four hour exposure.

There were indications of mild eye irritation in the washed and unwashed eyes of two rabbits after instillation of 10 mg technical grade DPX-L5300. Redness with vessels injected above normal was observed in both rabbits at the 1 and 4 hour observation periods.

Technical grade DPX-L5300 is not a dermal sensitizer.

b. Subchronic toxicity

In a three-month feeding study, groups of rats were given diets containing 0, 100, 1750, or 5000 ppm DPX L5300. The 100 ppm dietary level had no effect (NOEL). The two higher levels caused significant dose-related decreases in food consumption, body weight gain, and lower food efficiency. There were also significant decreases in absolute weights for the heart, brain, liver, and kidneys at the two highest dietary levels. Relative organ weights for the heart, liver, kidneys, testes, and spleen were increased significantly because of the decreased body weights observed. Serum glucose, globulin, and cholesterol concentrations were also decreased in the mid and high dose groups, but there were no treatment-related histopathological effects. These effects were described by the investigators as indications of cachexia. The LOEL is 1750 ppm.

In another 90-day feeding study, DPX-L5300 was given to dogs in their diet at levels of 0, 50, 500, or 2500 ppm. The highest dose tested caused no clearly treatment related effects. These results suggest a NOEL >2500 ppm in dogs.

c. Reproductive effects

As part of the subchronic feeding study in rats, a one-generation reproduction experiment was conducted. Six male and 6 female rats from each group were mated to produce the litters. Decreased pup viability and pup weights were observed in the 5000 ppm dose group. These results were consistent with the decreased body weights and generally poor condition of the dams given the highest dose level in the subchronic experiment. However, no further conclusions can be drawn from the reproduction experiment since there were only 3 to 6 dams with litters available for analysis and the experiment was carried out for only one generation.

006833

d. Developmental toxicity

Doses of 0, 20, 125, or 500 mg DPX-L5300 per kg body weight were administered to groups of pregnant rats on gestation days 6 through 16. The mid and high doses were found to cause maternal toxicity (decreased maternal body weight gain and food consumption, increased liver-to-body weight ratios at the highest dose, and excess salivation). Fetuses from dams given the two highest doses also had reduced body weights. The highest dose caused resorptions, fetal deaths, and incomplete ossification. These results indicated that the NOEL's and LOEL's for maternal and developmental toxicity are also 20 and 125 mg/kg/day, respectively.

e. Mutagenicity

No increase in the frequency of reverse mutations was observed in Salmonella typhimurium strains TA1535, TA97, TA98, and TA100 when exposed to levels as high as 500 ug/plate without metabolic activation or as much as 2000 ug/plate with metabolic activation. However, it should be noted that page 4 of the original report is missing, and there were no toxicity data presented to indicate that a sufficient dose range was tested. The study is considered unacceptable because the report is incomplete.

No mutagenic activity was observed in Chinese Hamster Ovary cells exposed in vitro to concentrations of 0.5 to 5.0 mM DPX-L5300-20 with and without activation.

Single oral doses of 50, 500, or 5000 mg DPX-L5300 per kg body weight had no effect on the incidence of chromosomal aberrations or mitotic index of bone marrow cells in male and female rats.

A single oral dose of 5000 mg DPX-L5300 per kg body weight was shown to be cytotoxic (reduced polychromatic/normochromatic erythrocyte ratio) in mice. However, that dose did not increase the incidence of polychromatic erythrocytes with micronuclei in treated mice.

Under the conditions of an in vitro unscheduled DNA sysnthesis assay, DPX-L5300 did not induce UDS in rat primary hepatocytes at concentrations of 0 to 2500 uM.

2. Toxicity data on Express® Herbicide (75% a. i.)

Acute toxicity results are summarized as follows:

Route of administration	Species	Sex	LD_{50} or LC_{50}	Toxicity Category
Oral	Rat	Female	5,700 mg/kg	IV
		Male	4,800 mg/kg	III
Dermal	Rabbit	Both	>2,000 mg/kg	III

In the listing of toxicity studies provided with a previous submission, the Registrant stated that an acute inhalation toxicity study with the

6

formulation was not conducted because <0.5% of the water-dispersible granules in the formulation are smaller than 105 um.

Most of the deaths observed in the acute oral toxicity study occurred 2 to 3 days after treatment, and some were noted as long as 9 days after dosing. Gross lesions observed at necropsy were not organ-specific. Signs of toxicity noted during post-dosing observation included staining of the face and perineum, chromodacryorrhea, and body weight loss.

An eye irritation study indicated that mean irritation scores for unwashed eyes 24, 48, and 72 hours after treatment were 14, 6, and 6, respectively. At 4 and 7 days after instillation the mean scores were 4 and 0. The results were sufficient to classify Express® Herbicide® into Toxicity Category III for eye irritation.

The Primary Irritation Score for dermal irritation was 0 which places the formulation into Toxicity Category IV.

No dermal sensitization was observed in guinea pigs.

II. Discussion

A. New Toxicology Data on Express® Herbicide

1. One-year feeding study - dogs

Groups of 5 male and 5 female beagle dogs were given diets containing 0, 25, 250, or 1500 ppm DPX-L5300 for one year. Effects associated with DPX-L5300 treatment included elevated blood levels of bilirubin and aspartate aminotransferase (AST), increased urinary volume and decreased body weight gain in males. Treated females exhibited elevated bilirubin, AST, creatinine and globulin levels along with decreased body weight gain. A no-observed-effect level was established for these effects at 25 ppm, and the LEL was 250 ppm.

2. Chronic feeding/oncogenicity study - rats

Groups of 72 male and 72 female Sprague-Dawley rats were given diets containing 0, 25, 250, or 1,250 ppm DPX-L5300 for up to 24 months.

Effects attributed to the test substance included decreased body weight and increased incidences of masses located on the shoulder, side, and under body regions in high dose group female rats. The masses were associated with the statistically significantly increased incidence of mammary gland adenocarcinomas observed in the high dose group females (see Section II. C. below). There was no significant effect on survival in treated animals.

By the end of the study, the mid and high dose group weight gains for males were 10.8 and 36.4% less than that for the control group. In female rats the mid dose group had a body weight gain that was 26.6% less than that for the control group at the end of the study, and the high dose group's weight gain was 53.8% less than controls.

Organ weights in treated animals reflected the observed decreases in body weight (i. e., significant increases in the majority of relative organ weights in the male and female rats at the 1,250 ppm dose level and female rats at the 250 ppm dose level along with statistically significantly decreased absolute organ weights).

In male rats given the high dose level, the incidence of polyarteritis in the pancreas, decreased secretion in seminal vesicles, lymphoid depletion in the spleen, and mineralization of the aorta and stomach were statistically significantly increased above control group incidences. The latter two lesions were associated with an increase in severity of glomerulonephropathy in the high dose group males. The incidence of dilatation of the renal pelvis, dilatation of the uterine horns, and retinal degeneration was statistically significantly increased in females given the highest dose level.

Based on the reduced body weights in treated male and female rats, a NOEL was established in the study at 25 ppm (1.25 mg/kg/day).

3. Oncogenicity study - mice

In a supplementary study, diets containing 0, 20, 200, or 1500 ppm Express were given to male and female Charles River Crl:CD-1(ICR) BR strain mice for 18 months.

By the end of the study the highest dose tested caused minimal effects on body weight (6 and 5% less than control group means for males and females, respectively) and body weight gain (24% and 20% less than controls for males and females, respectively) were observed. At 13 weeks, there was approximately a 10% decrease in body weight gain for the high dose group males, and female mice in that group gained the same amount of weight as the control group females.

Although mortality was not statistically significantly increased at the highest dose in male mice, it was 65% in the 1500 ppm dose group compared to 51% in the control group. The incidence of amyloidosis was statistically significantly increased in male and female mice at the highest dose level ($p < 0.01$; Fisher's Exact Test), and the incidence of bilateral seminiferous degeneration (atrophy) and oligospermia was statistically significantly increased in 200 and 1500 ppm group males. Amyloidosis was also increased in females from the 1500 ppm dose group. Thyroid inflammation was statistically significantly increased in both sexes at the highest dose.

Based on the increased incidence of bilateral seminiferous degeneration and oligospermia in mid dose group male mice, the suggested NOEL was 20 ppm (3 mg/kg/day), and the LEL was 200 ppm (30 mg/kg/day). However, the minimal body weight reductions and absence of compound-related lesions in female mice suggests that a dose range adequate for assessment of oncogenic potential of the test substance may not have been tested.

Under the limited conditions of the study, Express was not oncogenic.

4. Developmental toxicity - rabbits

Groups of 22 pregnant New Zealand White rabbits were given daily doses of 0, 5, 20, or 80 mg DPX-L5300 by gavage on gestation days 7 through 19.

Based on statistically significantly decreased feed consumption and increased incidence of abortions at the highest dose, the lowest-effect level for maternal toxicity was 80 mg/kg/day. The no-observed-effect level (NOEL) was 20 mg/kg/day. The LEL for fetal effects (10% reduction in fetal weight without statistical significance) was also 80 mg/kg/day, and the NOEL is 20 mg/kg/day. There were no fetal malformations or variations associated with administration of the test substance in pregnant rabbits.

5. Reproduction study - rats

In a two-generation reproduction study, male and female rats were given diets containing 0, 25, 250, or 1000 ppm DPX-L5300. There was no effect on fertility, gestation, or lactation, and effects associated with the test substance included reduced group mean body weight for the adult females and for offspring, reduced spleen weight in the second litter of the final generation in the study. The NOEL was established at 25 ppm, and the LEL was 250 ppm.

It should be noted that microscopic examinations of the reproductive organs of adult rats was limited to 10 adults of each sex in each group for the F_1 generation only, but results from subchronic and chronic feeding studies and the absence of effects on reproductive performance indicate that this deficiency is not sufficient to warrent classification of the study as supplementary.

6. Metabolism studies - rats

A series of limited experiments suggested that orally administered DPX-L5300 is readily absorbed by male and female rats. The excretion half-life (time required for excretion of half of the dose) for a low dose (20 mg/kg) was 26 to 33 hours. Half-life values were similar in male and female rats and in rats given repeated daily doses (100 ppm for 21 days followed by a single 20 mg/kg dose on day 22). At high single doses (1700 to 2000 mg/kg) the excretion half-life for male rats was 51 to 54 hours, and that value for female rats was 69 to 96 hours.

The major route of excretion in rats was the urine. Urine samples collected over a 168-hour period following a single 1700 mg/kg dose contained two to four times more of the administered radioactivity than the feces.

Tissue levels of DPX-L5300 and its metabolites increased with dose, but there was no concentration of radioactivity in any particular organ or tissue.

Major metabolites in the urine and feces included metsulfuron methyl, saccharin, and O-demethyl triazine amine. There was no evidence of glucuronide of sulfate conjugation.

Results from the single low dose indicated that approximately 35 to 40% of the recovered radioactivity in urine and feces samples collected during the 96 hours following dosing was associated with saccharin and approximately 15 to 20% was associated with metsulfuron methyl. Forty to 50% of the radiolabel recovered in excreta was unidentified.

The O-demethyl triazine amine was identified in the excreta of rats given the high dose, and it represented 40% of the recovered radioactivity in feces and urine from males and approximately 15% in female rats. Metsulfuron methyl accounted for approximately 20% of the radioactivity recovered during the 168 hours after the high dose in male and female rats. Approximately 25% of the radioactivity in excreta of males and 40% of that in females was not identified.

B. New Toxicology Data on DPX-L5296

DPX-L5296 is described by the Registrant (DuPont) as a moderately persistent soil metabolite of Express®. Its chemical name is 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine, and it has been identified as one of the possible minor metabolites in rats (see Section II. A. 6. above).

1. Acute toxicity

The acute oral LD_{50} for both sexes was 410 mg/kg with 95% confidence limits of 366 to 455 mg/kg. The calculated LD_{50} for males alone was reported to be 394 mg/kg with 95% confidence limits of 336 to 452 mg/kg, and those values for females were reported to be 427 and 306 to 548 mg/kg, respectively. Signs of toxicity included lethargy, decreased motor activity, hunched posture, ataxia, irregular breathing, colored ocular discharges, stained snout, and closed eyes. Survivors recovered from these signs during the first 4 days after dosing.

The results of an acute dermal toxicity study indicated that the dermal LD_{50} is greater than 2000 mg/kg in rats. No toxicity was observed in the treated animals, and 2000 mg/kg was the highest dose tested.

DPX-L5296 did not cause skin irritation in male rabbits. The metabolite caused slight eye irritation (conjunctival reactions) that reversed in 3 days in rabbits.

No delayed dermal sensitization reactions were observed in guinea pigs treated with DPX-L5296 in a Maximization test.

2. Subchronic toxicity - rats

In a four-week study, groups of male and female rats were given daily doses of 0, 8, 40, or 200 mg DPX-L5300 per kg body weight by gavage. The LEL was established at 40 mg/kg/day, and the effects associated with the test substance included reduced body weight and weight gain, decreased blood glucose levels, and reduced platelet counts. These effects were also observed in males and females given the highest dose tested. In addition, the high dose group females exhibited decreased spleen-to-body weight ratios and increased white blood cell counts. High dose group males and females also had decreased potassium, increased SGPT, and an

increased incidence of myocardial degeneration (often associated with fibrosis) in the ventricular apex. The 200 mg/kg dose group males also had elevated total serum protein. Based on these results, the suggested NOEL is 8 mg/kg/day.

3. Mutagenicity

DPX-L5296 did not increase the frequency of reverse mutations with or without metabolic activation in Salmonella typhimurium at concentrations up to 5000 ug/plate (a toxic dose level).

There were no structural or numerical chromosome aberrations induced by DPX-L5296 in human lymphocytes in vitro at concentrations up to toxic levels (100 ug/ml).

C. Oncogenic Potential of Express®

The following information is to be submitted to the Peer Review Committee for an assessment of the weight-of-the-evidence and classification of Express' oncogenic potential.

1. Chronic feeding study in Sprague-Dawley rats

a. Tumor results

As mentioned in Section II. A. above, DPX-L5300 was associated with a statistically significant increase in the incidence of malignant mammary gland tumors in female rats given diets containing 0, 25, 250, or 1250 ppm for 24 months. Mammary tumor results are summarized as follows:

Observation	Dose level (ppm) 0	25	250	1250
Mammary gland (number examined) †	60	60	57	61
adenoma	2	2	2	3
adenocarcinoma	9	9	13	26 **
fibroadenoma	16	12	12	8
Total with mammary tumors ††	24	17	20	34
Animals with tumor (any type)	58	56	52	57

† Excluding animals sacrificed at 12 months.
†† Animals having one or more of the types of tumor mentioned (animals are not counted more than once).

The time to observation of masses (median days on test) associated with mammary tumors was as follows:

Location	Dose level (ppm) 0	25	250	1250
Shoulders	614	502	552	474
Sides	404	551	502	530
Under body	530	558	502	502

The first mammary gland adenocarcinoma was microscopically diagnosed in a control group female examined at the 12-month interim sacrifice. The first of these tumors observed in the low, mid and high dose groups were diagnosed on days 574, 514, and 431, respectively. The respective median times to diagnosis of these tumors in the control, low, mid, and high dose groups were 542, 623, 592, and 578 days.

b. Toxicity

There was no significant effect on survival of treated animals in the study.

After 13 weeks of the study the high dose group male and female rats showed decreased body weight gain (approximately 21 and 34%, respectively). By the end of the study, the 250 and 1250 ppm dose group weight gains for males were 10.8 and 36.4% less than that for the control group. In female rats the 250 ppm dose group had a body weight gain that was 26.6% less than that for the control group at the end of the study, and the high dose group's weight gain was 53.8% less than controls.

In male rats given the 1250 ppm level, the incidence of polyarteritis in the pancreas, decreased secretion in seminal vesicles, lymphoid depletion in the spleen, and mineralization of the aorta and stomach were statistically significantly increased above control group incidences. The latter two lesions were associated with an increase in severity of glomerulonephropathy in the high dose group males. The incidence of dilatation of the renal pelvis, dilatation of the uterine horns, and retinal degeneration was statistically significantly increased in females given the 1250 ppm dose level.

c. Historical control information

In a supplement to the report on the rat chronic/oncogenicity study, the incidence of mammary gland tumors was compared with historical control data as follows:

> The malignant tumor incidence in the concurrent control and in the 25 and 250 ppm treatment groups were within the range of historical control data for Haskell Laboratory (1.5 to 23.4% with a mean of 12.6%; these data summarize results from 10 2-year feeding studies reported between 1980 and 1986).

The reported incidences of mammary gland adenocarcinomas in the control, low, mid, and high dose groups of the rat feeding study were 15, 15, 22.8, and 42.6%, respectively.

More detailed historical control data have been requested.

2. Oncogenicity study in Crl:CD-1 (ICR) strain mice

Diets containing 0, 20, 200, or 1500 ppm Express were given to male and female Charles River Crl:CD-1(ICR) BR strain mice for 18 months.

At 13 weeks, there was approximately a 10% decrease in body weight gain for the high dose group males, and the female mice in that group gained the same amount of weight as the control group. By the end of the study the highest dose tested was associated with minimal effects on body weight (6 and 5% less than control group means for males and females, respectively) and body weight gain (24% and 20% less than controls for males and females, respectively) were observed.

Although mortality was not statistically significantly increased at the highest dose in male mice, it was 65% in the 1500 ppm dose group compared to 51% in the control group. The incidence of amyloidosis was statistically significantly increased in male and female mice at the highest dose level ($p < 0.01$; Fisher's Exact Test), and the incidence of bilateral seminiferous degeneration (atrophy) and oligospermia was statistically significantly increased in 200 and 1500 ppm group males. Amyloidosis was also increased in females from the 1500 ppm dose group. Thyroid inflammation was statistically significantly increased in both sexes at the highest dose.

Based on the increased incidence of bilateral seminiferous degeneration and oligospermia in mid dose group male mice, the suggested NOEL was 20 ppm (3 mg/kg/day), and the LEL was 200 ppm (30 mg/kg/day).

Body weight results, mortality late in the study, and the incidence of age-related effects suggested that adequate dose levels for assessment of the oncogenic potential of Express® in male mice were used. However, results from female mice in the highest dosed group suggest that an adequate dose range was not tested.

Under the conditions of the study, Express was not oncogenic.

3. Mutagenicity

Express is not mutagenic in Chinese Hamster Ovary cells in vitro, and it did not induce chromosomal aberrations in rat bone marrow cells in vivo. Cytotoxic doses of the herbicide did not induce micronuclei in mice. Express also failed to induce unscheduled DNA synthesis in primary rat hepatocytes in vitro.

4. Structure-activity relationships

Express® is structurally similar to other herbicides including Londex, Harmony, and Beacon. The first two chemicals were not found to be oncogenic in rats or mice, and the third has been associated with an increased incidence of masses in the liver of mice (microscopic examinations have not been completed).

D. Data Gaps

As mentioned in section I. B. 1. e. above, page 4 of the original report on a mutagenicity study with Salmonella typhimurium is missing, and there were no toxicity data presented to indicate that a sufficient dose range was testei. The missing page is needed to complete an assessment of the study and its results.

Additional detailed historical control data for the rat are needed for the assessment of the significance of the mammary gland tumors observed. Those data should contain individual animal results and summaries showing the incidences of each type of mammary tumor (adenoma, adenocarcinoma, and fibroadenoma) as well as the time of diagnosis.

E. Reference Dose (ADI)

Using a safety factor of 100 and the NOEL of 25 ppm (6.25 mg/kg/day) established by the one-year dog feeding study, the Acceptable Daily Intake (ADI) is calculated as follows:

$$\frac{6.25 \text{ mg/kg/day}}{100} = 0.0625 \text{ mg/kg/day}$$

This ADI has not been verified.

III. References

Worthington, J. M. Memorandum dated October 18, 1985. To: R. Mountfort, PM No. 23. Registration Division. Subject: PP #5G3296. DPX-L5300 on wheat and barley. Comments on the analytical method and residue data. Accession Numbers 073785 and 073786. RCB #1427 and 1428.

006833

APPENDIX I

Toxicology Branch "One-Liners" for
Express (Tox. Chem. No. 419S)

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Reproduction-1 generation Species: rat Haskell Lab 413-83; 6/6/85	DPX-L5300 tech 96.8% ai	073790	Developmental NOEL = 2500 ppm, Developmental LEL = 5000 ppm (decrease pup viability and weight gain) . No final conclusion can be drawn because there were only 3 to 6 dams with liters available for analysis. Adult toxicity NOEL and LEL (see feeding phase of this study, Report no. 413-85, below) Doses tested: 0, 100, 2500, & 5000 ppm in the diet		Supplementary 004943
Teratology Species: rat Argus Research Labs HLO-513-85; 8/16/85	DPX-L5300 tech 96.8% ai	073790	Maternal NOEL = 20 mg/kg, Maternal LEL = 125 mg/kg (decreased body weight gain and food consumption, increased liver to body weight ratio), Developmental NOEL = 20 mg/kg, Developmental LEL = 125 mg/kg decreased body weight, at the HDT increased resorptions and fetal deaths, incomplete ossification. A/D ratio = 125/125 = 1. Doses tested = 0, 20, 125, and 500 mg/kg on gestations days 6-16		Guideline 004943
Reproduction-1 generation Species: rat Haskell Lab 413-83; 7/6/85	DPX-L5300 tech 96.8% ai	073790	Developmental NOEL = 2500 ppm,: Developmental LEL = 5000 ppm(decreased pup viability and weight gain) Doses tested: 0, 100, 2500, 5000 ppm in the diet. Adult toxicity NOEL and LEL (see subchronic feeding phase of this study, report no. 413-85 below)		Supplementary 004943
Feeding-3 month Species: dog Bio/dynamics Inc. HLO-514-85; 8/85	DPX-L5300 tech 96.8% ai	073788 073789	NOEL > 2500 ppm(HDT); Doses tested: 0, 50, 500, & 2500 ppm		Minimum 004943
Feeding-3 month Species: rat Haskell Lab 413-85; 7/6/85	DPX-L5300 tech 96.8% ai	073790	NOEL = 100 ppm, LEL = 1750 ppm(decrease body weight gain & food consumption and food efficiency; decrease absolute heart, brain, liver, and kidney weights; relative organ weights for heart, liver,kidneys, testes, and spleen were increased; serum glucose, globulin & cholesterol were decreased); Doses tested: 0,100, 1750, & 5000 ppm.		Minimum 004943
Dissimilation chemicals	Methyl-2-[[[[N-(4-methoxy 6-methyl-1,3,5-triazin-2-yl)N-methylaminocarbonyl] amino]sulfonyl]benzoate		Caswell # 419S		
Mutagenic-Ames Species: salmonella Haskell Lab 245-83; 5/25/85	DPX-L5300 tech 96.8% ai	073790	Incomplete report (Unacceptable)		Unacceptable 004943
Mutagenic-point mutation Species: cho cells Haskell Lab 58-85; 5/30/85	DPX-L5300 tech 96.8% ai	073790	Not mutagenic		Acceptable 004943

006833

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CONFGRADE/ DOCUMENT#
Mutagenic- cytogenetic Species: rat Haskell Lab 286-85; 6/14/85	DPX-L5300 tech 96.8% ai	073790	No genotoxicity		Acceptable 004943
Mutagenic-micronucleus assay Species: mouse Haskell Lab 420-85; 7/22/85	DPX-L5300 tech 96.8% ai	073790	No genotoxicity		Acceptable 004943
Mutagenic-unscheduled DNA synt Species: rat Haskell Lab 565-84; 7/18/85	DPX-L5300 tech 96.8% ai	073790	No genotoxicity		Acceptable 004943

Tox Chem No. 4195 - DPX-L5300 File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic (in vitro UDS) - rat ; Haskell Lab; Report no. 565-84; July 18, 1985	Technical (96.8% a. i.)	073790	No genotoxicity		Acceptable 004943
Acute oral LD_{50} - rat; Haskell Lab; Report No. 167-85; May 5, 1985	Technical (96.8% a. i.)		LD_{50} > 5000 mg/kg for both sexes (Limit Test)	IV	Minimum 004943
Acute oral LD_{50} - rat; Haskell Lab; Report No. 280-85; May 30, 1985	DPX-L5300 Herbicide (75%)		LD_{50} = 5700 mg/kg for males LD_{50} = 4800 mg/kg for females	IV III	Minimum 004943
Acute dermal - rabbit; Hazleton Lab; Report No. HLO-21-85; December 24, 1984.	Technical (96.8% a. i.)	073787	LD_{50} > 2000 mg/kg for both sexes (Limit test)	III	Minimum 004943
Acute dermal - rabbit; Hazleton Lab; Report No. HLO-234-85; April 11, 1985.	DPX-L5300 Herbicide (75% Dry Flowable)	073787	LD_{50} > 2000 mg/kg for both sexes (Limit test)	III	Minimum 004943
Acute inhalation -rat; Haskell Lab; Report No. 431-85; August 13, 1985	Technical (96.8% a. i.)	073787	LC_{50} > 6.7 mg/L for both sexes Four hour exposure. Concentrations tested = 6.7 mg/L and 1.3 mg/L. Nose only exposure.	III	Minimum 004943
Acute inhalation - rat;	DPX-L5300 Herbicide (75% Dry Flowable)	073787	Requirement waived because <0.5% of the formulations granules are <105 um in diameter.		004943
Primary eye irritation - rabbits: Hazleton Labs: HLO-305-85; May 24, 1985	DPX-L5300 Herbicide (75% Dry Flowable)	073787	Moderately irritating (no Primary Irritation Score given). Corneal opacity persisted for three days, redness persisted for 4 days.	III	Minimum 004943

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irritation-rabbit; Hazleton Lab; Report No. 233-85; April 11, 1985	DPX-L5300 Herbicide (75% Dry Flowable)	073787	Primary Irritation Score = 0	IV	Minimum 004943
Dermal sensitization - guinea pig; Hazleton Lab: Report No. HLO-295-85; May 23, 1985	DPX-L5300 Herbicide (75% Dry Flowable)	073787	Not a sensitizer		Minimum 004943

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation-rabbit,Haskell Lab. Report No. 604-86 9-19-86	Methyl 2-[[[[[N-(4-methyloxy-6-methyl-1,3,5,-triazin-2-yl)-N-methyl-amino]carbonyl]amino]sulfonyl]benzoate]	MRID No. 400498-07	Corneal opacity and irritation cleared by 72 hrs.	III	Guideline 006306
Acute dermal-rabbit Haskell Lab. Report No. 689-86 11-10-86	Methyl 2-[[[[[N-(4-methyloxy-6-methyl-1,3,5,-triazin-2-yl)-N-methyl-amino]carbonyl]amino]sulfonyl]benzoate]	MRID No. 400498-06	LD_{50} > 2000 mg/kg Dose 2000 mg/kg	III	Guideline 006306

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral-rat Haskell Lab ,Report No. 115-85, Feb. 25, 1985	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benezensulfo amide..62.5%. Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] amino]sulfonyl] benzoate 12 5%	258174	LD_{50} (M) = 5,600 mg/kg (4,600 - 6,200) LD_{50} (F) = 6,200 mg/kg (5,700 - 6,600)	IV	Guideline 006289
Acute dermal-rabbit; Hazleton Lab. Project No. 201-805 Jan. 1 1985	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benezensulfo amide..62 5%. Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl] amino]sulfonyl] benzoate. 12.5%	258174	LD_{50} > 2000 mg/kg	III	Guideline 006289

Study/Lab/Study #/Date	Material	EPA Accession No	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc No.
Dermal irri -rabbit Hazleton Lab. Project No. 201-806 Jan 8, 1985	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benezensulfo amide .62 5% Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl] amino]sulfonyl] benzoate 12 5%	258174	Slight to severe erythema at 30 to 60 minutes post-treatment At 24 hrs slight to well-defined irritation, had cleared by 72 hrs.	III	Guideline 006289
Eye irri -rabbit; Hazleton Lab ,Project No. #201-806, Jan. 8, 1985	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benezensulfo amide. 62.5%. Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl] amino]sulfonyl] benzoate. 12.5%	258174	Corneal opacity and other irritation had cleared at 72 hrs.	III	Guideline 006289

Tox Chem No. 419 S | File Last Updated | Current Date 3/3/88

Study/Lab/Study #/Date	Material	EPA Accession No	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Dermal sensitization- guinea pig Hazleton Lab ,Project No. 201-608, 3/12/85	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benezensulfo amide. 62.5% Methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]] amino]sulfonyl]] benzoate. 12 5%	258174	Nonsensitizing	-	Guideline 006289
Primary eye irritation - rabbit Hazleton. Rpt #201-616, 6/6/83	Technical	MRID # 403574-01	Levels tested = 10 mg instilled into the right eye of each of two rabbits. Redness with vessels injected more than normal were observed 1 and 4 hours after treatment.		Supplementary 006569
Dermal sensitization - guinea pig Hazleton, Rpt #201-617, 9/12/83	Technical	MRID # 403574-02	Not a sensitizer.		Minimum 006569
Primary dermal irritation - guinea pig Hazleton, Rpt #201-617 9/12/83	Technical	MRID # 403574-02	Levels tested = 7 and 70% solution in dimethyl phthalate Study was designed to determine a dose level for the dermal irritation study rather than assessing the dermal irritation potential of the undluited technical grade material		Supplementary 006569

APPENDIX II

Data Evaluation Records for Studies on DPX-L5300

Brock, W. J. October 10, 1986. One-year Feeding Study in Dogs with IN L5300. Unpublished report no. 565-86 prepared by Haskell Laboratory for Toxicology and Industrial Medicine. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-12.

Tobia, A. J. March 10, 1987. Combined Chronic Toxicity/Oncogenicity Study with IN-L5300: Long Term Feeding Study in Rats. Unpublished report no. 61-87 prepared by Haskell Laboratory for Toxicology and Industrial Medicine. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-11.

Tobia, A. J. March 6, 1987. Oncogenicity Study with INL-5300: Eighteen-Month Feeding Study in Mice. Unpublished report no. 60-87 prepared by Haskell Laboratory for Toxicology and Industrial Medicine. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-13.

Zellers, J. E. April 8, 1986. IN L5300. Developmental Toxicity Study in Rabbits Dosed by Gavage on Days 7-19 of Gestation. Unpublished report no. 150-86 prepared by Haskell Laboratory for Toxicology and Industrial Medicine. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-14.

Mullin, L. S. April 14, 1986. Two-Generation Study in Rats with IN-L5300. Unpublished report no. 193-86 prepared by Haskell Lab. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-15.

Hardesty, P. T. April 15, 1987. Fate of Radiolabeled DPX-L5300 in Rats. Unpublished report no. 31-98 prepared by Haskell Lab. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-16.

006833

Reviewed by: Sanford W. Bigelow, Ph.D. 7/14/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 7/14/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: chronic feeding - dog (83-1) CASWELL NO: 419S

ACCESSION NUMBER: MRID NO.: 402455-12

TEST MATERIAL: IN L5300

SYNONYMS: Express, INL-5300, benzoic acid, 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2yl)-N-methylamino]carbonyl]amino]sulfonyl]-, methyl ester, INL-5300-22.

STUDY NUMBER: Haskell Laboratory Report No. 565-86.

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine, P.O. Box 50, Elkton Road Newark, DE 19714

TITLE OF REPORT: One-Year Feeding Study in Dogs with IN L5300.

AUTHOR: W.J. Brock

REPORT ISSUED: October 10, 1986

CONCLUSIONS:

Male Dogs

NOEL = 25 ppm (0.79 mg/kg/day)
LEL = 250 ppm (8.16 mg/kg/day) based upon the following effects found in male beagle dogs: elevated bilirubin blood levels reported at 12, 26 and 52 weeks, elevated AST blood levels at 26 and 52 weeks, and increased urinary volume at 36 weeks.

Female Dogs

NOEL = 250 ppm (8.18 mg/kg/day)
LEL = 1500 ppm (52.02 mg/kg/day) based upon the following effects found in female beagle dogs: elevated serum creatinine levels at 4, 12, 26, 36, and 52 weeks, elevated blood bilirubin levels at 26 weeks, elevated serum AST levels at 4 weeks, elevated globulin levels at 12 weeks, and an 18.2% decrease in body weight gain at 371 days.

The elevated levels of serum creatinine, AST, albumin, or globulin observed in female dogs and increased serum levels of bilirubin, creatinine, and AST observed in male dogs indicated that kidney, heart or liver toxicity was present, however, no increased incidence of lesions in the kidney, heart or liver were reported in dogs exposed to INL-5300 in this study.

Other toxicological effects in male dogs exposed to 1500 ppm INL-5300 were:

- o elevated serum creatinine levels at 36 weeks,
- o increased urinary volume, and
- o a 20% decrease in body weight gain at 371 days.

Classification: core-minimum: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-1 have been satisfied.

II. MATERIALS:

A. Test Compound: INL-5300

Description: INL-5300, technical-grade
Batch #: not provided (Haskell Sample# 15,601).
Purity: The purity of INL-5300 used in this study was:
1) 95.8% used to feed from April 30, 1985 to July 1, 1985, and
2) 94.2% used to feed from July 8, 1985 to May 16, 1986.

B. Test Animals:

Species: Dogs (male and female)
Strain: Beagle
Age: median birthdate - December 9, 1984.
Weight (week 0): Mean, in kilograms (range)
females: 7.0 (6.0 to 7.8) males: 7.8 (6.7 to 8.5)
Source: Marshall Research Animals (North Rose, NY).

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study

Test Group	Dose in diet (ppm)	Main Study one year male	female	Least number of treatment weeks
1 Control	-	5	5	91
2 Low (LDT)	25	5	5	91
3 Mid (MDT)	250	5	5	91
4 High (HDT)	1500	5	5	91

Upon arrival from Marshall Research Animals, all dogs were quarantined for 17 days for observation prior to initiation of INL-5300 exposure. INL-5300 feeding started April 30, 1985 and ended May 16, 1986, a period reported as 371 days.

B. Diet Preparation:

The diet containing INL-5300 was prepared weekly and refrigerated prior to use. INL-5300 was dissolved in Mazola Corn Oil and added to ground Purina Certified Canine Diet #5007 at the following concentrations: 0, 25, 250, and 1500 ppm INL-3500. One day prior to the start of the study (day -1) and on days 181 and 363, 50 gram samples of the admixture was analyzed for stability and/or homogeneity on samples prepared fresh as well as fresh samples stored for 24 hours and 10 days. Samples tested for homogeneity were sampled from the top, middle and bottom of the mixing container. This analysis was performed in the Molecular and Genetic Toxicology Section at Haskell Laboratory.

Analytical results: The nominal INL-5300 dietary concentration for all of the admixtures ranged from 104% to 106% for the 25, 250, and 1500 ppm dietary levels. The difference in concentration between freshly frozen samples of the admixture and samples stored at room temperature for 16 days ranged from 92% to 108%. The homogeneity in the diet was within 13% of the nominally prepared concentrations (25, 250 and 1500 ppm); except on day 181, they were 16-19% higher than the nominal concentrations. Overall, minimal variability was reported in both batches of the admixture prepared from the two lots of test compound differing in purity regarding the homogeneity, stability and nominal dietary concentrations of test compound in the admixture.

Feeding schedule: Animals received about 350g daily of Purina Certified Canine Diet #5007) and tap water ad libitum throughout the 371-day feeding study.

C. Statistics:

The following statistical procedures were utilized in analyzing the numerical data:

The clinical laboratory results were compared with the Bartlett's test, Mann-Whitney U test, or the Kruskall-Wallis test. The incidences of clinical observations between control and treated group results were evaluated with Fisher's exact test. Body weights, body weight gains, organ weights and clinical laboratory measurements were analyzed for differences between control and treated groups by one-way analysis of variance (ANOVA).

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, the study was audited about 21 times during the course of the study. The dates when the study was audited were: 4/29/85, 5/16/85, 6/14,17-20/85, 8/12,13/85, 11/18-20/85, 2/3-7,10,11,19/86, 4/10,17,29/86, and 5/15,19-21/86.

IV. METHODS AND RESULTS:

A. Clinical Observations:

Animals were inspected twice daily for mortality and once daily for general appearance, behavior and excreta.

Toxicity/mortality (survival) results: No dogs died during the course of this study. One female dog in the 1500 ppm exposure group was found to have an umbilical hernia, reported in this study to be a common finding in dogs.

Clinical Results: No treatment-related clinical signs were reported in this study. Clinical signs most frequently observed were: isolated incidences of skin sores in both male and female dogs as well as alopecia.

Alopecia was found in 1 male dog in the 250 ppm exposure group, no females in the control group, 1 female in the 25 ppm exposure group and 2 females in both the 250 and 1500 ppm exposure groups. However, alopecia is viewed as unrelated to exposure to the INL-5300 because alopecia was not confined to any one region of the dogs' bodies, (alopecia was found on the front and rear legs as well as the chest and face). Furthermore, alopecia was not found in more than one body region in any one dog and, is therefore, viewed as toxicologically insignificant.

B. Body weight:

All animals were weighed weekly.

Results: No differences in absolute body weight between control and treated groups of dogs were found. No decreases in body weight gain were found in dogs fed 0, 25 or 250 ppm INL-5300.

A compound-related decrease in body weight gain was observed in male and female dogs fed 1500 ppm INL-5300. The results of the body weight gain are listed in Table 2 below. As an example of the data listed below, at day 371, male dogs exhibited a 20% decrease in body weight gain and female dogs exhibited an 18.2% decrease in body weight gain.

Table 2
Mean Body Weight Gain Changes in Dogs Fed 1500 ppm INL-5300
(taken from Tables 2 and 3)

	Percent Decrease (increase)			
	Day 14	Day 189	Day 203	Day 371
Males	25.0	9.8	11.8	20.0
Females	(33.0)	12.2	14.3	18.2

The reviewer calculated body weight gain by the following formula:

$$\%\ \text{Decrease (increase)} = 100 - \frac{\text{Mean body weight gain (test group)}}{\text{Mean body weight gain (control)}} \times 100$$

C. Food consumption and compound intake:

Food consumption was determined in all animals on a weekly basis. Mean daily diet consumption were calculated from these data. The dietary intake of INL-5300 was calculated and is reported in Table 4 below.

Food consumption results: No treatment-related reductions in mean food consumption or mean food efficiency (kg weight gain/kg diet consumed) were observed in this study.

Table 4
Dietary Intake of INL-5300 in Dogs
(taken from Tables 10 and 11)

		Dietary Concentration	Mean Daily Dose (mg/kg)[a]
Males:			
Group #	2	25	0.79
	3	250	8.16
	4	1500	51.46
Females:			
Group #	2	25	0.90
	3	250	8.18
	4	1500	52.02

Conclusion: On the basis of a daily dietary intake, female and male dogs received about the same amount of INL-5300 (Table 4).

D. Ophthalmological examination:

Ophthalmological examinations were performed on day 352 of the study on all male and female dogs.

Results and conclusions: Both eyes of all male and female dogs were reported to be normal on an ophthalmological basis.

E. Hematology:

Blood samples were obtained twice before INL-5300 exposure had commenced and at about week 4, 12, 24, 36, and 52. The dogs were fasted for about 16 hours before individual blood samples were taken for hematology analysis. Bone marrow smears were collected from all animals from a rib and not from the femur. The CHECKED (X) parameters were examined.

|X|	Hematocrit (HCT)*	|X|	Leukocyte differential count*
|X|	Hemoglobin (Hb)*	|X|	Mean corpuscular Hb (MCH)
|X|	Leukocyte count (WBC)*	|X|	Mean corpuscular Hb conc.(MCHC)
|X|	Erythrocyte count (RBC)*	|X|	Mean corpuscular volume (MCV)
|X|	Platelet count*+	| |	Reticulocyte count
| |	Plateletcrit	| |	Mean platelet volume
| |	Platelet dist. width	| |	Red cell dist. width
| |	Blood clotting msrmts.		
| |	(Thromboplastin time)		
| |	(Clotting time)		
| |	(Prothrombin time)		

* Required for subchronic and chronic studies
\+ Not required for oncogenicity studies

Results and conclusions: Selected hematological results from this study are presented in Table 5 below. All changes in hematological parameters discussed here are statistically significant. At 12 weeks, eosinophil levels were decreased in male dogs fed 1500 ppm INL-5300 and female dogs fed 250 ppm INL-3500. However, the variability in eosinophil levels within each group, whether treated or control, is excessively high to base toxicological significance on these findings (Table 5).

Other findings, decreases in monocyte levels in male dogs fed 250 ppm and increases in WBC and neutrophil levels in female dogs fed 250 ppm, were not found at higher exposure levels.

In conclusion, no toxicologically significant changes in hematological parameters were reported in this study.

Table 5
Selected Hematological Parameters in Dogs Fed INL-5300
(taken from Tables 13 and 14)

Group #:		1	2	3	4
Dose (ppm):		0	25	250	1500
Parameter (week):					
		Males			
Monocytes	(52w)	434 (56)	284 (124)	220 (112)[a]	502 (147)
Eosinophils	(12w)	877 (301)	1226 (644)	663 (239)	225 (217)[a]
		Females			
WBC	(52w)	4.8 (1.2)	6.1 (1.3)	8.0 (1.7)[a]	6.3 (1.5)
Neutrophils	(52w)	2698 (819)	3422 (832)	5392 (1631)[a]	3795 (1275)
Eosinophils	(12w)	724 (598)	84 (53)	65 (65)[b]	202 (161)

[a] $p = <0.05$, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.
[b] significantly different based on Mann-Whitney U-test criteria.

F. Clinical Chemistry:

Blood samples were obtained twice before INL-5300 exposure had commenced and at about week 4, 12, 24, 36, and 52. The dogs were fasted for about 16 hours before individual blood samples were taken for clinical chemistry analysis. The CHECKED (X) parameters were examined.

Electrolytes

|X| Calcium*
|X| Chloride*
| | Magnesium
|X| Phosphorus*
|X| Potassium*
|X| Sodium*

Other Analytes

|X| Albumin*
|X| Blood urea nitrogen*
|X| Glucose*
| | Triglycerides
|X| Serum protein electrophores
|X| Uric acid
|X| Blood creatinine*
|X| Cholesterol*
|X| Total bilirubin
|X| Total serum protein (TP)*

Enzymes

|X| Alkaline phosphatase (ALP)
| | Cholinesterase (ChE)#
| | Creatinine phosphokinase*^

| | Lactic acid dehydrogenase (LAD)
|X| Serum alanine aminotransferase (ALT)*
|X| Serum aspartate aminotransferase (AST)*

| | Gamma glutamyl transferase (GGT)
| | Glutamate dehydrogenase
| | Alpha-1-globulin

| | Alpha-2-globulin
| | Beta-globulins
|X| Globulin

* Required for subchronic and chronic studies
# Should be required for organophosphate pesticides
^ Not required for subchronic studies

Results and conclusions: Selected clinical chemistry results from this study are presented in Table 6 below. All changes in clinical chemistry parameters discussed here are statistically significant.

Male dogs. The toxicologically significant changes in clinical chemistry parameters in male dogs were elevated serum bilirubin, AST and creatinine levels. At 12 and 26 weeks, male dogs fed 250 or 1500 ppm INL-5300 exhibited elevated bilirubin levels. Elevated bilirubin levels were seen in male dogs fed 1500 ppm INL-5300 at 36 weeks as well as in male dogs fed 250 ppm INL-5300 at 52 weeks. No elevation in bilirubin blood levels were seen at 52 weeks in male dogs fed 1500 ppm INL-5300. Male dogs fed 1500 ppm INL-5300 had elevated serum creatinine levels at 36 weeks. In addition, AST blood levels were elevated in male dogs fed 250 or 1500 ppm INL-5300 at 26 and 52 weeks.

In addition, male dogs fed 25 ppm INL-5300 exhibited elevated albumin levels and diminished blood globulin levels at 26 weeks. However, no changes were observed at higher exposure levels (Table 6).

Female dogs. Toxicologically significant changes in female dogs involved elevated serum creatinine, AST and bilirubin levels. Female dogs fed 1500 ppm INL-5300 had elevated serum creatinine levels at 4, 12, 26, 36, and 52 weeks (every treatment time point measured). At 26 weeks, elevated blood bilirubin levels were seen in female dogs fed 1500 ppm INL-5300. Serum AST levels were elevated at 4 weeks in female dogs fed 1500 ppm INL-5300. In addition, elevated globulin levels were reported at 12 weeks in female dogs fed 1500 ppm INL-5300.

Elevated total serum protein levels were reported at 12 weeks in female dogs fed 250 or 1500 ppm INL-5300. However, these results are viewed as toxicologically insignificant because these levels were <10% higher than the control values for total serum protein (Table 6).

In conclusion, elevated serum levels of bilirubin, AST and creatinine in male and female dogs (and elevated globulin levels in females) are suggestive of liver, heart or perhaps renal toxicity.

Table 6
Selected Clinical Chemistry Parameters in Dogs Fed INL-5300
(taken from Tables 15 and 16)

Group #:		1	2	3	4
Dose (ppm):		0	25	250	1500
Parameter (week):					
Males					
AST	(26w)	18 (2)	22 (3)	26 (3)[a]	28 (5)[a]
AST	(52w)	19 (3)	22 (4)	28 (5)[a]	26 (2)[a]
Bilirubin	(-1w)	0.10 (0.01)	0.13 (0.02)	0.15 (0.02)[a]	0.13 (0.02)
Bilirubin	(12w)	0.18 (0.03)	0.22 (0.05)	0.28 (0.08)[a]	0.31 (0.06)[a]
Bilirubin	(26w)	0.27 (0.04)	0.33 (0.06)	0.37 (0.03)[a]	0.40 (0.08)[a]
Bilirubin	(36w)	0.21 (0.03)	0.22 (0.04)	0.26 (0.05)	0.28 (0.04)[a]
Bilirubin	(52w)	0.16 (0.03)	0.20 (0.03)	0.24 (0.04)[a]	0.23 (0.05)
Creatinine	(36w)	0.83 (0.09)	0.81 (0.08)	0.79 (0.07)	0.97 (0.08)[a]
Albumin	(26w)	3.6 (0.1)	3.9 (0.2)[a]	3.7 (0.2)	3.6 (0.1)
Globulin	(26w)	2.6 (0.2)	2.2 (0.2)[a]	2.5 (0.2)	2.5 (0.2)
Females					
AST	(4w)	30 (5)	32 (8)	40 (25)	38 (5)[b]
Bilirubin	(26w)	0.26 (0.16)	0.24 (0.07)	0.34 (0.05)	0.45 (0.09)[a]
Creatinine	(4w)	0.73 (0.03)	0.79 (0.06)	0.76 (0.03)	0.84 (0.05)[a]
Creatinine	(12w)	0.82 (0.04)	0.83 (0.11)	0.82 (0.03)	0.95 (0.06)[a]
Creatinine	(26w)	0.72 (0.08)	0.72 (0.07)	0.71 (0.04)	0.84 (0.07)[a]
Creatinine	(36w)	0.73 (0.07)	0.76 (0.08)	0.77 (0.05)	0.87 (0.02)[a]
Creatinine	(52w)	0.82 (0.08)	0.88 (0.12)	0.87 (0.10)	1.05 (0.06)[a]
Total Prot.	(12w)	5.6 (0.2)	5.7 (0.1)	6.0 (0.2)[a]	6.0 (0.3)[a]
Globulin	(12w)	1.9 (0.1)	1.9 (0.1)	2.2 (0.2)	2.3 (0.3)[a]

[a] $p = <0.05$, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.

G. Urinalysis:^

Urine samples were obtained twice before INL-5300 exposure had commenced and at about week 4, 12, 24, 36, and 52. The dogs were fasted for about 16 hours before individual urine samples were obtained for urinalysis.

One male from the 0 ppm exposure group and a male and a female from the 1500 ppm exposure group had no urinalysis test performed at 9 months (female) and 12 months (males). Also, another male dog at 12 months, as part of the urinalysis test, did not have his urinary appearance endpoint examined. One female dog in the 250 ppm INL-5300 exposure group at the 9-month urinalysis examination did not have the osmolality, occult blood, urinary glucose, protein, pH, bilirubin, uroporphyrinobilinogen, and ketone parameters measured. The CHECKED (X) parameters were examined.

|X| Appearance*	|X| Glucose*
|X| Volume*	|X| Ketones*
|X| Specific gravity*	|X| Bilirubin*
|X| pH	|X| Blood*
| | Nitrate	|X| Sediment (microscopic)*
|X| Protein*	|X| Urobilinogen

^Not required for subchronic studies
* Required for chronic studies

Results: The selected urinalysis results from this study are presented in Table 7 below. The only finding that was statistically significant in all of the urinalysis results was an increased urinary volume in male dogs fed 250 or 1500 ppm INL-5300 at 36 weeks.

Of interest to note, in the control and the 25 ppm exposure groups, female dogs had higher urinary volumes than male dogs at 36 weeks.

In conclusion, increased urinary volume in male dogs is suggestive of kidney toxicity from INL-5300 exposure.

Table 7
Selected Urinalysis Results in Dogs Fed INL-5300
(taken from Tables 17 and 18)

Group #:	1	2	3	4
Dose (ppm):	0	25	250	1500
Parameter (week):				
		Males		
Volume (ml) (36w)	206 (135)	179 (160)	446 (91)[a]	475 (51)[a]
		Females		
Volume (ml) (36w)	371 (147)	399 (123)	419 (134)	336 (161)

[a] $p = <0.05$, significantly different from control group when compared by the use of the two-tailed Dunnett t-test performed on the raw data.

F. Sacrifice, Gross Pathology and Histopathology:

All animals were fasted overnight prior to terminal necropsy. All animals that were sacrificed on schedule were examined for gross pathological and histological changes. Terminal necropsies began on day 377 and ended on day 381 of the study. Microscopic examinations were performed on all specified tissues and gross lesions from all animals in each group.

The CHECKED (X) tissues were collected for histological examination. The organs double-checked (XX) were weighed.

Digestive system	**Cardiovascular**	**Neurological**
| | Tongue	|X | Aorta*	|XX| Brain*+
|X | Salivary glands*	|XX| Heart*	|X | Periph. nerve (sciatic)*#
|X | Esophagus*	|X | Bone marrow*#	|X | Spinal cord (3 levels)*#
|X | Stomach*	|X | Lymph nodes*	|X | Pituitary*
|X | Duodenum*	|X | Spleen	|X | Eyes (optic n.)*#
|X | Jejunum*	|X | Thymus*	**Glandular**
|X | Ileum*	**Urogenital**	|XX| Adrenal gland*
|X | Cecum*	|XX| Kidneys*+	| | Exorbital lacrimal gland#
|X | Colon*	|X | Bladder*	|X | Mammary gland*#
|X | Rectum*	|XX| Testes*+	|X | Parathyroids*++
|XX| Liver*+	|X | Epididymides	|X | Thyroids*++
|X | Gall bladder*#	|X | Prostate	**Other tissues**
|X | Pancreas*	|X | Seminal vesicle	| | Bone (femur)*#
Respiratory	|X | Ovaries*+	|X | Skeletal muscle(thigh)*#
|X | Trachea*#	|X | Uterus*	|X | Skin*#
|X | Lung*	| | Cervix	|X | All gross lesions and masses*
| | Nose^	| | Fallopian tubes	|X | Rib (with costochondral junction)
| | Pharynx^	|X | Vagina	|X | Sternum
| | Larynx^		|X | Tonsil

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

1. Organ weight: Organ weights were determined for the liver, heart, kidneys, thyroids/parathyroids, testes, and brain in all dogs at the termination of INL-5300 feeding.

   Organ weight results: INL-5300 exposure caused no changes in (1) organ weight or in (2) organ weight relative to whole body weight as reported in this study. Therefore, no table is presented that lists these data.

2. Gross pathology results: INL-5300 exposure caused no changes in macroscopic or gross lesions as reported in this study. The incidence of selected gross lesions are listed in Table 8 below.

Table 8
Summary Incidence of Gross Lesions Observed in this Study
(taken from Table 21)

Sex:	Male Dogs				Female Dogs			
Group #:	1	2	3	4	1	2	3	4
Dose (ppm):	0	25	250	1500	0	25	250	1500
otal # of dogs:	5	5	5	5.	5	5	5	5
rgan or Site:								
arge Intestine: discolored	0	0	5	0	1	1	0	1
ungs: discolored	0	1	0	0	0	0	0	1
eart: thick	1	0	0	0	0	1	0	1

3. Histopathology results: These results are listed in Table 9 below.

a) Non-neoplastic lesions: No increased incidence of histopathologic lesions related to INL-5300 exposure to dogs was observed in this study.

Table 9
Summary Incidence of Microscopic Lesions Observed in this Study
(taken from Table 22)

Sex:	Male Dogs				Female Dogs			
Group #:	1	2	3	4	1	2	3	4
Dose (ppm):	0	25	250	1500	0	25	250	1500
Total # of dogs:	5	5	5	5	5	5	5	5
Organ or Site:								
Adrenal Cortex: accessory nodule	1	0	1	1	0	1	1	1
Duodenum: dilatation of gland	1	0	1	1	0	1	1	1
Gall Bladder: lymphoid hyperplasia	1	0	1	1	0	0	0	0
Heart: focal cartilaginous metaplasia	1	0	1	1	1	1	1	1
Kidney: glomerulonephropathy	1	1	1	1	2	1	1	1
focal mineralization	2	2	2	1	1	1	2	1
unilateral medullary inflammation	1	0	1	0	0	0	0	0
Liver: lymphoreticular foci	2	2	2	1	1	2	2	2
Parotid Salivary Gland: lymphocytic infiltration	1	1	1	0	1	2	1	1
Stomach: lymphoid hyperplasia	1	2	2	1	1	0	1	1
Thymus: atrophy/ fatty replacement	0	0	1	1	0	0	1	1

b) Neoplastic lesions: INL-5300 exposure did not cause a dose-related increased incidence of neoplasms in dogs in this study. Table 9 above shows that lymphoid hyperplasia was reported in the stomach and gall bladder.

V. DISCUSSION:

Male and female beagle dogs were fed either 0, 25, 250, or 1500 ppm INL-5300 for 371 days. After termination of feeding INL-5300, the dogs were examined for changes in hematology, clinical chemistry, urinalysis as well as the incidence of gross or histopathological lesions and ophthalmologic changes.

The LEL value in male dogs fed INL-5300 was 250 ppm based on elevated serum bilirubin levels reported at weeks 12, 26, and 52 as well as elevated AST serum levels at 26 and 52 weeks and increased urinary volume at 36 weeks. The NOEL value for INL-5300 in dogs is set at 25 ppm.

Female dogs fed 1500 ppm INL-5300 exhibited elevated creatinine levels in the serum at 4, 12, 26, 36, and 52 weeks, elevated blood bilirubin levels at 26 weeks, elevated serum AST levels at 4 weeks, elevated globulin levels at 12 weeks, and an 18.2% decrease in body weight gain at 371 days as well. Thus, the LEL value for INL-5300 is established at 1500 ppm in female dogs and the NOEL value is set at 250 ppm.

The elevated levels of serum creatinine, AST, albumin, or globulin observed in female dogs and increased serum levels of bilirubin, creatinine, and AST observed in male dogs indicated that kidney, heart or liver toxicity was present, however, no increased incidence of lesions in the kidney, heart or liver were reported in dogs exposed to INL-5300 in this study.

Other toxicological effects in male dogs exposed to 1500 ppm INL-5300 were:

- elevated serum creatinine levels at 36 weeks,
- increased urinary volume, and
- a 20% decrease in body weight gain at 371 days.

Classification: core-minimum: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-1 have been satisfied.

PC1/express/xprsqdog.035

006933

12viewed by: Roger Gardner Roger Gardner 8-19-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 8/19/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic feeding/Oncogenicity (Guideline §83-5)

MRID NUMBER: 402455-11

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): 61-87

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine

TITLE OF REPORT: Combined Chronic Toxicity/Oncogenicity Study with INL-5300: Long Term Feeding Study in Rats

AUTHOR(S): Tobia, A. J.

REPORT ISSUED: March 10, 1987

CONCLUSIONS: Groups of 72 male and 72 female Sprague-Dawley rats were given diets containing 0, 25, 250, or 1,250 ppm DPX-L5300 for up to 24 months. Twelve months after the study began, 10 animals of each sex from each group were sacrificed and necropsied.

Effects attributed by the investigators to the test substance included decreased body weight and increased incidences of masses located on the shoulder, side, and under body regions in high dose group female rats. The masses were associated with the statistically significantly increased incidence of mammary gland adenocarcinomas observed in the high dose group females.

By the end of the study, mean body weights for the mid and high dose group males were decreased from the control value by 8.6 and 29.2%, respectively, and group mean body weights for females in the low, mid, and high dose groups were 9.5, 21.3, and 42.5% less than the control group mean, respectively. Organ weights in treated animals reflected the observed decreases in body weight (i. e., significant increases in the majority of relative organ weights in the male and female rats at the 1,250 ppm dose level and female rats at the [illegible] ppm dose level along with statistically significantly decreased absolute organ weights).

44

In male rats given the high dose level, the incidence of polyarteritis in the pancreas, decreased secretion in seminal vesicles, lymphoid depletion

006833

CONCLUSIONS (continued)

in the spleen, and mineralization of the aorta and stomach were statistically significantly increased above control group incidences. The latter two lesions were associated with an increase in severity of glomerulonephropathy in the high dose group males. The incidence of dilatation of the renal pelvis, dilatation of the uterine horns, and retinal degeneration was statistically significantly increased in females given the highest dose level.

The only tumor incidence significantly increased by the test substance was mammary gland adenocarcinomas in female rats.

Based on the reduced body weights in treated male and female, a no-observed effect level (NOEL) was established in the study at 25 ppm (1.25 mg/kg/day).

Core classification: Minimum.

I. PROTOCOL

A. MATERIALS

1. Test species: Male and female 21-day-old Charles River Crl:CD®BR strain rats were used. Their weights ranged from 30 to 55 g. The animals were placed on test diets 17 days after receipt at the laboratory.

2. Diet preparation: Basal diet consisted of Purina Lab Chow #5002, and the test substance was added in corn oil in appropriate concentrations. (Corn oil was 1% by weight of the diet.) Test diets were prepared weekly 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 718 and 725. Diets were also analyzed for concentration and stability of test substance on days 180 and 369, and for concentration on days 381, 565, 676, and 685. The report noted that on test day 502 concentration and homogeneity analyses were made of test diets because of the reduction in amount of diet prepared and a change in the mixer used for diet preparations for the rest of the study.

B. STUDY DESIGN

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

Test groups		Dose	Animals per sex	
No.	Designation	(ppm)	Main study*	Interim Sacrifice**
1	Control	0	62	10
2	Low (LDT)	25	62	10
3	Mid	250	62	10
4	High (HDT)	1250	62	10

*24 months. **At 12 months

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day
Signs of toxicity	All	Twice a day*
Body weight	All	On day of arrival at lab, at weekly intervals through the first 6 months, bi-weekly thereafter, and on the day of necropsy.
Food consumption	All	For all weighing intervals during the study.**
Ophthalmology	High and low dose groups only	At the end of the study.
Blood samples	10***	At 3, 6, 9, 12, 18, and 24 months.
Urine samples	10***	At 3, 6, 9, 12, 18, and 24 months.
Necropsy	Animals found dead or moribund	When found.
	10 Survivors	At 12 months At 24 months

*Each rat was individually handled at least once each week during the first six months of the study and every other week during the remainder of the study. The gross presence of tissue masses and changes in appearance and behavior were noted.

**For each individual animal.

***The report stated that 10 animals of each sex were selected at random "...on the basis of freedom from any lesions which appeared to be of a spontaneous origin and which occurred with a similar frequency in control group rats."

C. METHODS

1. Observation of blood samples: Blood was collected by amputation of the distal portion of the tail from animals which were fasted for 16 hours prior to sampling.

006833

1. Observation of blood samples (continued):

a. Hematology

- X Hematocrit
- X Hemoglobin
- X Red cell count
- X Platelet count
- X Total white cell count
- X Differential white cell counts
- X Mean corpuscular hemoglobin concentration
- X Mean cell volume
- X Mean corpuscular hemoglobin

b. Blood chemistry

- X Total protein
- X Albumin
- X Globulin (calculated)
- ___ Albumin/globulin ratio
- X Blood urea nitrogen
- X Electrolytes
- X Creatinine
- ___ Uric acid
- X Glucose
- X Total cholesterol
- ___ Total bilirubin
- X Aspartate aminotransferase (AST)
- X Alanine aminotransferase (ALT)
- X Alkaline phosphatase
- ___ Lactate dehydrogenase
- ___ Triglycerides

2. Urine observations: Urine was collected from each animal during the 16 hour fasting period preceeding blood sample collection. The following observations were made:

- X Volume
- X pH
- X protein
- X glucose
- X ketones
- X bilirubin
- X occult blood
- X urobilinogen
- X osmolality
- X microscopic examination of centrifuged deposits

3. Necropsy Gross lesions were noted.

a. Weighed organs

- X Liver
- X Kidneys
- X Spleen
- X Heart
- X Brain
- X Testes

b. Tissues examined microscopically

Circulatory System

- X Aorta
- X Heart

Digestive System

- X Cecum
- X Colon
- X Duodenum
- X Esophagus
- X Ileum
- X Jejunum
- X Liver
- X Pancreas
- X Rectum
- X Salivary gland
- X Stomach

Hematopoietic System

- X Bone marrow
- X Lymph nodes
- X Spleen
- X Thymus

Musculoskeletal System

- X Bone
- X Skeletal muscle

Nervous System

- X Brain
- X Sciatic nerve
- X Spinal cord

Reproductive System

- X Epididymides
- X Mammary glands
- X Ovaries
- X Prostate
- X Seminal vessicles
- X Testes
- X Vagina
- X Uterus with cervix

Respiratory System

- X Lungs
- X Nasal turbanates
- X Trachea

006933

b. Tissues examined microscopically (continued)

Endocrine System	Other	Urinary System
X Adrenals	X All macroscopic abnormalities	X Kidneys
X Pituitary	X Eye	X Urinary bladder
X Thyroid with parathyroid	X Hardarian gland	
	X Skin and subcutis	

Tissue samples from the control and high dose groups as well as rats found dead or sacrificed in extremis were examined microscopically. The report stated that only the heart, liver, kidneys, lungs, and organs with gross lesions from rats in the low and mid dose groups were examined. After day 368 of the study, the mammary glands from all female rats in the low and mid dose groups were also examined.

D. STATISTICAL ANALYSIS

Observation	Statistical Test
	Continuous Variables
Body weights	One-way analysis of variance (ANOVA) Least Significant Difference test (LSD)
Organ weights	ANOVA with pair-wise comparisons by LSD and Dunnett's tests, and a test for linear trend
Clinical pathology	ANOVA and the Bartlett's test; if the F-test was significant, means of each treated group were compared with that of the appropriate control group by Dunnett's test; if results of the Bartlett's test were significant, the Kruskal-Wallis and Mann-Whitney tests were used to compare control group means with each treated group mean.
	Non-Parametric Variables
Survival among groups	Mantel-Haentzel and Fisher's Exact tests
Tumor incidence*	Fisher's Exact test
	Other Analyses
Survival probability	Kaplan-Meier procedure

*Tumors were analyzed by specific site, lesion, and benign-malignant classification.

006933

II. REPORTED RESULTS

A. Mortality and Signs of Toxicity: No treatment-related clinical signs were observed according to the report. Mortality during the last 9 months of the study is summarized as follows:

Dose (ppm)	Mortalities* during days: Males 0-365	Males 366-546	Males 547-730	Females 0-365	Females 366-546	Females 547-730
0	0 †	7	24	2	10	22
25	2 ††	4	24	2	9	25
250	2	11	24	4	7	15
1250	1	5	27	1	6	19

*Excludes those animals sacrificed at 12 and 24 months.
†Two animals died during the first two weeks of the study and were replaced
††One rat in this group died during the first two weeks of the study and was replaced.

The incidence of masses in the shoulder, side, and under body regions were associated by the investigators with administration of the test substance to female rats in the study. Those results are summarized as follows:

Location	Dose level (ppm): 0	25	250	1250
Shoulders	5	4	8	13
Sides	3	8	13	10
Under body	21	16	19	32

Colored discharges from the eyes and nose and hair loss were the most frequently observed clinical signs, but the report noted that these occurred at an incidence which was unrelated to treatment.

B. Body Weight and Food Consumption: The report noted that the test substance significantly affected body weight and body weight gain in both sexes. By the end of the study, mean body weights for the mid and high dose group males were decreased from the control value by 8.6 and 29.2%, respectively. Only the high dose group males were statistically significantly different from the control group, and the low dose group mean body weight was similar to that of the control group males. Group mean body weights for females in the low, mid, and high dose groups at the end of the study were 9.5, 21.3, and 42.5% less than the control group mean, respectively. The mid and high dose group values were statistically significantly different from the control value.

Reported group mean body weight gains were statistically significantly decreased in mid and high dose groups during the study. At six months, weight gains for mid and high dose group males averaged 6.5 and 22.5% less than that for the control group, respectively. By the end of

006833

B. Body weight and food consumption (continued):

the study, the mid and high dose group weight gains for males were 10.8 and 36.4% less than that for the control group. In female rats at six months, group mean body weights for the mid and high dose levels were also statistically significantly less than that for controls (8.5 and 39%, respectively). At the end of the study the mid dose group females had a body weight gain that was 26.6% less than that for the control group, and the high dose group's weight gain was 53.8% less than controls.

No statistically significant differences in group mean daily food consumption were observed during the study for male or female rats. Reported group mean daily food consumption values for males were 25.5, 25.7, 24.8, and 23.4 g/day in the control, low, mid, and high dose groups, respectively. The respective group mean food consumption values for female rats in the control, low, mid, and high dose groups 19.6, 18.9, 18.7, and 17.0 g/day.

Food efficiency results (g body weight gain/g food) are summarized as follows:

Weeks of observation	Dose level (ppm) 0	25	250	1250
		Males		
0-26	0.106	0.107	0.101	0.090
26-52	0.026	0.026	0.023	0.017
52-104	0.003	0.002	0.000	-0.005
0-104	0.033	0.033	0.030	0.023
		Females		
0-26	0.066	0.065	0.061	0.046
26-52	0.029	0.029	0.024	0.012
52-104	0.022	0.014	0.013	0.008
0-104	0.033	0.030	0.025	0.018

(No statistical analyses were reported.)

C. Test substance intake: According to the report, dietary analyses indicated that test substance concentrations were ±2% of the nominal concentrations, and homogeneity tests indicated a ±4% variation in concentration. Based on results of these analyses, body weight and food consumption measurements, the daily intake of test substance was calculated. For males the daily doses were reported to be 0.95, 10, and 55 mg/kg/day during the entire study. Those values for female rats were 1.2, 13, and 76 mg/kg/day.

D. Clinical Pathology

1. Hematology: The investigators noted that there were sporadic statistically significant differences between treated and control groups, but the differences were not dose-related, and they were within normal ranges.

D. Clinical Pathology (continued)

2. Clinical chemistry: The statistically significant differences between treated and control groups were not consistent with dose- or treatment-related effects, and the observations were within normal ranges for the age, sex, and strain of test species used.

E. Necropsy

1. Organ weights: Organ weight changes were consistent with the reduced body weight observed in the study. The absolute heart weight in high dose group male and female rats was statistically significantly less than that in the controls at 12 months and at termination. The heart-to-body-weight ratios were statistically significantly increased in males and females. These results are summarized as follows:

Time of observation	Dose level (ppm) Males 0	Males 1250	Females 0	Females 1250
	Absolute weight (g)			
12 months	1.891	1.656*	1.210	1.013*
Termination	2.194	1.895*	1.518	1.353*
	Relative weight (% of body weight)			
12 months	0.250	0.281*	0.280	0.345*
Termination	0.288	0.355*	0.266	0.395*

*Statistically significantly different from control ($p<0.05$; LSD and Dunnett's tests).

In addition to these results, the mid dose group males had a statistically significantly increased relative liver weight at 12 months.

High dose group female rats also had statistically significantly decreased liver and kidney weights at 12 months and at termination of the study. No significant differences were noted at the mid dose level. These results are summarized as follows:

Time of observation	Dose level (ppm) Liver 0	Liver 1250	Kidneys 0	Kidneys 1250
	Absolute weight (g)			
12 months	14.762	11.502*	2.749	2.377†
Termination	16.870	13.180*	3.459	3.017*
	Relative weight (% of body weight)			
12 months	3.467	3.917*	0.639	0.804*
Termination	2.907	3.816*	0.616	0.884*

*Statistically significantly different from control ($p<0.05$; LSD and Dunnett's tests).

1. Organ weights (continued)

Organ-to-body-weight ratios for all weighed organs were statistically significantly greater in the high dose group than those values for control group animals.

2. Non-neoplastic lesions: The microscopic observations associated by the investigators with administration of the test substance are presented in Table 1 below.

The investigators noted that the incidence of male rats with glomerulonephropathy was similar in all groups, but the severity of the lesion increased with the dose level (see Table 1). The incidence of mineralization in the aorta and stomach of mid and high dose group males was also considered to be a secondary effect associated with the increase in severity of the kidney lesions.

3. Neoplastic lesions: The incidences of the most frequently observed tumors are summarized in Table 2. below. The incidence of adenocarcinomas in the mammary glands was statistically significantly increased in female rats at the 1250 ppm dose level (highest dose tested), and the incidence of hepatocellular tumors was statistically significantly decreased in male rats given the highest dose. NO other tumor incidences were statistically significantly changed in a dose related or treatment related manner.

006833

E. Necropsy (continued)

Table 1

Incidence of selected non-neoplastic lesions in male and female rats treated with Express® in their diets for up to 24 months. †

Observation	Dose level (ppm) 0	25	250	1250
Males				
Liver (number examined)	62	60	60	61
Fatty changes (focal/multifocal)	17	14	12	8 *
Pancreas (number examined)	62	34	38	60
polyarteritis	4	8	4	15 *
Seminal vessicles (number examined)	62	33	39	61
decreased secretion	4	7	7	20 *
Spleen (number examined)	62	31	38	61
lymphoid depletion	0	2	3	8 *
Kidney (number examined)	62	60	60	61
glomerulonephropathy	59	56	57	59
Minimum	13	18	11	6
Mild	20	9	16	10
Moderate	15	14	12	14
Severe	11	15	18	29
Aorta (number examined)	62	29	35	61
Mineralization	2	2	7 *	9 *
Stomach (number examined)	62	30	35	61
mineralization	3	2	8 *	11 *
Females				
Kidney (number examined)	60	60	58	61
dilatation renal pelvis	4	8	10	13 *
Uterus (number examined)	60	36	28	61
dilatation	10	7	4	27 *
Eye (number examined)	59	33	23	61
retinal degeneration	33	4	9	42 *

† Excluding animals sacrificed at 12 months.
* Statistically significantly different from the control group according to the report ($p<0.05$; Fisher's Exact Test).

E. Necropsy (continued)

Table 2

Incidence of selected neoplastic lesions in male and female rats treated with Express® in their diets for up to 24 months. †

Observation	Dose level (ppm) 0	25	250	1250
Males				
Pancreas (number examined)	62	34	38	60
islet cell adenoma	9	5	2	3
islet cell carcinoma	-	2	-	-
Pituitary (number examined)	62	41	44	60
adenoma	36	32	26	22 *
Adrenal medulla (number examined)	62	32	40	61
pheochromocytoma (benign)	5	5	3	3
pheochromocytoma (malignant)	4	3	3	4
Animals with tumor (any type)	48	51	44	41
Females				
Pituitary (number examined)	60	52	54	61
adenoma	48	44	47	45
carcinoma	2	4	-	4
Mammary gland (number examined)	60	60	57	61
adenoma	2	2	2	3
adenocarcinoma	9	9	13	26 **
fibroadenoma	16	12	12	8
Animals with tumor (any type)	58	56	52	57

† Excluding animals sacrificed at 12 months.
* According to the report, the incidence is statistically significantly different from the control group (Fisher's Exact test, $p < 0.05$).
** According to the report, the incidence is statistically significantly different from the control group (Fisher's Exact test, $p < 0.01$).

III. DISCUSSION

A. Authors' Conclusions

The investigators concluded:

> Effects attributable to the dietary intake of INL-5300 by rats in this study were considered to be minimal. Mean body

A. Authors' conclusions (continued)

> weights for both male and female rats in the 250 and 1,250 ppm groups were decreased when compared to controls...The only important clinical sign observed was a higher incidence of masses located on the shoulder(s), side(s), and under body regions in the female 1,250 ppm group when compared to controls. These masses are consistent with the increased incidence of mammary gland adenocarcinomas observed in this same group.
>
> There was a significant decrease in the mean absolute heart weights in the male 1,250 ppm group rats and a decrease in mean absolute liver, heart, and kidney weights in the female 1,250 ppm rats when compared to controls. The significant increase in the majority of relative organ weights in the male and female rats at the 1,250 ppm dose level and female rats at the 250 ppm dose level had no significant evidence of microscopic lesions, and the weight differences were considered to be related to the lower final body weights observed. These findngs are consistent with those observed at the one-year interim sacrifice.
>
> Administration of INL-5300 was associated with a significant increase in mammary gland adenocarcinomas in the 1,250 ppm female rats...A specific target organ was not identified for non-neoplastic effects.
>
> ..., the no-observable-effect level for dietary intake of INL-5300 in this study was 25 ppm.

B. Reviewer's Discussion

The report noted that the time to observation of masses (median days on test) associated with mammary tumors was as follows:

Location	Dose level (ppm) 0	25	250	1250
Shoulders	614	502	552	474
Sides	404	551	502	530
Under body	530	558	502	502

The first mammary gland adenocarcinoma was microscopically diagnosed in a control group female examined at the 12-month interim sacrifice. The first of these tumors observed in the low, mid and high dose groups were diagnosed on days 574, 514, and 431, respectively. The respective median times to diagnosis of these tumors in the control, low, mid, and high dose groups were 542, 623, 592, and 578 days. The incidence of mammary gland adenomas and adenocarcinomas in female rats according to time of diagnosis is summarized in Table 3.

B. Reviewer's Discussion (continued)

In a supplemental report, the incidence of mammary gland tumors was compared with historical control data as follows:

> The malignant tumor incidence in the concurrent control and in the 25 and 250 ppm treatment groups were within the range of historical control data for Haskell Laboratory (1.5 to 23.4% with a mean of 12.6%; these data summarize results from 10 2-year feeding studies reported between 1980 and 1986).

The incidences of mammary gland adenomas and adenocarcinomas combined in the control and high dose groups were reported to be 15.5 and 43.1%, respectively.

Thr group mean body weight results were used to determine effects on body weight gain at 13 weeks during the study. The results of those calculations are summarized as follows:

Observation	Dose level (ppm) 0	25	250	1250
		Males		
Body weight at Week 0	155.9	155.6	158.1	157.3
Week 13	530.9	542.7	513.4	453.2
Weight gain	375.0	387.1	355.3	295.9
% difference *	---	+ 3.2	- 5.3	-21.1
		Females		
Body weight at Week 0	122.0	122.1	122.4	122.8
Week 13	286.6	279.8	271.8	231.7
Weight gain	164.6	157.7	149.4	108.9
% difference *	---	- 4.2	- 9.2	-33.8

* Calculated as follows:

$$\% \text{ difference} = \frac{(\text{control weight gain} - \text{test group weight gain})}{(\text{control weight gain})} \times 100$$

The body weight and body weight gain decreases observed in male and female rats given the 1250 ppm diet indicated that adequate dose levels were tested.

B. Reviewer's Discussion (continued)

Table 3

Summary of the incidence of dose-related mammary gland tumors in female rats according to time of diagnosis.

Observation	Dose level (ppm) 0	25	250	1250
In 12-month interim sacrifice animals				
Adenoma	0/10	0/1	0/2	0/10
Adenocarcinoma	1/10	0/1	0/2	0/10
Combined adenoma/adenocarcinoma	1/10	0/1	0/2	0/10
In animals dying on test (days 368 - 735)				
Adenoma	2/28	2/36	2/25	3/26
Adenocarcinoma	6/28 †	4/36	5/25	17/26 *
Combined adenoma/adenocarcinoma	7/28 †	5/36	7/25	20/26 **
Terminal sacrifice animals				
Adenoma	2/33	1/26	1/35	2/36
Adenocarcinoma	3/33 ††	5/26	8/35	9/36 ***
Combined adenoma/adenocarcinoma	3/33 ††	5/26	9/35	11/36 ****
Total				
Adenoma	4/71	3/63	3/63	5/72
Adenocarcinoma	10/71 †	9/63	13/63	26/72 †††
Combined adenoma/adenocarcinoma	11/71 †	10/63	16/63	31/72 ††††

* Statistically significantly different from controls (p = 0.0012; Fisher's Exact Test).

** Statistically significantly different from controls (p = 0.00015; Fisher's Exact Test).

*** Not statistically significantly different from controls (p = 0.075; Fisher's Exact Test).

**** Statistically significantly different from controls (p = 0.026; Fisher's Exact Test).

† Statistically significant trend (p < 0.005; Cochran-Armitage trend test).

†† No statistically significant trend (p > 0.005; Cochran-Armitage trend test).

††† Statistically significantly different from controls (p = 0.0025; Fisher's Exact Test).

†††† Statistically significantly different from controls (p = 0.0002; Fisher's Exact Test).

006833

Reviewed by: [redacted]r Gardner Roger Gardner 8-19-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 8/19/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity (Guideline §83-2)

MRID NUMBER: 402455-13

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): 60-87

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medic[redacted]

TITLE OF REPORT: Oncogenicity Study with INL-5300: Eighteen-Month Feeding Study in Mice

AUTHOR(S): Tobia, A. J.

REPORT ISSUED: March 6, 1987

CONCLUSIONS: Diets containing 0, 20, 200, or 1500 ppm Express were given to male and female Charles River Crl:CD-1(ICR) BR strain mice for 18 months.

At the end of the study the highest dose tested was associated with minimal effects on body weight (6 and 5% less than control group means for males and females, respectively) and body weight gain (24% and 20% less than controls for males and females, respectively) were observed. At 13 weeks, there was approximately a 10% decrease in body weight gain for the high[redacted] group males, and the female mice in that group gained the same amo[redacted] weight during the first three months of the experiment.

Although mortality was not statistically significantly increased at the highest dose in male mice, it was 65% in the 1500 ppm dose group compared to 51% in the control group. The incidence of amyloidosis was statistically significantly increased in male and female mice at the highest dose level ($p < 0.01$; Fisher's Exact Test), and the incidence of bilateral seminiferous degeneration (atrophy) and oligospermia was statistically significantly increased in 200 and 1500 ppm group males. Amyloidosis was also increased in females from the 1500 ppm dose group. Thyroid inflammation was statistically significantly increased in both sexes at the highest dose.

006933

CONCLUSIONS (continued)

Based on the increased incidence of bilateral seminiferous degeneration and oligospermia in mid dose group male mice, the suggested NOEL was 20 ppm (3 mg/kg/day), and the LEL was 200 ppm (30 mg/kg/day).

Under the conditions of the study, Express was not oncogenic.

Core classification: Supplementary. Body weight results, mortality late in the study, and the incidence of age-related effects suggested that adequate dose levels for assessment of the oncogenic potential of Express® in male mice were used. However, results from female mice in the highest dosed group suggest that an adequate dose range was not tested.

I. PROTOCOL

A. MATERIALS

1. Test species: Male and female 29-day-old Charles River Crl:CD-1(ICR) BR strain mice were used. Their weights ranged from 16 to 24 g. for males and 13 to 22 g. for females. The animals were placed on test diets 17 days after their arrival at the laboratory.

2. Diet preparation: Basal diet consisted of Purina Lab Chow #5002, and the test substance was added in corn oil in appropriate concentrations. (Corn oil was 1% by weight of the diet.) Test diets were prepared weekly 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 174, 363, and 545. The report noted that on test day 440 concentration and homogeneity analyses were made of test diets because a change was made in the mixer used for diet preparations for the rest of the study.

B. STUDY DESIGN

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

Test Group No.	Designation	Dose (ppm)	Animals per sex: Main study*	Animals per sex: Interim Sacrifice**
1	Control	0	80	10
2	Low (LDT)	20	80	10
3	Mid	200	80	10
4	High (HDT)	1500	80	10

*24 months. **At 12 months

2. Observation schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day
Signs of toxicity	All	Twice a day*
Body weight	All	On day of arrival at lab, at weekly intervals through the first 6 months, bi-weekly thereafter, and on the day of necropsy.
Food consumption	All	For all weighing intervals during the study.**
Ophthalmology	High and low dose groups only	At the end of the study.
Blood samples	10***	At 3, 6, 9, 12, and 18 months.
Necropsy	Animals found dead or moribund	When found.
	10 Survivors	At 12 months At 24 months

*Each mouse was individually handled at least once each week during the first six months of the study and every other week during the remainder of the study. The gross presence of tissue masses and changes in appearance and behavior were noted.

**For each individual animal.

***The report stated that 10 animals of each sex were selected at random "...on the basis of freedom from any lesions which appeared to be of a spontaneous origin and which occurred with a similar frequency in control group mice."

C. METHODS

1. Observations of blood samples: Blood was collected by amputation of the distal portion of the tail from animals which were fasted for 16 hours prior to sampling.

Hematology

X	Hematocrit	X	Differential white cell counts
X	Hemoglobin	X	Mean corpuscular hemoglobin concentration
X	Red cell count	X	Mean cell volume
X	Platelet count	X	Mean corpuscular hemoglobin
X	Total white cell count		

2. Necropsy Gross lesions were noted.

a. Weighed organs

X Liver	X Spleen	X Brain
X Kidneys	X Heart	X Testes

The kidneys were weighed with adrenals attached, and the testes were weighed with epididimydis attached.

b. Tissues examined microscopically

Circulatory System

X Aorta
X Heart

Digestive System

X Cecum
X Colon
X Duodenum
X Esophagus
X Ileum
X Jejunum
X Liver
X Pancreas
X Rectum
X Salivary gland
X Stomach
X Gallbladder

Endocrine System

X Adrenals
X Pituitary
X Thyroid with parathyroid

Hematopoietic System

X Bone marrow
X Lymph nodes
X Spleen
X Thymus

Musculoskeletal System

X Bone
X Skeletal muscle

Nervous System

X Brain
X Sciatic nerve
X Spinal cord

Other

X All macroscopic abnormalities
X Eye
X Hardarian gland
X Skin and subcutis

Reproductive System

X Epididymides
X Mammary glands
X Ovaries
X Prostate
X Seminal vessicles
X Testes
X Vagina
X Uterus with cervix

Respiratory System

X Lungs
X Nasal turbanates
X Trachea

Urinary System

X Kidneys
X Urinary bladder

Tissue samples from the control and high dose groups as well as mice found dead or sacrificed in extremis were examined microscopically. The report stated that only the heart, liver, kidneys, lungs, and organs with gross lesions from mice in the low and mid dose groups were examined.

D. STATISTICAL ANALYSIS

Observation	Statistical Test
Continuous Variables	
Body weights	One-way analysis of variance (ANOVA) Least Significant Difference test (LSD)
Organ weights	ANOVA with pair-wise comparisons by LSD and Dunnett's tests, and a test for linear trend
Clinical pathology	ANOVA and the Bartlett's test; if the F-test was significant, means of each treated group were compared with that of the appropriate control group by Dunnett's test; if results of the Bartlett's test were significant, the Kruskal-Wallis and Mann-Whitney tests were used to compare control group means with each treated group mean.
Non-Parametric Variables	
Survival among groups	Mantel-Haentzel and Fisher's Exact tests
Tumor incidence*	Fisher's Exact test
Other Analyses	
Survival probability	Kaplan-Meier procedure

*Tumors were analyzed by specific site, lesion, and benign-malignant classification.

II. REPORTED RESULTS

A. Mortality and Signs of Toxicity: Mortality during the study is summarized as follows:

Dose (ppm)	Mortalities during days: Males 0-365	Males 366-553	Males Termination	Females 0-365	Females 366-546	Females Termination
0	3	38	39	4	40	36
20	3	45	32	4	45	31
200	7	36	37	2	39	39
1500	5	47	28	2	46	32

One female mouse from the 200 ppm group died during the first week of the study and was replaced by a pretest mouse. The report also noted that one male from the 200 ppm dose group and one female from the 20 ppm dose group were accidently killed during the study.

62

006833

A. Mortality and signs of toxicity (continued)

According to the report the following clinical signs were slightly elevated but not attributed to treatment:

Location	Dose level (ppm) * 0	20	200	1500
	Males			
Irregular respiration	1	2	0	6
Weakness	13	15	13	20
Hunched appearance	2	8	1	7
Swollen eyes	6	10	13	11
	Females			
Cyanosis	9	11	9	18
Exophthalmus	6	8	9	18
Colored occular discharges	3	7	3	10
Pallor	13	19	24	20
Ruffled fur	5	10	8	13

* No statistical evaluations of these results were conducted according to the report.

B. Body Weight and Food Consumption:

The report noted that at 18 months the respective group mean body weights for the high dose group males and females were 6.8 and 10.3% less than control group means. The report indicated that the weight decreases were observed from days 133 to 517 in males and from day 182 to 546 for female mice.

The investigators noted statistically significantly decreased group mean body weight gains in male and female mice at the 1500 ppm dose level in comparison with that of the control groups during the first six months of the study. The high dose group females also had statistically significantly decreased body weight gains during the last year of the feeding period. These differences are summarized as follows:

006833

B. Body Weight and Food Consumption (continued)

Weeks of observation	Dose level (ppm) 0	20	200	1500
		Males		
0-26	12.0	12.4	12.8	9.9*
26-52	1.6	0.9	0.8	1.0
52-81	-1.9	-2.1	-1.7	-1.6
0-81	13.6	12.3	12.4	10.4
		Females		
0-26	10.3	10.7	9.9	9.2*
26-52	3.9	3.2	4.4	3.1
52-81	0.7	0.2	-0.5	0.5
0-81	15.8	13.7	13.9	12.6*

* Statistically significantly different from control ($p < 0.05$; LSD test).

There were no significant differences in group mean food consumption or food efficiency observed according to the report.

C. Test substance intake: According to the report, dietary analyses indicated that test substance concentrations were within ±12% of the nominal concentrations, and homogeneity tests indicated a ±15% variation in concentration. Based on results of these analyses, body weight and food consumption measurements, the daily intake of test substance was calculated. For males the daily doses were reported to be 2.5, 25, and 197 mg/kg/day during the entire study. Those values for female mice were 3.1, 31, and 247 mg/kg/day.

D. Ophthalmology: No significant effects were noted by the investigators.

E. Clinical Pathology - Hematology: The investigators noted that there were sporadic statistically significant differences between treated and control groups, but the differences were described as unrelated to dose, and they were reported to be within normal ranges.

F. Necropsy

1. Organ weights: Organ weight changes were consistent with the reduced body weight observed in the study. The mean values are summarized as follows:

Time of observation	Dose level (ppm) Males 0	Males 1500	Females 0	Females 1500
Males				
Body weight (g)	44.2	44.2	44.5	41.5*
Liver weight (g)	2.273	2.303	2.446	2.574
% Body weight	5.195	5.234	5.493	6.182*
Females				
Body weight (g)	41.1	39.2	39.1	37.3*
Liver weight (g)	2.098	2.020	2.113	2.179
% Body weight	5.142	5.195	5.480	5.814 *

*Statistically significantly different from control ($p<0.05$; LSD and Dunnett's tests).

2. Non-neoplastic lesions: The investigators described the microscopic lesions they observed as follows:

> Histopathology data revealed several minor modifications in the normal lesions of aging within the male and female 1,500 ppm dose groups. These included a slight increase in the severity of amyloidosis and marginal changes in some background inflammatory lesions. A specific target organ was not identified...In general, both the incidence and severity of amyloidosis was slightly greater in the 1,500 ppm male group than in other male groups. Examination of the organs which were most consistently infiltrated with amyloid (kidneys, liver, heart, thyroid, jejunum, ileum, and adrenal cortex) indicates that the increased incidence was not statistically significant. The incidence of amyloid in other organs (i.e., secondary target organs) is more an indication of the severity within the individual rather than the incidence within a group. A statistically significant increase of amyloid was observed in a few of these secondary target organs (glandular stomach, mesenteric lymph nodes, and testes) in the male 1,500 ppm dose group. Amyloidosis was also slightly increased in incidence and severity in the female 1,500 ppm group, although only two organs (glandular stomach and mandibular salivary glands) demonstrated statistically significant increases.

2. Non-neoplastic lesions (continued)

Other lesions the investigators associated with the increased severity of amyloidosis included testicular atrophy and oligospermia. The reported incidences of amyloidosis and testicular effects are summarized in Table 1 below.

The report also noted a statistically significant increase in the incidence of thyroid inflammation in the high dose group male and female mice. The severity of these lesions was slight in male mice and slight to mild in female mice in the test group, and the authors characterized the lesions as possible indications of the catabolic condition of the animals in the highest dosed group.

3. Neoplastic lesions: The incidences of the most frequently observed tumors are summarized in Table 2. below. According to the report there was no signiifcant increase in the incidence of any tumors in mice treated with the test substance.

006833

Table 1

Incidence of amyloidosis and related lesions in male and female mice treated with Express® in their diets for up to 18 months. †

Observation	Dose level (ppm) 0	20	200	1500
Males				
Liver	40/80	44/80	48/80	49/79 *
Jejunum	47/78	43/73	51/78	52/74
Ileum	41/74	40/70	46/73	48/71
Kidney (amyloid)	48/80	48/80	52/80	56/80
Focal atrophy (secondary to amyloid deposition)	11/80	13/80	15/80	21/80 *
Adrenal cortex	44/80	44/78	50/80	52/80
Heart	44/80	44/80	50/80	52/80
Glandular stomach	31/78	35/79	39/78	48/78 **
Lymph node (mesenteric)	28/77	26/70	35/78	44/76 **
Testes (amyloid)	20/79	21/80	34/80 *	42/78 **
Edema	0/79	6/80**	13/80***	6/78 **
Seminiferous degeneration (bilateral)	38/79	41/80	46/80	54/78 **
Epididymides (amyloid)	17/80	11/80	18/80	18/78
Oligospermia (bilateral)	22/80	26/80	36/80 *	43/78 ***
Thyroid (amyloid)	42/80	46/80	47/79	49/79
Inflammation	0/80	0/80	0/79	8/79 **
Females				
Liver	47/80	50/80	49/79	56/80
Jejunum	44/74	52/76	47/74	50/71
Ileum	48/72	52/74	50/75	57/74
Kidney	54/80	54/80	54/80	60/80
Adrenal cortex	45/80	46/80	50/80	56/80
Heart	47/80	51/80	49/80	58/80
Glandular stomach	39/79	44/78	46/78	51/79 *
Salivary gland (mandibular)	11/80	13/80	15/80	25/80 **
Salivary gland (parotid)	45/80	51/80	50/80	53/80
Thyroid (amyloid)	47/79	53/79	50/80	56/79
Inflammation	9/79	5/79	14/80	27/79**

* Statistically significantly different from control ($p<0.05$; Fisher's Exact test).
** Statistically significantly different from control ($p<0.01$; Fisher's Exact test).
*** Statistically significantly different from control ($p<0.001$; Fisher's Exact test).

006833

E. Necropsy (continued)

Table 2

Incidence of selected neoplastic lesions in male and female mice treated with Express® in their diets for up to 24 months.

Observation	Dose level (ppm) 0	20	200	1500
Males				
Liver (number examined)	80	80	80	79
Hepatocellular adenoma	6	3	4	5
Hepatocellular carcinoma	4	2	6	3
Lungs (number examined)	80	80	80	80
Broncho-alveolar adenoma	15	4 **	4 **	3 **
Broncho-alveolar adenocarcinoma	3	1	2	1
Hardarian gland (number examined)	80	80	80	80
Adenoma	5	7	9	3
Animals with tumor (any type)	34	22	27	21
Females				
Lungs (number examined)	79	80	80	80
Broncho-alveolar adenoma	5	5	5	6
Broncho-alveolar adenocarcinoma	1	0	2	2
Miscellaneous (number examined)	20	19	25	18
Lymphoma (lymphocytic)	5	4	8	3
Lymphoma (histiocytic)	20	19	25	18
Animals with tumor (any type)	28	17	27	22

** According to the report, the incidence is statistically significantly different from the control group (Fisher's Exact test, $p < 0.01$).

III. DISCUSSION

A. Authors' Conclusions

The investigators concluded:

> Effects attributable to the dietary intake of INL-5300 by mice in this study were minimal. Mean body weights for both male and female mice in the 1,500 ppm group were lower when compared to controls. Evaluation of mean final body weight at sacrifice confirmed a statistically and/or biologically

A. Authors' Conclusions (continued)

significant decrease in body weights at the 1,500 ppm level in both sexes. Organ weight data revealed an increase in relative liver weights in these same groups. However, this effect was interpreted to have no major biological significance and was considered to be related to the lower mean final body weights observed.

Histopathology data revealed several minor modifications in the normal lesions of aging within the male and female 1,500 ppm dose groups when compared to their respective control groups. These included a slight increase in the severity of amyloidosis and some marginal changes in some background inflammatory lesions. A specific target organ was not identified. In addition, secondary changes observed in a few organs (thyroid, testes, and epididymus) were considered to be directly related to the amyloidosis observed and to the slightly catabolic condition seen in these groups.

INL-5300 was not carcinogenic in mice under the conditions of this study.

No other effects observed in this study could be considered compound related. Therefore, the no-observed-effect level (NOEL) for the dietary intake of INL-5300 for mice this study was 200 ppm.

B. Reviewer's Discussion

The decrease in group mean body weights for male and female mice in the 1500 ppm dose group were approximately 6 and 5% below control values at the end of the study, respectively. Overall weight gains for the high dose group were decreased in comparison to controls by 24% for males and 20% for females. Food consumption results and clinical signs observed in the study suggested that the body weight decreases were not associated with other effects such as diarhea, anorexia, emaciation, or poor palatability of test diets. There were also no statistically significant absolute organ weight decreases reported.

Thr group mean body weight results were used to determine effects on body weight gain at 13 weeks during the study. The results of those calculations are summarized as follows:

B. Reviewer's Discussion (continued)

Observation	Dose level (ppm) 0	20	200	1500
		Males		
Body weight at				
Week 0	29.2	29.6	29.5	29.1
Week 13	38.7	39.4	39.8	37.8
Weight gain	9.5	9.8	10.3	8.5
% difference *	---	+ 3.2	+ 8.4	-10.5
		Females		
Body weight at				
Week 0	23.2	23.1	23.3	22.9
Week 13	30.6	30.8	30.3	30.9
Weight gain	7.4	7.7	7.0	7.4
% difference *	---	+ 4.1	- 5.4	0.0

* Calculated as follows:

$$\% \text{ difference} = \frac{(\text{control weight gain} - \text{test group weight gain})}{(\text{control weight gain})} \times 100$$

By the end of the study survival in the control, low, mid, and high dose group male mice was 49, 40, 46, and 35%, respectively. Survival rates in those groups of female mice were 45, 39, 49, and 40%, respectively. These survival rates were not statistically significantly different according to the report, and the most frequently identified probable cause of death in the study was amyloidosis.

The only microscopic observations associated with the administration of the test substance were increases in the incidence and severity of lesions indicative of aging (amyloidosis, seminiferous degeneration, and oligospermia in males, and amyloidosis in females). The investigators suggested that the effects in the testes and epididymus were a result of severe amyloidosis, but the incidence of those effects (see page 9 above) is much greater than that of amyloid in the testes or epididymus. Edema in the testes was also statistically significantly increased in all dose groups, but most of those lesions occurred with amyloid in the testes and their incidences were not dose related.

The incidence of oligospermia was statistically significantly greater in the mid and high dose groups than the control group. A review of individual animal data indicated that oligospermia occurred in animals with moderate to severe seminiferous degeneration, and the proportion of animals with seminiferous degeneration in each group having oligospermia increased with dose (58, 63, 78, and 80% for the control, low, mid, and high dose groups, respectively). These results support the investigator's conclusion that a dose-related increase in severity of effects on the

B. Reviewer's Discussion (continued)

testes of mice treated with Express. Since these effects occurred during the last 6 months of the study, they are probably related to the age of the animals.

Based on the increased severity of seminiferous degeneration as indicated by a statistically significantly increased incidence of oligospermia in mid and high dose group male mice, a no-observed-effect level of 20 ppm (3 mg/kg/day; lowest dose tested) is suggested. The lowest-effect level (LEL) was 200 ppm, and the reduced body weight gain in male mice indicated that adequate dose levels were tested in that sex.

The incidence of age-related effects and the absence of significant weight loss during the first 13 weeks of the study in female mice suggests that an adequate dose range for assessment of the oncogenic potential of Express® was not used in this study.

The report stated that a four-week range-finding study and a 90-day feeding study were conducted to provided a basis for selection of the doses used in the oncogenicity study. Results from the 90-day study were described in the report as follows:

> The dose levels for the ninety-day study were 0, 125, 500, 1,250, and 2,500 ppm...
>
> - No compound-related effects on mean body weight, mean body weight gains, food consumption, food efficiency, or clinical signs.
> - No compound-related effects on measured hematological parameters.
> - Significantly elevated mean absolute and relative liver weights in female mice in the 2,500 ppm group.
> - Elevated mean relative liver weights in male mice in the 2,500 ppm group.
> - Elevated mean relative liver weights in male and female mice in the 1,250 ppm group.
> - No gross or histological changes attributed to dietary administration of IN L5300.
>
> The no-observed-effect level for the ninety-day study was considered to be 500 ppm.

Since no data were submitted to support these conclusions, the toxicological significance of the results can not be determined, and the adequacy of the dose levels tested in the oncogenicity study is not supported.

The incidence of tumors was not increased in male or female mice under the limited conditions of the study.

006833

Reviewed by: Roger Gardner RGM Gardner 9-19-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 8/19/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Rabbits (Guideline §83-3)

MRID NUMBER: 402455-14

TEST MATERIAL: Technical grade INL-5300 with a stated purity 94.2% 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): 150-86

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine

TITLE OF REPORT: IN L5300. Developmental Toxicity Study in Rabbits Dosed by Gavage on Days 7-19 of Gestation.

AUTHOR(S): Zellers, J. E.

REPORT ISSUED: April 8, 1986

CONCLUSIONS: Groups of 22 pregnant New Zealand White rabbits were given daily doses of 0, 5, 20, or 80 mg DPX-L5300 by gavage on gestation days 7 through 19.

Based on statistically significantly decreased feed consumption and increased incidence of abortions at the highest dose, the lowest-effect level for maternal toxicity was 80 mg/kg/day. The no-observed-effect level (NOEL) was 20 mg/kg/day. The LEL for fetal effects (10% reduction in fetal weight without statistical significance) was also 80 mg/kg/day, and the NOEL is 20 mg/kg/day. There were no fetal malformations or variations associated with administration of the test substance in pregnant rabbits.

Core classification: Minimum

I. PROTOCOL

72

A. Materials

Test species: Six-month old female New Zealand White strain rabbits were used. The Day the animals were artificially inseminated was designated Day 0 of gestation.

B. Experimental procedures: The test substance was suspended in aqueous 0.5% methyl cellulose and administered by gavage on Days 7 through 19 of gestation. Doses of 0, 5, 20, or 80 mg test substance per kg body weight were given to groups of 22 inseminated does.

C. Maternal observations: Each doe was observed at least once daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0, 7 through 20, 24 and 29 of gestation, and food consumption was measured daily during gestation.

Surviving rabbits were weighed and sacrificed on day 29 of gestation. Internal organs were examined for gross lesions, and liver weights were obtained. The numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted.

Does dying prior to the end of the study were examined for gross pathological changes and to determine the cause of death when possible. The pregnancy status of these animals was also noted.

D. Litter observations: The number of implantation sites, live and dead fetuses, and resorptions were counted.

E. Fetal observations: The position of each live fetus in utero was recorded, and they were weighed and examined for external abnormalities. The fetuses were then internally sexed, and their viscera were examined for variations and malformations. The brain was examined through a transverse section between the parietal and frontal bones of the unfixed fetal head.

Fetuses were then fixed in 70% ethanol and macerated in potassium hydroxide. The skeletons were then stained with alizarine red S for examination.

F. Evaluation of observations: The report stated that the litter was considered the experimental unit. The proportion of affected fetuses/litter or the litter mean of each parameter was subjected to statistical analysis, and differences were considered to be statistically significant at $p < 0.05$.

Statistical analyses were listed in the report as follows:

Observation	Test for Linear Trend	Between Groups
Incidence of pregnancy Clinical signs Maternal death Litters with fetal resorptions	Cochran-Armitage	Fisher's exact
Maternal body weight Maternal body weight change Feed consumption Liver weight (absolute & relative)	Orthogonal polynomial of dose ranks *	Dunnett's, when one-way ANOVA was significant *

E. Evaluation of Observations (continued):

Observation	Test for Linear Trend	Between Groups
Implantations	Jonckheere's **	Mann Whitney U **
Live fetuses		
Dead fetuses		
Resorptions		
Corpora lutea		
Male fetuses		
Female fetuses		
Fetal weight		
Percent resorptions		
Incidences of fetal alterations		

* If Bartlett's test is significant ($p < 0.05$), then Jonckheere's test is used to determine significance of trends, and the Fisher's exact test is used to determine the significance of differences between groups.

** When more than 75% ties occurred in reproductive and fetal parameters, the Cochran-Armitage test was used to detect significant linear trends, and the Fisher's exact test was used to determine the significance of differences between groups.

II. REPORTED RESULTS

A. Maternal observations: The only sign observed during gestation and attributed to the test substance was red discharges on the cageboard. This effect was significantly increased in the high dose group in comparison to the control group during gestation days 20 through 29. The overall incidence of red discharge on cageboards according to individual animal data was 5/22 in the control group compared to 9/22 in the high dose group.

One female from the high dose group was found dead on gestation day 17 with hepatization of the lungs. Another female from the high dose group, which previously aborted a fetus and had four more in utero, was found dead on day 29 of gestation. A female from the mid dose group died just prior to scheduled sacrifice and was found to have multiple mucosal hemorrhages of the stomach associated with a trichobezoar. All three of these animals were pregnant.

During the dosing period, the high dose group exhibited statistically significant decreases in body weight gain in comparison to the control group. After dosing the high dose group had a statistically significantly increased body weight gain when compared with that of the control group. These results are summarized as follows:

A. Maternal observations (continued)

Dose (mg/kg/day)	Mean weight (kg) on gestation† Day 0	Day 29††	Weight change (kg) for Days 7-20	Days 0-29
0	3863.8	3846.9	114.4*	-16.9
5	3822.9	3721.5	12.1**	-101.4
20	3868.9	3816.1	63.4	-52.8
80	3853.2	3684.9	-325.0**	-168.3

†The report did not include statistical analyses of these results.
††Body weights at day 29, corrected for gravid uterine weight.
†††These values were calculated from group mean body weights. No statistical analyses were conducted on these data.
*Statistically significant trend ($p<0.05$).
**Statistically significantly different from the control group ($p<0.05$).

The report noted that feed consumption was decreased in the high dose group during the dosing period, and in the low dose group it was decreased during the post dosing period. These results are summarized as follows:

Dose (mg/kg/day)	Feed Consumption (g/day) on Days 7-10	10-13 *	13-16 *	16-20 *	20-24
0	147.9	144.3	133.7	132.4	135.0
5	147.4	139.0	111.2	100.4	116.0**
20	148.4	131.5	110.3	101.4	129.1
80	114.7**	80.8**	61.1**	44.3**	90.8**

*Statistically significant trend ($p<0.05$).
**Statistically significantly different from the control group ($p<0.05$).

According to the report there were no compound related effects observed at gross necropsy, and there were no significant effects on absolute or relative liver weights.

A. Maternal observations (continued)

Pregnancy status in each of the test groups is summarized as follows:

Parameter	Dose (mg/kg/day) 0	5	20	80
Number inseminated	22	22	22	22
Number pregnant	18	18	20	19
Deaths	0	0	1	2*
Abortions	1	0	1	7** ***
Total resorptions	1 †	1 †	1 †	0
Number of litters	16	17	17	11

*Both were pregnant.
**One of these also died before the end of the study.
***Statistically significantly different from controls ($p < 0.05$; Fisher's exact test).
†Pregnancy determined by ammonium sulfide staining.

B. Litter observations: The report summarized these observations (means per litter) follows:

Parameter	Dose (mg/kg/day) 0	5	20	80
Number of litters	16	17	17	11
Live fetuses				
Males	4.0	4.4	5.0	3.5
Females	4.1	3.7	4.0	2.4*
Total	8.1	8.1	9.0	5.8*
Dead fetuses	0.0	0.0	0.0	0.0
Resorptions	0.8	0.6	0.6	0.6
Implantations	8.9	8.7	9.6	6.5*
Corpora lutea	11.2	12.8	12.4	11.3
Fetal weight (g)				
Males	43.79	42.73	42.69	39.19
Females	42.96	43.26	41.25	38.57
Both sexes combined	43.32	43.36	42.53	39.13

*Statistically significantly different from controls ($p<0.05$).

C. Fetal observations: The report noted that the mean percentage of fetuses per litter with malformations was statistically significantly increased at the highest dose level. These results are reproduced from the report in Addendum A below.

C. Fetal observations (continued)

The mean percentage of fetuses per litter with variations in the low dose group was also statistically significantly increased. These results are reproduced from the original report in Addendum B below.

III. DISCUSSION

A. Authors' Conclusions

The investigators discussed the results of the study as follows:

> No significant differences in mortality were observed among the groups, but three females...were found dead during the study. Whether these deaths were associated with the abortion process, disease, INL-5300 treatment or a combination of these factors could not be determined.
>
> ...On the basis of the high variability in body weight change and feed consumption among these rabbits, the lack of a dose-response relationship, and the absence of a rebound effect following cessation of treatment at the lower dose levels, the test substance did not appear to have any clear maternal toxicity at the low or intermediate INL-5300 levels.
>
> ...In addition to a significant increase in abortions, the high dose group also had significant decreases in the number of nidations and live fetuses per litter. As no significant difference in the number of corpora lutea per litter was observed, the reason for this decrease in nidations was unclear. The reduction in litter size resulted in a reduction of both males and females...The difference in the number of males and females in the group was...due to normal variation and not to be compound related.
>
> ...In conjunction with the decrease in nidations in this group, reductions in fetal weight were considered to be biologically significant. The reduced fetal weights were probably related to maternal toxicity, but a direct test substance effect can not be ruled out.
>
> The percentage of malformed fetuses was significantly increased in the high dose group, but not in the other test substance groups. The mean percentage of fetuses with developmental variations was significantly increased at the low dose level, but the percentages of the intermediate and high dose groups were not significantly different from the control group. No significant differences in the rates of variations due to retarded development, or in the mean percent of fetuses with variations were observed. As no dose-response relationship was evident and total variations were not significantly increased for the low dose group, the increase in variations for this dose group was not demonstrated to be due to the test substance.

77

A. Authors' Conclusions

The authors concluded:

> On the basis of significant increases in abortions, significant effects on feed consumption and maternal body weight, lower fetal weights, and a significant increase in the percentage of malformed fetuses, both maternal and embryo-fetal toxicity occurred at the 80 mg/kg level. At lower levels, INL-5300 did not cause definitive maternal or embryo-fetal toxicity.
>
> Thus, the apparent no-effect level for the female rabbit and fetus is 20 mg/kg.

B. Reviewer's Discussion

Maternal toxicity: Individual animal data suggested that the variability in body weight change results for each group was large. The standard deviations for means in all groups were greater than the mean changes determined from day 7 onward in the study. Therefore, these data are not a reliable indication of maternal toxicity by themselves.

Feed consumption results were not as variable. In the high dose group consistently lower feed consumption was observed in comparison to control group values at the beginning of the dosing period (20% less). By the end of the dosing period feed consumption was 67% less in the high dose group animals than in controls, and during the post-dosing period it rebounded somewhat (33% less than controls) (see Page 4 above). Despite these changes in feed consumption, group mean maternal body weight for the high dose group at the end of the study was only 4% less than that for the control group (based on terminal body weights corrected for the weight of gravid uteri). These results suggest that the maternal weight changes observed during the study may have been related to effects on fetal weights.

Feed consumption and body weight results for the low dose group were slightly less than those values for the control group during later portions of the dosing and post-dosing periods. The difference between the control and low dose groups with respect to feed consumption was less than 1% for the first 6 days of the dosing period and 16% less during days 13-16 of gestation. At the end of the dosing period feed consumption in the low dose group was 24% less than the control group, and during the post dosing period it was 14 to 10% less than the control group values. Group mean maternal body weight for the low dose group at the end of the study was 3.2% less than that for the control group.

The pattern for body weight and feed consumption results at the mid dose level was similar to the low dose group's results (see Page 4 above). The control group also exhibited a minimally decreased feed consumption during the dosing period with a minimal increase at the beginning of the post-dosing period.

B. Reviewer's Discussion (continued)

Maternal toxicity (continued)

These feed consumption results suggested that the test substance enhanced the decreases in feed consumption in a dose-related manner such that statistically significant changes were found at the 80 mg/kg/day dose level (highest dose tested).

Since the number of dead fetuses and resorptions per litter was not affected by treatment, the statistically significantly increased incidence of abortions in the high dose group is an indicator of maternal toxicity.

Based on statistically significantly decreased feed consumption and increased incidence of abortions at the highest dose, the lowest-effect level for maternal toxicity in rabbits is 80 mg/kg/day. The no-observed-effect level (NOEL) is 20 mg/kg/day.

Fetal effects: Malformations occurred in isolated instances (one fetus per group) or data were not available from all test groups (see Addendum A below) to assess the toxicological significance of their incidences. In addition, the litter size in the highest dosed group was significantly reduced, which exaggerated the percentage of malformed fetuses per litter observed. Therefore, the significance of the increased proportion of malformed fetuses per litter in the highest dose group is more likely to be statistical rather than toxicological.

Since there was no increase in the number of resorptions or dead fetuses, data suggest that the reduced litter size in the highest dosed group is the result of reduced implantations in that group. Because administration of the test substance began at or soon after the time of implantation, the reduction is unlikely to be the result of the test substance.

Individual animal data indicated the following pattern of variability for litter size and sex distribution:

Observation	Control	High dose group
Number of litters	16	11
No. litters with <50% female fetuses	9	6
Total fetuses/litter		
Mean	8	5.8
Range	5 - 13	2 - 11
Female fetuses/litter		
Mean	4.0	2.4
Range	2 - 7	0 - 5

The proportion of litters with fewer female than male fetuses (56% in the control and 55% in the high dose group), a similar range for litter sizes (8 in the control group and 9 in the high dose group), and a similar range

B. Reviewer's Discussion (continued)

Fetal effects

for the number of female fetuses per litter in the control and high dose groups (5 for both groups) are similar. In addition, fetal weight for males and females in the high dose group was similarly decreased below those in the control group (10 to 11% without being statistically significant). These results suggest that the reported significant decrease in female fetuses at the high dose level is not toxicologically significant.

Reduced fetal weights were not associated with an increase in the incidence of developmental variations (see Addendum B), andt. These results suggest that fetal effects were the result of reduced maternal feed consumption. Therefore, the LEL for fetal effects is also 80 mg/kg/day, and the NOEL is 20 mg/kg/day.

ADDENDUM A

Incidence of Malformations in Fetuses from Rabbits Treated with Express Herbicide

MR-7409-002
H-15601

TABLE 5

INL-5300. DEVELOPMENTAL TOXICITY STUDY IN RABBITS
DOSED BY GAVAGE ON DAYS 7-19 OF GESTATION

FETAL MALFORMATIONS

	DAILY DOSE (mg/kg) 0.0	5.0	20.0	80.0
NO. EXAMINED [Fetuses(Litters)]	130(16)	138(17)	153(17)	64(11)
External				
No. Affected	0	0	1(1)	1(1)
Abdomen - Gastroschisis	...	...	...	1(1)[c]
Head - Exencephaly with Open Eye	...	...	...	1(1)[c]
Paw - Clubbed	...	...	...	1(1)[c]
Umbilicus - Hernia	...	...	1(1)	...
Visceral				
No. Affected	0	0	0	1([redacted]
Kidney - No Papilla (Size 0)	...	...	...	1(1)[d]
Head				
No. Affected	0	1(1)	1(1)	3(2)
Brain - Hydrocephaly	...	...	1(1)[b]	...
Eye - Cataract	...	1(1)	...	2(1)[d]
Skeletal				
No. Affected	0	1(1)	1(1)	3(2)
Rib - Fused	...	1(1)[a]	...	1(1)[a]
Sternebra - Fused	...	...	1(1)[b]	...
Vertebra - Hemivertebra	...	1(1)[a]	...	3(2)[a]
TOTAL NUMBER AFFECTED	0(0)	2(2)	2(2)	6(4)
MEAN PERCENT AFFECTED PER LITTER[+] (±S.E.M.)	0.0	1.6 (1.11)	1.3 (0.91)	13.4* (6.28)

* Significantly different from control values, $p \leq 0.05$.
\+ Significant trend in groups, $p \leq 0.05$.
a-e Same fetus affected.
... No data.

82

006833

ADDENDUM B

Incidence of Variations in
Fetuses from Rabbits Treated with
Express Herbicide

MR-7409-002
H-15601

TABLE 6 (CONT.)

INL-5300. DEVELOPMENTAL TOXICITY STUDY IN RABBIT
DOSED BY GAVAGE ON DAYS 7-19 OF GESTATION

FETAL VARIATIONS

	DAILY DOSE (mg/kg) 0.0	5.0	20.0	80.0
DEVELOPMENTAL VARIATIONS (Cont.)				
Skeletal				
No. Affected	45(13)	72(15)	54(15)	37(9)
Hyoid - Bent	2(2)	3(3)	3(2)	...
Rib - Rudimentary (Lumbar 1)	14(10)	20(10)	19(11)	7(7)
- Thickened	...	1(1)	...	...
- Extra Ossification Site (Lumbar 1)	2(2)	4(3)	5(5)	..
- Extra (Lumbar 1)	32(10)	55(14)	34(14)	27( )
Sternebra - Bipartite	2(2)	5(3)	1(1)	..
- Extra	...	...	...	1( )
Vertebra - Extra (Thoracic 13 with ribs)	...	...	...	4(1)
TOTAL WITH DEVELOPMENTAL VARIATIONS	90(16)	125(16)	109(16)	52(11)
MEAN PERCENT AFFECTED PER LITTER (±S.E.M.)	69.5 (5.54)	83.9* (6.12)	66.7 (6.62)	78.4 (6.98)
VARIATIONS DUE TO RETARDED DEVELOPMENT				
No. Affected	30(13)	47(12)	66(13)	33(8)
Skeletal				
Femur - Partially Ossified	...	2(1)	3(2)	10(2)
Humerus - Partially Ossified	...	1(1)	...	3(2)
Hyoid - Partially Ossified	4(3)	2(2)	11(5)	6(4)
- Unossified	...	...	5(1)	...
Pubis - Partially Ossified	...	...	...	2(2)
- Unossified	...	1(1)	...	1(1)
Rib - Partially Ossified	...	1(1)	...	...

84

006833

MR-7409-002
H-15601

TABLE 6 (CONT.)

INL-5300. DEVELOPMENTAL TOXICITY STUDY IN RABBITS DOSED BY GAVAGE ON DAYS 7-19 OF GESTATION

FETAL VARIATIONS

	DAILY DOSE (mg/kg) 0.0	5.0	20.0	80.0
VARIATIONS DUE TO RETARDED DEVELOPMENT (Cont.)				
Skull - Frontal Partially Ossified	4(1)	3(2)	3(1)	7(2)
- Interparietal Partially Ossified	...	1(1)	2(1)	...
- Parietal Partially Ossified	...	...	1(1)	1(1)
- Nasal Partially Ossified	...	1(1)	...	...
Sternebra - Partially Ossified	18(11)	38(12)	43(13)	21([redacted])
- Unossified	6(4)	7(5)	16(6)	5([redacted])
TOTAL WITH VARIATIONS DUE TO RETARDED DEVELOPMENT	30(13)	47(12)	66(13)	33(8)
MEAN PERCENT AFFECTED PER LITTER (±S.E.M.)	23.8 (5.65)	30.0 (6.99)	38.5 (7.34)	43.3 (9.79)
TOTAL WITH VARIATIONS	99(16)	128(16)	123(16)	56(11)
MEAN PERCENT FETUSES WITH VARIATIONS (±S.E.M.)	77.2 (5.40)	85.9 (6.24)	75.2 (6.56)	84.2 (5.99)

* Significantly different from control values, $p \leq 0.05$.
... No data.

85

006833

Reviewed by: Sanford W. Bigelow, Ph.D. 8/19/88
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Roger Gardner, M.S.
Section VI, Tox. Branch (TS-769C)
Tertiary Reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 8/19/88
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 2 generation repro. - rat (83-4) TOX. CHEM NO: 419S

ACCESSION NUMBER: MRID No.: 402455-15

TEST MATERIAL: IN L5300

SYNONYMS: Express, INL-5300, benzoic acid, 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2yl)-N-methylamino]carbonyl]amino]sulfonyl]-, methyl ester, INL-5300-22, DPX L5300.

STUDY NUMBERS: Haskell Laboratory Report No. 193-86.

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine, P.O. Box 50, Elkton Road Newark, DE 19714

TITLE OF REPORT: Two-Generation Study in Rats with IN L5300.

AUTHOR: Linda S. Mullin

REPORT ISSUED: April 14, 1986

CONCLUSION:

Parental Systemic Toxicity NOEL = 25 ppm (2.0 mg/kg/day)[a]
LEL = 250 ppm (21.0 mg/kg/day)[a] based on decreased body weight gain in the F_{1a} adult females.

Reproductive NOEL = 25 ppm (2.5 mg/kg/day)[b]
LEL = 250 ppm (25.0 mg/kg/day)[b] based on decreased body weight gain in F_{1b} pups on day 7 and in F_{2b} pups on postpartum days 14 and 21.

Developmental Toxicity NOEL = 25 ppm (2.5 mg/kg/day)[b]
LEL = 250 ppm (25.0 mg/kg/day)[b] based on decreased absolute splenic weights in F_{2b} male and female pups.

[a] These mg/kg/day values are calculated from the body weight, dietary analyses, and the food consumption results.

[b] These mg/kg/day values are calculated by multiplying the ppm value by 0.1.

Core classification: **Minimum** This classification is based on the fact that the methodology and data reporting requirements established in the Pesticide Assessment Guidelines, Subdivision F §83-4 have been adequately satisfied.

The reproductive tissues were microscopically examined for 10 male and 10 female parental animals from the F_1 generation only. To fully satisfy the data requirements in Subdivision F §83-4, histological examination is required for:

- reproductive and target organs of all control and high dose F_0 parental animals,
- reproductive and target organs of all control and high dose F_1 (in this case F_{1a}) parental animals, and
- organs demonstrating pathology in the high dose group that should be examined in all F_0 and F_1 parental animals in the mid and low dose groups.

However, based on the absence of significant toxicological effects on reproductive parameters (i.e., mating, fertility, and gestation indices) in this study, the deficiency is not sufficient to reduce the classification of the study to supplementary or invalid.

Special Review Criteria: (40 CFR 154.7) Not triggered by this study.

A. MATERIALS:

1. Test compound:

The test compound, IN L5300, was described as an off-white solid, purity was 94.2%, whose lot number was not provided in this report.

2. Test animals:

Male and female Crl:CD (SD) BR rats, obtained from the Charles River Breeding Laboratories Kingston, NY were used. The males weighed from 23.3 to 59.2 g on receipt at the laboratory. The animals were 38 days of age when placed on test diets.

3. Mating procedure:

Four groups each containing 23 male and 23 female rats were designated the first parental generation (F_o) in the study. After 70 days on test diets, one F_o male was cohabited with a female from the same test group up to 7 days. Each day after pairing the investigators examined each female for the presence of a copulatory plug. The day a plug was discovered was designated day 0 of gestation for the appropriate animal. If no copulatory plug was observed in the 7-day mating period, the female was paired with another male in the same treatment group.

The offspring from the first mating of F_o animals were designated F_{1a} litters, and F_1 parental animals were selected from those litters when the pups were 21 days of age (23 per sex per test group).

F_o animals were mated a second time with different pairings 7 days after the F_{1a} pups were weaned. Those offspring were designated F_{1b} animals. Pregnancy confirmation and premating procedures were the same as those in the first mating of F_1 animals.

After feeding the test diets to F_1 parental animals for approximately 80 days, they were mated in the same manner as the F_o animals to produce the F_{2a} and F_{2b} litters.

4. Diet preparation

The test diet was prepared every week and refrigerated until use. Appropriate amounts of test substance were suspended or dissolved in corn oil, and then mixed with feed to obtain the desired test levels. Corn oil was 1% (w/w) of the feed. Animals received food (Purina Certified Rodent Chow No. 5002) and water ad libitum.

Actual storage time before use did not exceed 13 days. Samples of freshly prepared treated food were analyzed for chemical stability and concentration by liquid chromatography. Samples tested for homogeneity were sampled from the top, middle and bottom of the mixing container. This analysis was performed in the Molecular and Genetic Toxicology Section at the Haskell Laboratory.

B. STUDY DESIGN AND METHODS:

1. Animal assignment

Animals were assigned randomly to test groups (see Table 1). The study was started on January 3, 1985 and was finished on November 11, 1985. The number of animals assigned to each group in each generation was as follows:

Table 1
Animal Assignment in this Study

Test Group	Conc. in diet (ppm)	F_0 generation (#/group) male	F_0 generation (#/group) female	F_{1a} generation (#/group) male	F_{1a} generation (#/group) female
1 Cont	0	23	23	23	23
2 Low	25	23	23	23	23
3 Mid	250	23	23	23	23
4 High	1000	23	23	23	23

2. Dosing schedule:

The F_0 parental animals were given test diets from age 38 days to sacrifice, and the F_1 parental animals were given test diets from weanling (21 days of age) until they were sacrificed.

3. Observations schedule:

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day
Signs of toxicity	All	Twice a day[a]
Body weight	All	On day of arrival at lab, at weekly intervals through the premating period.
	All males and females from unsuccessful matings.	Weekly
	Pregnant females	On days 0, 7, 14, and 21 of gestation and lactation
Food consumption	All	For all weighing interval during the study[b]
Ophthalmology	High and low dose groups only.	At the end of the study.
Necropsy	All animals found dead or moribund[c]	When found.

[a] During the premating period, gestation, and lactation each animal was handled individually and signs of toxicity were noted.
[b] Except the last week of gestation and during lactation for rats bearing litters.
[c] Schedule for other animals is as follows:

Animals	Generation	Scheduled sacrifice
Adult males	F_o	After siring F_{1b} litter
	F_{1a}	After siring F_{2b} litter
Pregnant females	F_o	After weaning of all F_{2b} litter
	F_{1a}	Lactation day 21 of F_{2b} litter
Nonpregnant females	F_o	Same day as pregnant F_o females
	F_{1a}	Same day as 2nd lot of pregnant F_o females.
Weanlings	F_{1a}, F_{1b} F_{2a}, F_{2b}	Lactation day 21 (except F_{1a} weanlings selected for breeding)

CO

4. Necropsy observations:

Parental animals found dead or sacrificed moribund before the end of the study were sacrificed and subjected to gross necropsy. The report stated that for both generations, 10 animals of each sex in each group were examined grossly and the testes, epididymis, and prostate or the corpus and cervix uteri, and vagina were collected for examination. The testes were weighed, and histopathological examination of collected tissues was conducted for the F_{1a} parental animals only.

Ten F_{2b} weanlings of each sex from each group were randomly selected for necropsy. The following organs from all test groups were weighed:

Liver	Kidneys	Spleen
Heart	Thymus	Testes
Lungs		

Tissues from the F_{2b} weanlings in all groups were examined grossly, and those from the control and high dose groups were examined microscopically. The tissues sampled for these observations included the following:

Adrenals	Epididymis	Lungs	Stomach
Bladder	Esophagus	Mesenteric lymph nodes	Testes
Bone	Eye		Thymus
Bone marrow	Heart	Ovaries	Thyroid
Brain	Ileum	Pancreas	Trachea
Cecum	Jejunum	Pituitary	Uterus with cervix
Colon	Kidneys	Rectum	
Duodenum	Liver	Spleen	Vagina

5. Reproductive performance:

The following reproductive indices were calculated:

$$\text{Fertility Index (\%)}^{a} = \frac{\text{number of females bearing litters}}{\text{number of females mated}} \times 100$$

$$\text{Gestation Index (\%)} = \frac{\text{number of females bearing litters with at least one live pup}}{\text{number of females bearing litters}} \times 100$$

$$\text{Percent Pups Born Alive (per litter)}^{b} = \frac{\text{total number of pups born alive}}{\text{total number of pups born}} \times 100$$

$$\text{Viability}^{b} = \frac{\text{total number of pups alive at 4 days postnatal (prior to litter production)}^{c}}{\text{total number of pups born alive}} \times 100$$

$$\text{Lactation Index (\%)}^{b} = \frac{\text{total number of pups alive at weaning (21 days postnatal)}^{c}}{\text{number of pups alive after litter reduction (4 days postnatal)}} \times 100$$

$$\text{Litter Survival (\%)} = \frac{\text{number of litters at weaning}}{\text{number of litters delivered}^{c}} \times 100$$

$$\text{Average Number of Pups/Litter}^{b} = \frac{\text{total number of pups born alive}}{\text{total number of litter delivered}^{c}}$$

a excluding female rats that died during the mating phase or before the last litter of that generation's test group was delivered.

b determined for each litter with a mean and standard deviation calculated for dose level.

c excluding litters that were sacrificed due to death of the maternal rat prior to the weaning date (21 days postnatal).

It should be noted that litters were culled to 4 of each sex when possible 4 days after birth. This was done after calculation of the viability index and before the lactation index were calculated.

6. Statistics and reproduction indices

The following statistical procedures used for analyzing the numerical data:

One-way analysis of variance (ANOVA) was used to analyze the results concerning body weight, body weight changes, food consumption, and organ weight changes. When the F-test results were found to be significant, with the use of the Least Significant Difference (LSD), pairwise comparisons were performed between the means of the exposure and control groups; significance was determined at the $p \leq 0.05$ level.

Fisher's Exact Test with the Bonferroni correction and the Cochrane-Armitage test for trend were used to analyze the incidence of clinical observations.

Fisher's Exact, Kruskal-Wallis, and Mann-Whitney U tests were employed for analyzing the results concerning reproductive and lactation performance.

006833

Reviewed by: Sanford W. Bigelow, Ph.D. 8/2/88
Section VI, Toxicology Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 8/3/88
Section VI, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY:

STUDY TYPE: Metabolism - rat (85-1) CASWELL NO: 419S

ACCESSION NUMBER: MRID NO.: 402455-16

TEST MATERIAL: DPX-L5300

SYNONYMS: Express, INL-5300, benzoic acid, 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester, INL-5300-22.

STUDY NUMBER: Haskell Laboratory Report No. 31-87.

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

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine, P.O. Box 50, Elkton Road Newark, DE 19714

TITLE OF REPORT: Fate of Radiolabelled DPX-L5300 in Rats.

AUTHOR: P.T. Hardesty.

REPORT ISSUED: April 15, 1987.

CONCLUSIONS:

DPX-L5300 is rapidly excreted, does not remain in any one tissue or organ, and is demethylated and/or hydrolyzed to form the same urinary and fecal metabolites identified in both male and female rats.

The distribution, metabolism and the elimination of DPX-L5300 was described in this report. To this end, male and female rats were treated with 3 dose regimens of DPX-L5300. These regimens were: (1) a single low dose (20 mg/kg), (2) a repeated low dose (fed an admixture containing 100 ppm DPX-L5300 for 21 days and then given a single dose of 20 mg/kg), and (3) a single high dose (1800 mg/kg of phenyl-based radiolabel or 2000 mg/kg of a triazine-based radiolabel) of DPX-L5300. A number of DPX-L5300 metabolites were identified in the urine and feces of these rats for a period 96 hours after exposure. Also, the levels of a

number of the identified DPX-L5300 metabolites were measured in a variety of tissues at 96 hours in these rats. In addition, another group of female rats were treated with a single high dose (1700 mg/kg) of either the triazine- or phenyl-based radiolabeled DPX-L5300 and urine and feces were collected for measuring identified DPX-L5300 metabolites for a period of 7 days.

Absorption of DPX-L5300 in rats. No data was provided in this summary report regarding the absorption of DPX-L5300 in rats.

Distribution of DPX-L5300 in rats. No more than 1% of the total applied dose was found in any one tissue or organ 168 hours after exposure. At 96 hours, 28% of the applied dose was found in the gastrointestinal tract of rats treated with a single high dose (1800 mg/kg) of DPX-L5300.

Excretion of DPX-L5300 in rats. At total of 98% of the administered radiolabeled was excreted within 7 days in the urine and feces of male and female rats given DPX-L5300. No radiolabel was found in the expired air of these animals. Within 96 hours after exposure, in the rats treated with a single low dose, an average of 99% is found in the excreta (females: urinary, 69%, fecal, 26%; males: urinary, 65%, fecal, 15%). In rats given repeated low doses at 96 hours, an average of 97% of the applied radiolabel was excreted (females: urinary, 84%, fecal, 12%; males: urinary, 83%, fecal, 14%). Female rats given a single high dose of DPX-L5300 excreted an average of 74% of the radiolabel (urinary, 67%, fecal, 6%). An average of 15% of the remaining radiolabel was found in the gastrointestinal tract (at 96 hours but not at 168 hours). Male rats treated with a single high dose of DPX-L5300 excreted an average of 97% of the applied radiolabel within 96 hours (urinary, 83%, fecal, 14%; the same urinary:fecal differential as male rats given repeated low doses of DPX-L5300).

Metabolism of DPX-L5300 in rats. A number of DPX-L5300 metabolites were identified in groups of rats treated with the 3 dosage regimens. The identified urinary and fecal metabolites were the same in male and female rats, and tissue metabolites of DPX-L5300 identified in a variety of tissues were the same as those found in the urine and feces of male and female rats. The major metabolites of DPX-L5300 in male and female rats were saccharin (a known tumor promoter), metsulfuron methyl, and O-demethyl triazine amine. The two major routes of DPX-L5300 metabolism are the demethylation of the carbamoyl methyl group and the hydrolysis of the carbamate moiety. Saccharin and metsulfuron methyl are the major metabolites of the demethylation route whereas O-demethyl triazine amine and metsulfuron methyl are the major metabolites of the hydrolysis route.

Classification: core-minimum: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied. FIFRA testing guidelines Subdivision F § 85-1 (d)(2)(iv) dictates that 5 animals of each sex be used for the metabolism study and not the 2 animals of each sex that were used in this study. However, considering that the (1) urinary, and fecal metabolites of DPX-L5300 were identified in the male and female rats treated with the 3 dosage regimens and (2) a number of identified DPX-L5300 metabolites were measured in a variety of tissues, testing with 5 animals for each sex is not required.

II. MATERIALS:

A. Test Compound: DPX-L5300

Description: DPX-L5300, technical-grade
Batch #: not provided
Purity: The purity of nonradiolabeled DPX-L5300 used in this study was 94.2%.

Two types of radiolabeled DPX-L5300 were used in this study, radiolabeled either on the molecule, the phenyl moiety or on the triazine moiety. The purity of [phenyl-^{14}C (U)]-radiolabeled DPX-L5300 used in this study was 97.0% with a specific activity of 20.5 uCi/mg. The purity of [triazine-2-^{14}C]-DPX-L5300 was 97.5% with a specific activity of 41.2 uCi/mg.

B. Test Animals:

Species: Rats (male and female)
Strain: Crl:CD BR
Age: 6-8 weeks upon arrival
Weight (week 0): Mean, in grams (range)
females: 215 (212 to 258) males: 271 (214 to 384).
Source: Charles River Breeding Laboratories Kingston, NY.

III. STUDY DESIGN:

A. Animal Assignment:

Animals were assigned randomly to the following test groups:

Table 1
Animal Assignment in this Study

Dosage Group	Daily Oral Dose Given[a] (mg/kg)	Rats male	Rats female	Duration of Exposure
Phenyl-labeled				
1 Low single	20.0	2	2	4 days
2 High single	1800.0	2	2	4 days
3 Repeated	20.0[b]	2	2	25 days
4 High single	1700.0	5	0	7 days
Triazine-labeled				
5 High single	2000.00	2	2	4 days
6 High single	1700.0	5	0	7 days

[a] After the last oral dose was given, the urinary and fecal levels of radioactivity were measured for 4 or 7 days.

[b] The rats were exposed to 100 ppm of test compound in their diet for 21 days, and 24 hours later, given a single dose of 20 mg/kg radiolabeled compound.

B. Diet Preparation: DPX-L5300 was given orally to the rats (via a stomach tube) as a radiolabeled active ingredient. Animals were allowed free access to animal feed (Purina) and tap water. The animals were allowed a one-week acclimation period prior to initiation of experimentation.

In the repeated exposure experiments, 2 male and 2 female rats were given Purina chow containing 100 ppm DPX-L5300 to eat for 21 days.

Analytical results: The analytical results of the admixture containing 100 ppm DPX-L5300 were not reported in this study.

Feeding schedule: Animals received regular rat chow (except for those rats given the 100 ppm admixture to eat for 21 days) and tap water ad libitum throughout the treatment period.

C. Statistics:

The use of statistical procedures were not reported in this study.

D. Quality Assurance:

A signed quality assurance statement was provided by a quality assurance inspector. According to the statement, the study was audited about 21 times during the course of the study. The dates when the study was audited were: 5/30/85, 7/24/85, 9/19/85, 4/18,24,28/86, 5/29/86 and 4/1-4,6-9/87.

IV. METHODS:

A. Observations: The frequency of clinical observations made on these rats was not provided in this summary report.

Toxicity/mortality (survival) results: There were no treatment-related deaths reported in this study.

Metabolite collection and identification: After treatment with the radiolabeled test compound, rats were put in metabolism cages designed to trap urine and feces. To measure the amount of radiolabel in the expired air, one male rat treated with 20 mg/kg of the phenyl-radiolabeled DPX-L5300 and one male rat treated with 2000 mg/kg of the triazine-label were placed in cages with traps to collect expired CO_2 and volatile metabolites of DPX-L5300.

At 6 and 24 hours after dosing, urine and feces were collected and also at 24 hour intervals thereafter (for 4 or 7 days depending on the particular exposure group). The trapped CO_2 and volatile metabolites were collected at the same time intervals as for urine and feces.

Metabolites in the urine and feces of the female and male rat were determined by the following analytical chemistry steps: chemical cleanup procedure, thin layer chromatography, high performance liquid chromatography and mass spectral determination.

In the groups of rats whose excreta was collected for 4 or 7 days, the animals were killed and the following tissues were excised for determined of the amount of radiolabel present (Figure A).

FIGURE A

	Digestive system		Cardiovascular		Neurological
	Tongue		Aorta*	X	Brain*@
	Salivary glands*	X	Heart*@		Peripheral nerve*#
	Esophagus*		Bone marrow*#		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*	X	Spleen@		Eyes (optic n.)*#
	Jejunum*		Thymus*		
	Ileum*		Red blood cell		Glandular
	Cecum*		Urogenital		Adrenal gland*
	Colon*	X	Kidneys*+@		Exorbital lacrimal gland#
	Rectum*		Bladder*		Mammary gland*#
X	Liver *+@	X	Testes*+@		Parathyroids*++
	Gall bladder*#		Epididymides		Thyroids*++
	Pancreas*		Prostate		Other tissues
	Respiratory		Seminal vesicle	X	Bone (femur)*#
	Trachea*#		Ovaries*+	X	Muscle*#@
X	Lung*@		Uterus*		Skin*#
	Nose^		Cervix		All gross lesions and masses*
	Pharynx^		Fallopian tubes	X	Residual Carcass@
	Larynx^			X	Fat@
				X	Plasma (blood)@

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
\# In subchronic studies, examined and preserved only if indicated by signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
@ Required for determining distribution in metabolism studies.

V. RESULTS:

A. Elimination of DPX-L5300 and Its Metabolites

DPX-L5300 is rapidly excreted in the urine and feces of male and female rats. Male and female rats were treated with DPX-L5300 with the radiolabel based either on the phenyl moiety or the triazine moiety (see Figure 39 below, taken from the report, for the positions of the radionuclides in DPX-L5300). The tables mentioned below were taken directly from the report. Within 96 hours:

- an average 99.0% of the radiolabel was excreted in the urine and feces of the male (urinary, 65.2%; fecal, 15.2%) and female (urinary, 69.0%; fecal, 26.0%) rats treated with a single dose of 20 mg/kg DPX-L5300 (Table 2),
- an average of 97% of the radiolabel was excreted in male (urinary, 83.2%; fecal, 13.8%) and female (urinary, 83.5%; fecal, 11.8%) rats in the repeated exposure regimen (Table 3) (fed an admixture containing 100 ppm for 21 days and then treated with 20 mg/kg radiolabel), and
- in the high dose groups (both the phenyl and triazine radiolabeled compounds), male (urinary, 73.2%; fecal, 16.4%) rats excreted an average of 97% and female (urinary, 67.1%; fecal, 6.1%) rats excreted only an average of 74% within this time period (Tables 4 and 5). (The values presented in this paragraph are from Table 4.) Another 15% of the applied radiolabel was found in the gastrointestinal tract of female rats (Table 11).

After 168 hours, groups of female rats treated with a single high dose of 1700 mg/kg (both phenyl- or triazine-labels) of DPX-L5300, an average of 98% of the radiolabel was found in the urine and feces (Tables 6 and 7). An average of 15% of the remaining radiolabel found in the gastrointestinal tract at 96 hours (Table 11) had decreased to <0.4% by 168 hours (Table 13).

Whole body half-lives. In general, as shown below in Table 8 taken from the report, both male and females rats exposed to either single or repeated low doses of DPX-L5300 exhibit 96-hour whole body half-lives that range from 26 to 33 hours post exposure. The 96-hour half-life was reported as 68 hours (triazine-labeled) and 81 hours (phenyl-labeled) in female rats, and 54 hours (triazine-labeled) and 51 hours (phenyl-labeled) in male rats treated with a single high dose of DPX-L5300. The 168-hour half-lives were

reported as 88 hours (phenyl-labeled) and 96 hours (triazine-labeled) for female rats treated with a single high dose of DPX-L5300 (Table 8).

Some interesting conclusions regarding comparisons of the whole body half-lives can be made (Table 8). The 96- and 168-hour half-lives are quite similar for female rats treated with a single high dose of DPX-L5300 labeled in the phenyl moiety, however, dissimilar half-lives were reported (68 and 96 hours) for the groups treated with the triazine-labeled DPX-L5300 for 96 or 168 hours, respectively. Also, dissimilar half-lives were found between groups of females treated with single high doses of triazine- or phenyl-labeled DPX-L5300 (Table 8). Similar half-lives were found for:

- male and female rats treated with single or repeated doses of phenyl-labeled DPX-L5300, and
- groups of male rats treated with a single high dose of triazine- or phenyl-labeled DPX-L5300.

B. Tissue Distribution of Applied Radiolabel

The tables below, Tables 9 through 14, depict the tissue distribution results and were taken from the report. Male and female rats were treated with DPX-L5300 with the radiolabel based either on the phenyl moiety or the triazine moiety.

Concerning the single high dose of the phenyl-labeled DPX-L5300 only, the gastrointestinal tract of female rats retained an average of 28.3% of the applied dose at 96-hours post exposure (Table 11), however, after 168 hours, the percentage of applied dose had dropped to less than 0.4% (Table 13). At 168 hours, the tissue with the next highest percentage of applied dose, the blood constituted only less than 1.0%, and the remaining tissue had lower percentages of the applied dose. This observation was the only sex difference regarding the tissue distribution of DPX-L5300.

The remaining tissues with the highest percentages at 96 hours were the skin at an average of about 1.5%, followed by the gastrointestinal tract at <1.5% and the liver at <1.0% in rats treated with a single high dose of the triazine-labeled DPX-L5300 (Table 12). Every other tissue at 96 hours had a percentage less than 1.0%. At 168 hours post exposure, the percentage of applied dose in any one tissue was lower than 0.4% for both the phenyl- or triazine-label treated groups of rats (Tables 13 and 14).

Regarding the single and repeated low dose groups, no tissue had more than 1.1% of the applied dose in male or female rats (Tables 9 and 10).

C. The Metabolism of DPX-L5300

Male and female rats were treated with DPX-L5300 with the radiolabel based either on the phenyl moiety or the triazine moiety. The identified urinary and fecal metabolites (as well as the tissue-borne metabolites) were the same in male and female rats.

The major urinary and fecal metabolites of phenyl-labeled DPX-L5300 were saccharin and metsulfuron methyl in both male and female rats treated with a single high or low dose, and the repeated low dose of DPX-L5300 as well (see Tables 15, 16, and 17 below). The major urinary and fecal metabolites of triazine-labeled DPX-L5300 were O-demethyl triazine amine and metsulfuron methyl (and lower amounts of N-demethyl triazine amine, DPX-L5300 acid and triazine amine) in both male and female rats treated with a single high dose or low dose as well as a repeated dose of DPX-L5300. See Figure 1 below for the chemical structures and the nomenclature for the urinary and fecal metabolites; this figure was taken from the report as well.

Sex differences. The percentage of urinary and fecal levels of saccharin produced in male and female rats were similar (Tables 15 and 17). Male rats produced almost four times more urinary metsulfuron methyl than female rats, especially in those animals treated with repeated low or single high doses of DPX-L5300 (Table 15). Male rats also produced three times more O-demethyl triazine amine than female rats exposed to the single high dose of triazine-labeled DPX-L5300 (Table 16). The increased production of urinary metabolites of metsulfuron methyl and O-demethyl triazine amine in male rats comprised the bulk of the differences between sexes in the metabolism of DPX-L5300.

Dosage differences. A higher percentage of urinary DPX-L5300 levels was found in male and female rats that were repeatedly exposed than those who received a single low dose (Table 15). With an increase in dosage (those male rats who received repeated low or single high doses), a higher percentage of urinary levels of metsulfuron methyl were produced (in females, the percentage remained the same) (Table 15). No differences in the levels of fecal metabolites of DPX-L5300 were seen between dosages (Table 17). The triazine-based radiolabel was administered at roughly the same dose (1700 and 2000 mg/kg), therefore, no comparisons in dosage difference have been attempted.

DPX-L5300 metabolic routes. Figure 39 below taken from the report shows the proposed metabolic routes for DPX-L5300 in the rat. The 3 major metabolites of DPX-L5300 in rats were identified as: saccharin, metsulfuron methyl, and O-demethyl triazine amine. The parent compound is demethylated at the carbamate moiety to form saccharin and metsulfuron methyl. Metsulfuron methyl, the demethylated product of DPX-L5300, is shown in Figure 39 as an intermediate for saccharin. The major products of the hydrolysis of the carbamate moiety are metsulfuron methyl and O-demethyl triazine amine (Figure 39).

D. Tissue Distribution of Identified DPX-L5300 Metabolites

The tissue levels of a number of the metabolites were measured at 96 hours post treatment. Figures 40, 41, and 42 presented below (and taken from the report) show the relative percentages of all of the identified metabolites in a particular tissue studied, be it the blood, liver, kidney, gastrointestinal tract (and lumen), muscle, fat, skin or the remaining carcass. (For each tissue, the percentage values for the metabolic residues should total 100%.)

The 3 major metabolites found in the tissues, saccharin, O-demethyl triazine amine and metsulfuron methyl, were found in the urine and feces as well. The metabolites found in the tissues were the same as those found in the urine and feces.

As noted earlier in Tables 4 and 5, female rats given a single high dose of the phenyl-labeled DPX-L5300 had 28.4% of radiolabeled residing the gastrointestinal tract at 96 hours (Table 11). To this end, Table 40 shows that the major metabolites found at 96 hours in the gastrointestinal tract (with relative percentages) are: the hydroxylated sulfonamide (29.0%), saccharin (21.6%), and DPX-L5300 acid (21.3%). Saccharin is the proposed terminal metabolite of the metabolism of the DPX-L5300 acid (Figure 39). Surprisingly, little metsulfuron methyl (2.2%), sulfonamide (4.6%) and acid sulfonamide (9.1%) were found, 3 of the intermediate metabolites that are thought to be metabolized eventually into saccharin and the hydroxylated sulfonamide (Figure 39). Although the levels of DPX-L5300 metabolites in the gastrointestinal tract are relatively high at 96 hours and these levels diminish to <0.4% after 168 hours post exposure, it is important to evaluate the tissue levels to saccharin since it is a known tumor promoter.

V. DISCUSSION:

DPX-L5300 is rapidly excreted, does not remain in any one tissue or organ, and is demethylated and/or hydrolyzed to form the same urinary and fecal metabolites identified in both male and female rats.

The distribution, metabolism and the elimination of DPX-L5300 was described in this report. To this end, male and female rats were treated with 3 dose regimens of phenyl-based radiolabeled DPX-L5300. These regimens were: (1) a single low dose (20 mg/kg), (2) a repeated low dose (fed an admixture containing 100 ppm DPX-L5300 for 21 days and then given a single dose of 20 mg/kg), and (3) a single high dose (1800 mg/kg of phenyl-based radiolabel or 2000 mg/kg of a triazine-based radiolabel) of DPX-L5300. A number of DPX-L5300 metabolites were identified in the urine and feces of these rats for a period 96 hours after exposure. Also, the levels of a number of the identified DPX-L5300 metabolites were measured in a variety of tissues at 96 hours in these rats. In addition, another group of female rats were treated with a single high dose (1700 mg/kg) of either the triazine- or phenyl-based radiolabeled DPX-L5300 and urine and feces were collected for measuring identified DPX-L5300 metabolites for a period of 7 days.

Absorption of DPX-L5300 in rats. No data was provided in this summary report regarding the absorption of DPX-L5300 in rats.

Distribution of DPX-L5300 in rats. No more than 1% of the total applied dose was found in any one tissue or organ 168 hours after exposure. At 96 hours, 28% of the applied dose was found in the gastrointestinal tract of rats treated with a single high dose (1800 mg/kg) of DPX-L5300.

Excretion of DPX-L5300 in rats. At total of 98% of the administered radiolabeled was excreted within 7 days in the urine and feces of male and female rats given DPX-L5300. No radiolabel was found in the expired air of these animals. Within 96 hours after exposure, in the rats treated with a single low dose, an average of 99% is found in the excreta (females: urinary, 69%, fecal, 26%; males: urinary, 65%, fecal, 15%). In rats given repeated low doses at 96 hours, an average of 97% of the applied radiolabel was excreted (females: urinary, 84%, fecal, 12%; males: urinary, 83%, fecal, 14%). Female rats given a single high dose of DPX-L5300 excreted an average of 74% of the radiolabel (urinary, 67%, fecal, 6%). An average of 15% of the remaining radiolabel was found in the gastrointestinal tract (at 96 hours but not at 168 hours). Male rats treated with a single high dose of DPX-L5300 excreted an average of 97% of the applied radiolabel within 96 hours (urinary, 83%, fecal, 14%; the same urinary:fecal differential as male rats given repeated low doses of DPX-L5300).

Metabolism of DPX-L5300 in rats. A number of DPX-L5300 metabolites were identified in groups of rats treated with the 3 dosage regimens. The identified urinary and fecal metabolites were the same in male and female rats, and tissue metabolites of DPX-L5300 identified in a variety of tissues were the same as those found in the urine and feces of male and female rats. The major metabolites of DPX-L5300 in male and female rats were saccharin (a known tumor promoter), metsulfuron methyl, and O-demethyl triazine amine. The two major routes of DPX-L5300 metabolism are the demethylation of the carbamoyl methyl group and the hydrolysis of the carbamate moiety. Saccharin and metsulfuron methyl are the major metabolites of the demethylation route whereas O-demethyl triazine amine and metsulfuron methyl are the major metabolites of the hydrolysis route.

Classification: core-minimum: This classification is based on the fact that the methodology requirements established in the Pesticide Assessment Guidelines, Subdivision F §85-1 have been satisfied. FIFRA testing guidelines Subdivision F § 85-1 (d)(2)(iv) dictates that 5 animals of each sex be used for the metabolism study and not the 2 animals of each sex that were used in this study. However, considering that the (1) urinary, and fecal metabolites of DPX-L5300 were identified in the male and female rats treated with the 3 dosage regimens and (2) a number of identified DPX-L5300 metabolites were measured in a variety of tissues, testing with 5 animals for each sex is not required.

Express science review

Page _____ is not included in this copy.

Pages 106 through 118 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ **The document is a duplicate of page(s) _____**

___ **The document is not responsive to the request**

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Table 15
Percent of Total Applied Dose Recovered as Urinary Metabolites
Rats Treated With Phenyl-Based Radiolabel

Dosage[a]:	Sex	Single Low	Repeated Low	Single High
Compound:				
DPX-L5300	Male	2.2	8.7	1.1
	Female	7.2	9.3	0.8
Saccharin	Male	27.4	21.3	19.5
	Female	35.1	36.1	20.5
Metsulfuron methyl	Male	15.7	21.5	46.0
	Female	10.8	7.2	12.2
Sulfonamide acid	Male	6.5	8.4	4.3
	Female	3.8	6.0	6.2
DPX-L5300 acid	Male	2.8	5.2	2.8
	Female	2.5	6.3	2.9
% of total in urine	Male	53.6	65.1	73.7
	Female	59.4	64.9	42.6

[a] Dosages are: single low - a single dose of 20 mg/kg of DPX-L5300
repeated low - exposure to 100 ppm DPX-L5300 in the feed fo
21 days, then a single dose of 20 mg/kg DPX-L5300
single high - a single high dose of 1800 mg/kg DPX-L5300

Table 16
Percent of Total Applied Dose Recovered as Urinary or Fecal Metabolites
Rats Treated With Triazine-Based Radiolabel

Dosage: Sex / Compound:		Single High (Fecal)	Single High (Urinary)	Totals
DPX-L5300	Male	0.8	0.1	0.9
	Female	0.2	3.5	3.7
Metsulfuron methyl	Male	4.1	18.7	22.8
	Female	1.1	20.2	21.3
O-demethyl triazine amine	Male	4.5	35.4	39.9
	Female	1.9	15.9	17.8
DPX-L5300 acid	Male	0.9	6.3	7.2
	Female	0.8	10.5	11.3
N-demethyl triazine amine	Male	0.8	1.3	2.1
	Female	0.4	3.3	3.7
Triazine amine	Male	0.5	2.3	2.8
	Female	0.4	1.8	2.2
% of total in excreta	Male	11.6	64.1	75.7
	Female	4.8	55.2	60.0

[a] Dosage was: single high - a single high dose of 2000 mg/kg DPX-L5300

Table 17
Percent of Total Applied Dose Recovered as Fecal Metabolites
Rats Treated With Phenyl-Based Radiolabel

Dosage[a]:	Sex	Single Low	Repeated Low	Single High
Compound:				
DPX-L5300	Male	0.5	0.6	0.4
	Female	1.2	0.3	0.2
Saccharin	Male	6.8	1.9	2.1
	Female	4.7	2.3	1.5
Metsulfuron methyl	Male	3.9	1.2	2.9
	Female	4.1	1.1	1.4
Sulfonamide acid	Male	4.0	2.3	2.0
	Female	4.0	2.9	0.5
DPX-L5300 acid	Male	1.7	1.9	1.5
	Female	3.6	2.0	1.1
% of total in feces	Male	16.9	7.9	8.9
	Female	17.6	8.6	4.7

[a] Dosages are: single low - a single dose of 20 mg/kg of DPX-L5300
repeated low - exposure to 100 ppm DPX-L5300 in the feed fo
21 days, then a single dose of 20 mg/kg DPX-L5300
single high - a single high dose of 1800 mg/kg DPX-L5300

Express science review

Page _____ is not included in this copy.

Pages 122 through 130 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ **The document is a duplicate of page(s)** _____

___ **The document is not responsive to the request**

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX III

Data Evaluation Records for Studies on DPX-L5296

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. A. Acute Oral Toxicity in the Rat. Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. B. Acute Percutaneous Toxicity in the Rat. Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

Cummins, H. A. January 22, 1987. Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. C. Acute Dermal Irritation/Corrosivity Test in Rabbits Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. D. Acute Eye Irritation/Corrosivity Test in Rabbits. Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. E. Delayed Contact Hypersensitivity Study in Guinea Pigs. Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. F. Four-Week Toxicity Study by Oral Administration to CD Rats. Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. G. Assessment of Mutagenic Potential in Histidine Auxotrophs of Salmonella typhimurium (The Ames Test). Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

APPENDIX III (continued)

Cummins, H. A. January 22, 1987. L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. H. In vitro Assessment of the Clastogenic Activity of L5296 in Cultured Human Lymphocytes. Unpublished report no. LSR 86/DPR001-013/525 prepared by Life Science Research, Ltd., Suffolk, England. Submitted by E. I. DuPont de Nemours and Company, Inc., Newark, DE. MRID No. 402455-17.

006833

Reviewed by: Roger Gardner Roger Gardner 8-19-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W Hauswirth 8/19/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Acute oral - Rats (Guideline §81-1)

MRID NUMBER: 402455-17

TEST MATERIAL: L-5296 with unspecified purity was used.

SYNONYMS: 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine.

STUDY NUMBER(S): ISR 86/DPR001-013/525

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

TESTING FACILITY: Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. A. Acute Oral Toxicity in the Rat

AUTHOR(S): Cummins, H. A.

REPORT ISSUED: January 22, 1987

CONCLUSIONS: The results of the study indicated that the acute oral LD_{50} is 410 mg/kg for both sexes, 394 mg/kg for male rats, and 452 mg/kg for female rats. These results indicate that L5296 should be classified into Toxicity Category II.

Core classification: Minimum

I. PROTOCOL

A. Test species: Five-week old Charles River male and female CD (remote Sprague-Dawley origin) rats were used. Male rats weighed from 86 to 154 g, and females weighed from 91 to 133 g at the start of the experiment.

B. Experimental procedure: Groups of 5 male and 5 female rats were given single oral doses of 202, 285, 402, or 567 mg test substance per kg body weight. The test substance was administered in 0.5% aqueous methyl cellulose by gavage. The rats were fasted overnight before treatment. The animals were checked three times during the first hour and twice later in the first day following treatment. They were also observed for mortality and appearance of toxicological signs twice daily for the 14 days that followed dosing. Body weights were measured on the day before dosing and on Days 1 (dosing day), 8, and

B. Experimental procedure (continued)

15 of the study. Necropsies were done (where possible) on animals from each group that died during the observation period and on survivors sacrificed 14 days after dosing.

The report stated that a probit analysis was used to calculate an LD_{50}, 95% confidence limits, and the slope of the dose-response curve.

II. REPORTED RESULTS

Signs of toxicity observed by the authors included lethargy, decreased motor activity, hunched posture, ataxia, irregular breathing, colored occular discharges, stained snout, and closed eyes. The report stated that survivors recovered from these signs during the first 4 days of the observation period, and they appeared normal until day 15 of the study.

Three of 5 males given the 402 mg/kg dose died, and only one of the females in that group died. All rats given the 567 mg/kg dose died. Most of the deaths observed during the study occurred during the first 24 hours following dosing. One male from the 402 mg/kg dose group died on day 2 and another on day 3.

The investigators noted no significant effects on bodyweight during the study.

The authors reported that the only gross lesion observed was intussusception of the small intestine in 4 of 10 rats given the 402 mg/kg dose and 2 of the 10 given the 567 mg/kg dose.

The reported LD_{50} for both sexes was 410 mg/kg with 95% confidence limits of 366 to 455 mg/kg. The calculated LD_{50} for males alone was reported to be 394 mg/kg with 95% confidence limits of 336 to 452 mg/kg, and those values for females were reported to be 427 and 306 to 548 mg/kg, respectively.

III. DISCUSSION

There was adequate information presented in the report to support the conclusions of the investigators.

006833

Reviewed by: Roger Gardner Roger Gardner 8-19-86
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 8/19/86
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal - Rats (Guideline §81-2)

MRID NUMBER: 402455-17

TEST MATERIAL: L-5296 with unspecified purity was used.

SYNONYMS: 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine.

STUDY NUMBER(S): LSR 86/DPR001-013/525

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

TESTING FACILITY: Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. B. Acute Percutaneous Toxicity in the Rat.

AUTHOR(S): Cummins, H. A.

REPORT ISSUED: January 22, 1987

DISCUSSION AND CONCLUSIONS: The results of the study indicated that the acute dermal LD_{50} is greater than 2000 mg/kg in rats, and L5296 should be classified into Toxicity Category III.

Core classification: Minimum

I. PROTOCOL

A. Test species: Five-week old Charles River male and female CD (remote Sprague-Dawley origin) rats were used. Male rats weighed from 234 to 261 g, and females weighed from 212 to 229 g at the start of the experiment.

The report stated that on the day before treatment the hair on the dorsum between the limb girdles of each animal was clipped as close to the skin as possible. Rats showing signs of skin irritation on the prepared test sites were replaced.

B. Experimental procedure: The report stated that 5 animals of each sex were used, and the clipped skin of each was moistened. The test substance was applied directly to the site at a dose of 2000 mg per kg body weight.

B. Experimental procedure (continued)

After the application of test substance, the test site was covered with aluminum foil which was held in place by a bandage wrapped twice around the trunk of the animal. At the end of the 24-hour exposure period the dressings were removed, and the test sites were gently brushed and gently wiped clean with a moist cloth.

All animals were observed three times on the day of application and twice daily for the next 14 days for the appearance of toxic signs and mortality. The rats were weighed on the day of dosing and on days 7 and 14 of the observation period. Surviving rats were sacrificed at the end of the 14-day observation period, and gross postmortem examinations were conducted.

The report stated that a median lethal dose was not calculated.

II. REPORTED RESULTS

The authors noted no deaths and no sign of compound-related effects. All animals gained weight during the study.

Reviewed by: Roger Gardner Roger Gardner 1-19-84
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 6/19/85
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Irritation - Rabbits (Guideline §81-4)

MRID NUMBER: 402455-17

TEST MATERIAL: L-5296 with unspecified purity was used.

SYNONYMS: 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine.

STUDY NUMBER(S): LSR 86/DPR001-013/525

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

TESTING FACILITY: Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. C. Acute Dermal Irritation/Corrosivity Test in Rabbits

AUTHOR(S): Cummins, H. A.

REPORT ISSUED: January 22, 1987

DISCUSSION AND CONCLUSIONS: The results of the study indicated that L5296 is not a skin irritant in male rabbits, and it should be classified into Toxicity Category IV.

Core classification: Minimum

I. PROTOCOL

A. Test species: Two-and-one-half to 3 month old New Zealand White strain rabbits were used. They weighed from 2.17 to 2.71 kg on arrival at the laboratory.

B. Experimental procedure: Twenty-four hours before the beginning of the study, three male rabbits were prepared by clipping their backs free of hair.

On the day of dosing two 6 X 6 cm test sites were marked and moistened with 0.2 ml distilled water. Five-hundred mg test substance was placed on 3 X 2 cm gauze patches which were placed on one of two sites on each animal, and untreated patches were placed on the other site. The gauze patches were secured with cotton pads, adhesive tape, and bandages around each animal.

B. Experimental procedure (continued)

Four hours after application of the test substance the dressings were removed, and the test sites were rinsed and gently wiped clean. The test sites were scored for edema and erythema at that time, and they were scored again 48 hours after removal of the dressings. A scheduled 72-hour evaluation of the test sites was not done because of a procedural error according to the report, and a 6-day examination was substituted.

Erythema and eschar formation as well as edema were scored on a 5-point scale (0-4) with a maximum possible score of 8 for any site. Scoring was done according to the following classifications:

Erythema and eschar		Edema	
No erythema	0	No edema	0
Slight erythema	1	Very slight edema	1
Well-defined erythema	2	Slight edema	2
Moderate to severe erythema	3	Moderate edema	3
Severe erythema to slight eschar formation	4	Severe edema	4

II. REPORTED RESULTS

According to the report, no animal showed signs of edema or erythema during the study, and no deaths were observed.

006833

Reviewed by: Roger Gardner Roger Gardner 8-19-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 8/19/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Eye Irritation - Rabbits (Guideline §81-5)

MRID NUMBER: 402455-17

TEST MATERIAL: L-5296 with unspecified purity was used.

SYNONYMS: 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine.

STUDY NUMBER(S): LSR 86/DPR001-013/525

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

TESTING FACILITY: Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. D. Acute Eye Irritation/Corrosivity Test in Rabbits

AUTHOR(S): Cummins, H. A.

REPORT ISSUED: January 22, 1987

DISCUSSION AND CONCLUSIONS: There were adequate data included in the report to support the conclusion that L5296 should be classified into Toxicity Category IV for primary eye irritation. Conjunctival involvement was reversed by 3 days following instillation of the test substance, and there was no corneal or iridial involvement.

Core classification: Minimum

I. PROTOCOL

A. Test species: Two-and-one-half to 3 month old New Zealand White strain rabbits were used. They weighed from 2.16 to 2.68 kg on arrival at the laboratory.

B. Experimental procedure: Three male rabbits previously examined and found without signs of eye irritation were used in the experiment. One-hundred mg of the test substance was instilled into the right eye of each rabbit, and the eyelids were gently held together for one second. The left eye remained untreated. The treated eyes were not rinsed after instillation of the test substance.

C. Observations: All eyes were examined 1, 24, 48, and 72 hours after instillation of the test substance and 4 and 7 days after treatment. Ocular reactions were scored according to the following scales:

Assessment of Pain Response

Reaction to treatment	Score	Descriptive Rating
No response.	0	No initial pain
A few blinks only, normal within one or two minutes.	1	Practically no initial pain
Rabbit blinks and tries to open eye but the reflexes close it.	2	Slight initial pain
Rabbit holds eye shut and puts pressure on lids; may rub eye with paw.	3	Moderate initial pain
Rabbit holds eye shut vigorously, may squeal.	4	Severe initial pain
Rabbit holds eye shut vigorously, may squeal, claw at eye, and try to escape.	5	Very severe initial pain

Cornea

Degree of density

1 - scattered or diffuse area, details of iris visible
2 - easily discernible transluscent areas, details of iris slightly obscured
3 - opalescent areas, no details of iris visible, size of pupil barely discernible
4 - opaque, iris invisible

Area of cornea involved

1 - one-quarter (or less but not zero)
2 - greater than one-quarter to less than one-half
3 - greater than one-half to less than three-quarters
4 - greater than three-quarters

Iris

1 - folds above normal, congestion, swelling, circumcorneal injection (any one or a combination of these), iris still reacting to light (sluggish reaction is positive)

2 - no reaction to light, hemorrhage, gross destruction (any one or all of these)

C. Observations (continued)

Conjunctivae

Redness

1 - vessels definitely injected above normal
2 - more diffuse, deeper crimson red, individual vessels not discernible
3 - diffuse beefy red

Chemosis

1 - any swelling above normal (including nictitation membrane
2 - obvious swelling with parital eversion of the lids
3 - swelling of lids about half closed
4 - swelling of lids about half to completely closed

Discharge

1 - any amount different from normal (does not include small amount in inner canthus of normal animals)
2 - discharge with moistening of the lids and hairs just adjacent to the lids
3 - discharge with moistening of the lids and considerable area around the eye

Criteria for classification of the test substance as an irritant were described in the report as follows:

"A test material is considered irritant if, when applied to the eye, significant ocular lesions which are caused are present 24 hours or more after the instillation procedure. Ocular lesions are considered significant if two or more of the rabbits have mean values at or above the limit values following

Lesion	Limit Value
Corneal opacity	2
Iris lesions	1
Redness of conjunctivae	2.5
Chemosis	2

"Mean values are calculated using all scores recorded 24, 48 and 72 hours after treatment. If the limit values for corneal opacity or iridial lesions equal or exceed 3 and 2 respectively, the material is considered to have potential to cause serious damage to the eye."

II. REPORTED RESULTS

The report stated that there was practically no response to pain in treated rabbits.

None of the three rabbits exhibited corneal opacity in treated eyes during the study. Two of the three rabbits had a score of 1 for the iris at the first examination (1 hour after treatment), but scores thereafter were 0.

All treated eyes had redness of the conjunctivae which persisted through the 48-hour examination. Chemosis was noted in two rabbits and discharge was observed in one during the first hour after treatment.

The mean scores for the 24, 48 and 72 hour observations were reported to be 0.7 for redness in all 3 rabbits. No other reactions showed means above 0.

006833

Reviewed by: Roger Gardner Roger Gardner 8-19-88
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 8/19/88
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Skin Sensitization - Guinea Pigs (Guideline §81-6)

MRID NUMBER: 402455-17

TEST MATERIAL: L-5296 with unspecified purity was used.

SYNONYMS: 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine.

STUDY NUMBER(S): LSR 86/DPR001-013/525

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

TESTING FACILITY: Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: L5296: Technical Dossier Concerning Toxicological Properties. Final Reports on "Base Set" Studies. E. Delayed Contact Hypersensitivity Study in Guinea Pigs.

AUTHOR(S): Cummins, H. A.

REPORT ISSUED: January 22, 1987

DISCUSSION AND CONCLUSIONS: No delayed dermal sensitization reactions were observed in guinea pigs treated with L5296 in a Maximization test.

Core classification: Minimum

I. PROTOCOL

A. Materials

1. Test species: Male and female Dunkin-Hartley strain guinea pigs were used. They weighed from 292 to 416 g on the first day of the study.

   On the day before treatment began, the hair was clipped from an area of skin over the scapulae (4 X 6 cm).

2. Test materials: The test substance was added to olive oil, paraffin oil, or 50% olive oil/Freund's Complete Adjuvant (FCA). Doses were prepared on the morning of their use in the study.

B. Experimental procedure: A preliminary study to determine the primary skin irritation potential of the test substance was conducted. The dermal sensitization study followed the Magnusson-Kligman Maximization procedure.

B. Experimental procedure (continued)

1. Preliminary irritation study: A series of intradermal injections of the test material at concentrations of 0.3, 0.5, 1, 3, 5, and 10% (w/v) in olive oil were administered to two guinea pigs. A series at the same concentrations in olive oil/FCA were given to two other guinea pigs, and the skin reactions of all animals were assessed 24 and 48 hours after treatment.

   Preliminary studies for topical application of the test substance were described as follows:

   Topical application

   Five guinea-pigs were subjected to single intradermal injection (0.1 ml) of FCA. Two were used to ascertain the maximum tolerable concentration of test material, and three to determine the maximum non-irritant concentration of test material following occluded application to the skin. The intervals between administration of adjuvant and occluded application of the test material were arranged to be similar on the preliminary and main studies.

   First phase

   The hair was shaven from both flanks of two guinea-pigs. Topical application of 0.03 ml of 5%, 10%, 30% and 50% w/v L5296 in paraffin oil were administered to the four test sites on each guinea pig. Each dose was used to saturate a 10 mm diameter absorbant patch and after application to the skin, the patch was covered with an air-tight occlusive dressing for 24 hours. Reactions of the skin were assessed 24 and 48 hours after removal of the patches.

   Second phase

   The irritation screen was repeated by the same method using three guinea-pigs to assess the irritancy of 5%, 10%, 30% and 50% w/v L5296 in paraffin oil.

2. Main study: As the result of an error noted in the report, there were 12 male and 8 female guinea pigs assigned to the control group and 8 males and 12 females assigned to the treated group..

   There were two phases to the induction portion of the experiment. The primary phase (Day 1 of the study) was described in the report as follows:

   Three pairs of injections (0.1 ml) were made, deep into the dermis, such that on either side of the dorsal median line there were three injection sites in a row parallel

2. Main study (continued)

to the spinal column...The anterior and middle sites were placed close together and distant from the posterior site.

Injection sites	Test Group	Control Group
Anterior	FCA	FCA
Middle	5% w/v in olive oil	Olive oil
Posterior	35% w/v in olive oil/FCA	Olive oil/FCA

The second phase (Day 8 of the study) was described as follows:

On Day 7 the clipped dorsa of all animals were subjected to inunction with 10% w/v sodium lauryl sulphate in petrolatum. This was intended to enhance the absorption of formulations administered on the following day.

On Day 8 the dermal test sites defined by the intradermal injections were wet shaven and treated by topical application of 0.6 ml 50% w/v L5296 in paraffin oil in test animals while controls received 0.6 ml paraffin oil. Each dose was absorbed onto a 40 X 25 mm absorbant patch which was applied to the skin and covered by an occlusive dressing for 48 hours.

Challenge doses were applied to an area of shaven skin (50 X 59 mm) on either side of the trunk on the 22nd day of the study. The left side was treated with paraffin alone, and the right side was treated with 0.03 ml of 50% w/v test substance in paraffin oil in a manner similar to the topical applications made earlier in the study.

C. Observations: All injection sites and topical application sites were examined and scored for skin reactions approximately 24 and 48 hours after each dose was administered or after removal of occlusive dressings.

Erythema and eschar formation as well as edema were scored on a 5-point scale (0-4) with a maximum possible score of 8 for any site. Scoring was done according to the following classifications:

No response	0
Barely perceptible erythema	+
Slight confluent erythema	1
Moderate confluent erythema	2
Severe confluent erythema	3

The investigators' interpretation of results was described in the report as follows:

C. Observations (continued)

The incidence of significant erythematous reactions (grade 1 or above) were tabulated for each treatment regime. Barely perceptible erythema (grade ±) is often a non-specific response to the dosing procedure and is not considered to be a significant or conclusive indication of delayed contact hypersensitivity. The test is considered positive when more than two of the twenty test group animals exhibit a significant erythematous reaction following challenge with a sub-irritant concentration of the test material.

In addition to these observations, the animals were weighed at weekly intervals during the study.

II. REPORTED RESULTS

Results of the preliminary studies indicated that concentrations of 5 and 10% w/v test substance in olive oil/FCA could not be dissolved, and the 10% concentration could not be dissolved in the oil without the adjuvant. Slight confluent erythema was observed in animals receiving injections of the test substance in the oil/adjuvant vehicle.

No reactions were observed after topical applications of 5, 10, 30, and 50% concentrations of test substance in paraffin oil in the preliminary study.

Based on the preliminary results the following dosing regime was used in the main study:

First induction:	5% in olive oil
	3% in olive oil/FCA
Second induction:	50% in paraffin oil
Challenge:	50% in paraffin oil

None of the animals exhibited skin reactions following challenge applications of the test substance.

006833

Reviewed by: Marion P. Copley, D.V.M., D.A.B.T. Marion P. Copley
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth
Section VI, Tox. Branch (TS-769C) 6/24/88

DATA EVALUATION REPORT

STUDY TYPE: 4 week oral - rat (82-1) TOX. CHEM NO: 550A

MRID NO.: 402455-17

TEST MATERIAL: DPX-L5296

SYNONYMS: 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine

STUDY NUMBER: LSR report No.:86/DPR006/525

SPONSOR: DuPont

TESTING FACILITY: Dept. of Short-term Toxicology, Life Science Research, Eye, Suffolk, IP23 7PX

TITLE OF REPORT: Four-week toxicity study by oral administration to CD rats

AUTHOR(S): Study Director - HA Cummins

REPORT ISSUED: January 22, 1987

CONCLUSION: NOEL = 8 mg/kg/day
LEL = 40 mg/kg/day (males) based on decreased body weight and weight gain, decreased glucose and decreased platelets.
In addition in the HDT (200 mg/kg/day) females there were decreased: body weight, weight gain, glucose, platelets and relative spleen weight; increased: WBC. In the HDT males and females there were decreased potassium; increased SGPT; and histologically an increased incidence of myocardial degeneration in the ventricular apex, often with fibrosis. In HDT males only, total serum protein was increased.

Classification: core-supplementary due to study design (4 weeks)

Special Review Criteria (40 CFR 154.7) There are no special review triggers triggered by the results of this study.

A. MATERIALS:

1. Test compound: Crude L5296, DPX-L5296, Description - white, crystalline solid, lot # - E308-110 Batch 3, Purity - not given, stability - not given.

2. Test animals: Species: Rats, Strain: CD (remote Sprague-Dawley), Age: 3-5 weeks old, Weight: 64-81 g, Source: Charles River U.K., Margate, England, animals were acclimated for 5 days prior to study initiation.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to test groups as noted in table 1.

Table 1

Group	dosage mg/kg/day	Numbers of rats male	female
1 Cont.	0	5	5
2 Low (LDT)	8	5	5
3 Mid (MDT)	40	5	5
4 High (HDT)	200	5	5

2. Treatment preparation and administration

DPX-L5296 was ground and suspended in the appropriate concentrations in methylcellulose in distilled water (0.5 % w/v). Animals received 5 ml/kg-bodyweight of the appropriate concentration by gavage daily for 29 days based on the most current body weight. Controls received vehicle only. The suspension was prepared daily. Samples of the suspensions were collected on days 1 and 30 for possible analysis.

Results - Compound formulation analysis was not reported.

3. Animals received food (LAD1. from Labsure) and water ad libitum.

4. Statistics

Statistical procedures are in Appendix 1, taken from page 189 of the report.

5. There is a signed quality assurance statement dated Jan. 22, 1987.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected three times daily for signs of toxicity and mortality. A complete physical examination was completed prior to and weekly during the study.

Results - Mortality - There were two deaths during the study, 1 LDT female and 1 MDT male. The LDT female was sacrificed in extremis (day 23). Signs prior to death included hunched posture, distended abdomen, thinness, and urinalysis with high urine volume and the presence of total reducing substances, glucose, ketones and blood pigments. Changes noted at necropsy included dark areas on the gastric mucosa with abnormal gastro-intestinal contents, thickened duodenal wall, small spleen and hydronephrosis. Histopathological changes were not considered remarkable. The MDT male was found dead on day 28. There were no abnormal signs noted prior to death. Macroscopic signs included dark-red fluid in the urinary bladder, small seminal vesicles and hydronephrosis. Histologic renal changes included, papillary necrosis, proximal tubular necrosis and hydronephrosis.
Toxicity - Clinical signs of toxicity at 200 mg/kg/day (HDT) included brief periods of salivation immediately after dosing at the 200 mg/kg/day. As the study progressed, several animals in this group also started salivating prior to dosing. After day 23, all HDT animals had periods of lethargy after dosing and piloerection. By day 29, all HDT rats were unsteady on their feet. One female also had slight clonic convulsions (duration - a few minutes) on days 20 and 21. There were no signs noted at the mid and low doses, except for irritability in the mid dose females post dosing on one occasion.

2. Body weight

Animals were weighed twice weekly throughout the study.

Results - Body weight and weight gain were decreased in the high and to a lesser extent, the mid dose males and females (see table 2). The decrement in weight gain, as compared to controls for the 28 day period was 16 % and 40 % for mid and high dose males, respectively, and 8 % and 23 % for mid and high dose females, respectively.

TABLE 2 Body weight (G) and weight gain (G)

(mg/kg/day) Period	MALES 0	8	40	200	FEMALES 0	8	40	200
Day 0	119	112	120	123	110	114	111	114
Day 28	314	294	283	245	202	205	194	187
0-28 gain	195	182	165**	122***	92	91	84	73**
% gain[a]	164	163	138	99	83	80	76	64

** $p<0.01$; *** $p<0.001$

[a] % gain = $\frac{\text{Day 28 - day 0}}{\text{day 0}}$

3. Water consumption, food consumption and food efficiency (conversion)

Water consumption was observed but not quantitatively determined. Food consumption and food conversion (consumption/unit of bodyweight gain) were calculated weekly.

Results - There were no obvious treatment-related adverse effects on water consumption or food consumption. Food conversion was decreased throughout the study in the 200 mg/kg/day males and for week 1 in the 200 mg/kg/day females.

4. Ophthalmological examination was not performed.

5. Blood was collected after 3 weeks of treatment for hematology and clinical analysis from all animals. They were fasted overnight prior to anesthesia with ether and blood was obtained from the retro-orbital sinus. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc.HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements	X	RBC morphology
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic and chronic studies

Results - As can be seen in table 3, platelets were decreased in the mid and high dose males and high dose females, 11, 21 and 17 %, respectively. There was also a 90 % increase in WBCs in the high dose females. This was due to an absolute increase in both neutrophils and lymphocytes. Small (< 9 %), but statistically significant decreases in HGB, RBC and prothrombin clotting times were also observed.

TABLE 3 Selected hematologic results

	MALES				FEMALES			
(mg/kg/day) Parameter	0	8	40	200	0	8	40	200
Platelet 1000/cmm	777	739	692*	615***	798	703	717	662*
WBC 1000/cmm	NO CHANGE				10.9	13.8	13.3	18.0**

* p<0.05; ** p<0.01; *** p<0.001

b. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium*		Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*		Cholesterol*
X	Potassium* (K)		Globulins
X	Sodium* (Na)	X	Glucose*
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
	Creatinine phosphokinase*^	X	Serum protein electrophoreses
	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

Results - As can be seen in table 4, there was a statistically significant increase in: SGPT (HDT males and females) and total protein (HDT males); and decrease in: glucose (MDT and HDT males), sodium (LDT and HDT males and females) and potassium (MDT and HDT males and females). Although there was a 22 % increase in SGOT

(HDT females), there was a large variation between the groups for this parameter.

TABLE 4 Selected clinical chemistry results

	MALES				FEMALES			
(mg/kg/day) Parameter	0	8	40	200	0	8	40	200
SGPT (iu/l)	23	23	30	41***	22	18	26	42***
SGOT (iu/l)	82	77	68	86	70	77	75	86*
Protein (g%)	6.2	6.4	6.4	6.8***	6.8	6.6	6.6	7.0
Glucose (mg%)	140	129	119*	105***	113	117	134	124
Na (mEq/l)	148	146*	147	145**	151	147*	150	146**
K (mEq/l)	3.8	3.5	3.4*	3.2**	3.9	3.5	3.4*	2.9***

* p<0.05; ** p<0.01; *** p<0.001

6. Urinalysis

Urine was collected from fasted animals after 3 weeks. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*	X	Nitrate
X	Protein*	X	Urobilinogen
	Osmolality	X	Total reducing substances

* Required for chronic studies

Results - There were no treatment-related changes in urinalysis.

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed and relative weights calculated.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
	Tongue		Aorta*		Brain*+
	Salivary glands*	XX	Heart*		Periph. nerve*#
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*#
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*	XX	Spleen		Eyes (optic n.)*#
	Jejunum*		Thymus*	Glandular	
	Ileum*	Urogenital		XX	Adrenals(with kid.)*
	Cecum*	XX	Kidneys*+		Lacrimal gland#
	Colon*		Urinary bladder*		Mammary gland*#
	Rectum*	XX	Testes*+		Parathyroids*++
XX	Liver *+		Epididymides		Thyroids*++
	Gall bladder*		Prostate	Other	
	Pancreas*		Seminal vesicl		Bone*#
Respiratory			Ovaries*+		Skeletal muscle*#
	Trachea*		Uterus		Skin*#
	Lung*			X	All gross lesions and masses*
	Nose^				
	Pharynx^				
	Larynx^				

* Required for subchronic and chronic studies
^ Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
\+ Organ weight required in subchronic and chronic studies
++ Organ weight required for non-rodent studies

a. Organ weight changes included a decrease in absolute (but not relative) heart and liver weights in the HDT males. As can be seen in table 5, there was a decrease in absolute and relative spleen weight in the HDT males.

TABLE 5 Spleen weight

	MALES				FEMALES			
(mg/kg/day) Parameter	0	8	40	200	0	8	40	200
Absolute (G)	.807	.694	.603*	.468**	.510	.560	.521	.457
Relative (% of body wt)	.256	.237	.213	.192*	.256	.276	.273	.257

* $p<0.05$; ** $p<0.01$

b. Gross pathology - There were no treatment related changes in gross pathology.

c. Microscopic pathology

1) Non-neoplastic treatment related alterations were limited to myocardial degeneration of the ventricular apex with or without fibrosis in 3/5 males and 5/5 females in the HDT.

2) There were no neoplastic lesions observed in this study.

D. DISCUSSION

This compound produced toxicity in males and females at 200 mg/kg/day and to a lesser extent in males at 40 mg/kg/day. Clinical signs were non-specific and included salivation, lethargy and piloerection at the HDT. Deaths at the LDT and MDT were probably not treatment related since the accompanying signs did not occur at the HDT and there were no HDT deaths.

Body weight and weight gain decreases were treatment related in the mid and high dose males and high dose females. Although the slight decrease (8 %) in the mid dose females may have been treatment-related, this could not be confirmed. Food and water consumption were not affected by treatment. There was a decrease in food utilization associated with decreased weight gain.

There was a treatment related decrease in platelets in the MDT and HDT males and HDT females and an increase in WBCs, limited to the HDT females. The pathogenesis of these changes was not proposed. Other hematologic changes were of small magnitude and were therefore, not treatment-related.

Treatment-related changes in clinical chemistries were primarily limited to the HDT males and females and included increased SGPT, increased protein (males only), decreased glucose (MDT and HDT males only), and decreased potassium. Since increases in SGOT were small, within the normal range, and only present in the females, they were probably not treatment-related. Although there were statistical changes in sodium values, these were too small and erratic to be considered treatment-related. The decrease in potassium was also small at the MDT and therefore, probably not treatment-related.

It can not be definitively determined whether the decreased relative splenic weight in the HDT males was treatment related. Although this change did not occur in the females, the spleen can contract due to stress, and the HDT males appeared to be more sensitive to treatment than the females for several parameters. The changes in absolute weights in

the other organs appeared to be a function of decreased body weight rather than direct effects on the organ.

The NOEL for this study is 8 mg/kg/day and the LEL is 40 mg/kg/day based on decreased body weight and weight gain and decreased glucose and platelets in the males. In addition in the HDT (200 mg/kg/day) females there were also decreased body weight and weight gain and glucose and an increase in WBCs. In the HDT males and females there were salivation, lethargy and piloerection along with increased SGPT and decreased potassium. Total serum protein was increased in the HDT males only. There was also a decreased relative spleen weight (females) and myocardial degeneration in the ventricular apex often with fibrosis (males and females) at the HDT.

This was a well conducted study. Although the study protocol for this study is not specified in the guidelines, it provides useful toxicologic information and is therefore classified as core-supplementary.

COPLEY, PC5\DPXL5296.4WK, 550A, 6/28/88

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch/HED (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch/HED (TS-769C)

006833

006833

DATA EVALUATION REPORT

I. SUMMARY

Study Type: Mutagenicity - Reverse Gene Mutation in Bacteria (Salmonella/Ames)

TB Project No.: 7-0828B
Caswell No.: 550A,
Metabolite of 419S

MRID No.: 402445-17

Chemical: L5296, 4-methoxy-N,6-dimethyl-1,3,5-triazin-2-amine, a metabolite of DPX-L5300 (EXPRESS®)

Synonyms: (None known)

Sponsor: Dupont

Testing Facility: Life Science Research, Ltd.
Suffolk (UK)

Title of Report: Assessment of Mutagenic Potential in Histidine Auxotrophs of Salmonella typhimurium (The Ames Test)

Authors: J. Bootman and K. May

Study No.: 86/DPR 007/525

Date of Issue: (Not given)

TB Conclusions:

In replicate (separate) experiments, the test article did not induce any increase in revertent colonies (i.e., was negative) in Ames testing with S. typhimurium strains exposed to concentrations up to 5000 ug/plate (at which dose toxicity was found), in either the presence or absence of a mammalian activation system (rat S9)

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - L5296

Description: Fine off-white powder
Batch (Lot): Batch 3
Purity (%): (Not stated)
Solvent: Dimethylsulfoxide (DMSO)

B. Test Organisms - Bacterium

Species: Salmonella typhimurium
Strains: TA1535, TA1537, TA98, TA100 (all his⁻)
Source: Derived from cultures originally provided by Bruce Ames, UCal.

C. Study Design (Protocol) - A standard protocol (designated (ITP162) was included in the Report, and was stated to conform with OECD Guideline No. 471 for Ames testing.

A Quality Assurance statement (unsigned and not dated) was present, attesting to periodic inspections of procedures as well as the data generated.

D. Procedures/Methods of Analysis - A preliminary toxicity test was conducted in Strain TA98 cells (unactivated) exposed to eight concentrations of test material ranging from 2.5 to 5000 ug/plate, in order to select doses for Ames testing. From the results of this screening test, triplicate plates per treatment were exposed to test concentrations of 50, 158, 500, 1580, and 5000 ug/plate, both in the absence as well as presence of the postmitochondrial fraction (S9) of livers from young male CD rats whose microsomal enzyme activity had been induced by Aroclor 1254 treatment, and to which NADP-generating cofactors were added (S9 Mix). Solvent controls (DMSO) and positive controls appropriate for each strain and activation condition* were run concurrently. Two separate assays were performed with each strain.

---

*Sodium azide (NaAz, 2 ug), for nonactivated TA1535 and TA100 cultures.
2-Aminoanthracene (2-AA, 5 ug), for activated TA1535 cultures.
9-Aminoacridine (2-AAc, 50 ug), for nonactivated TA1537 cultures.
2-Nitrofluorene (2-NF, 5 ug), for nonactivated TA98 cultures.
Benzo[a]pyrene (BaP, 5 ug), for activated TA1537, TA100, and TA98 cultures.

Results:

In the cytotoxicity test, thinning of the background lawn (evidence of general toxicity) and decrease in revertent colony counts occurred only at 5000 ug/plate, which was thus chosen as the highest test dose.

In neither mutagenicity experiment were revertent colony counts increased in cultures treated with test article over solvent controls, in contrast to the expected response in the positive controls, in which increases 5 to 100X background were found (see data summary on page following). Hence, the authors concluded that "L5296 was devoid of mutagenic activity under the conditions of the test."

TB Evaluation:

ACCEPTABLE. This study was carried out in replicate with adequate procedures and controls (including sterility and viability checks, and confirmation of spontaneous reversion rates) such as to render the negative results obtained valid. Characterization of the test material (description, purity, etc.) was described elsewhere in the submission.

Table: Mean Revertent Counts in Salmonella typhimurium Cultures Exposed to L5296 (5000 ug/plate) and Known Mutagens*

Treatment		Test 1 TA 1535	TA 1537	TA 98	TA 100	Test 2 TA 1535	TA 1537	TA 98	TA 100
DMSO (Control)	-S9	14	8	30	123	14	8	32	127
	+S9	14	6	31	120	16	7	30	123
L5296	-S9	11	7	28	97	8	6	27	106
	+S9	8	6	29	107	10	3	30	110
2AA	-S9	12	--	--	--	13	--	--	--
	+S9	403	--	--	--	533	--	--	--
NaAz	-S9	829	--	--	693	607	--	--	497
BaP	-S9	--	5	28	115	--	7	27	120
	+S9	--	267	510	496	--	202	358	325
9 AAc	-S9	--	536	--	--	--	636	--	--
2 NF	-S9	--	--	529	--	--	--	306	--

*Extracted from Tables 1 to 4 of the Final Report. Responses at concentrations of L5296 less than 5000 ug/plate were also not different from solvent control (DMSO) values.

Reviewed By: Irving Mauer, Ph.D., Geneticist
Section VI, Toxicology Branch/HED (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch/HED (TS-769C)

7-26-88

7/21/89

DATA EVALUATION REPORT

006833

I. SUMMARY

Study Type: Mutagenicity - Cytogenetic Damage in vitro (Human Lymphocytes)

TB Project No.: 7-0828B
Caswell No.: 550A,
Metabolite of 419S

MRID No.: 402455-17

Chemical: L5296, 4-methoxy-N-6-dimethyl-1,3,5-triazin-2-amine, a metabolite of DPX-L5300 (EXPRESS®)

Synonyms: (None known)

Sponsor: Dupont

Testing Facility: Life Science Research, Ltd.
Suffolk (UK)

Title of Report: In Vitro Assessment of the Clastogenic Activity of L5296 in Cultured Human Lymphocytes

Authors: J. Bootman and G. Hodson-Walker

TB Conclusions:

The test article did not induce structural or numerical chromosome aberrations in cultured primary human lymphocytes when tested up to toxic levels (100 ug/mL).

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - L5296

Description: Fine off-white powder
Batch (Lot): 3
Purity (%): (Not stated)
Solvent: Dimethylsulfoxide (DMSO)

B. Test Organisms - Lymphocytes from human volunteer blood donors.

C. Study Design (Protocol - A formal protocol (designated (ITPICI) was included in the report, employing methods for this type of assay said to conform to EEC Directive No. 79/381 (Annex V), as well as to OECD Guideline #473.

Quality Assurance inspections during the course of the study were also carried out, as reported in an unsigned and undated statement.

D. Procedures/Methods of Analysis - Peripheral blood was obtained from a nonsmoking male, reportedly healthy and medication/drug-free. Small aliquots (0.5 mL) were dispensed to tubes containing RPMI-1640 buffered culture medium fortified with fetal calf serum, glutamine, heparin and antibiotics, as well as phytohemagglutinin to stimulate lymphocytes to divide. After a 48-hour incubation at 37 °C, cultures were centrifuged and cell pellets resuspended in fresh medium to which was added 25 uL of test article solutions, or DMSO, or positive control solutions (chlorambucil [CB] and cyclophosphamide [CP]). Mammalian metabolic activation, provided by S9 mix from rat livers which had been induced with Aroclor 1254, was added to designated tubes. All cultures were then incubated for a further 2 hours, following which they were centrifuged and cells resuspended in fresh medium to remove test compound and S9. Test article, solvent or positive control substances were again added to reestablish the previous exposure concentrations, and all cultures were reincubated for a further 22 hours.

Three hours before cultures were to be harvested, 0.4 ug/mL Colcemid was added to each tube, in order to arrest cell division and accumulate cells in the metaphase stage. At harvest, cultures were centrifuged, cells resuspended in hypotonic (0.56%) potassium chloride (to expand the cells) and then fixed in Carnoy's fluid (methanol:glacial acetic acid, 3:1 v/v). After further changes of fresh fixative, single drops of cell suspension were applied to microscope slides, allowed to air-dry, then stained with Giemsa (1:10 in Sorensen's buffer).

After air-drying,the slide preparations were cleared in xylene and mounted under DPX. From one to four slides were made from each culture.

A preliminary toxicity test was carried out, in which the mitotic index (MI) in 1000 cells per culture was determined (i.e., the percentage of lymphocytes in metaphase) in cultures exposed to five concentrations of L5296: 7.81, 15.63, 31.25, 62.5, and 125.0 ug/mL. The highest dose which produced a decrease in MI was selected as the HDT for the main cytogenetic assay.

In the main test, 100 metaphases from each of two coded slides per culture were scored for chromosome number, aberrant cells, and specific types and numbers of structural aberrations according to recognized (published) standards,* as follows:

Gap - Achromatic region(s) in chromatid(s) no greater than the width of a chromatid. Scored as singlestranded (SSG) or double-stranded (DSG).

Break - Achromatic region in chromatid(s) greater than the width of a chromatid, or a discontinuity with displacement. Scored as chromatid (SSB) or chromosomal (DSB).

Fragment - Any free, displaced portion of chromosome material, without a centromere (F), etc.

Exchange - Aberration arising from an exchange between two or more chromosomes which results in the products reuniting to form a dicentric or polycentric structure. These may be chromosome or chromatid interchanges. In studies of this type, where full karyotyping is not undertaken and chromosome banding has not been performed, only asymmetrical or chromatid exchanges will normally be recognized (E).

Multiple aberrations - Cells with more than eight aberrations (M).

*Ad Hoc Committee of the Environmental Mutagen Society and the Institute for Medical Research Report, Chromosome Methodologies in Mutation Testing. Toxicol. Appl. Pharmacol., 22, 269-275, 1972.

Endoreduplication - Diplochromosomes or quadruple chromosomes (etc.) in which 4, 8 (or more) chromatids are held together at the centromere (ENDO).

Pulverization - Extreme fragmentation of chromosomal material; recorded separately since no accurate chromosome count can be performed (PULV).

Polyploidy - Any multiple of the normal chromosome number; these are recorded separately and excluded from the total of cells scored (P).

Frequencies of aberrant metaphases (i.e., cells with one or more aberrations) were calculated for each culture, both with and without gaps. Fisher's Exact Probability Test was used to compare data from replicate cultures with and without S9 to determine any significant differences. Where no significant differences were found, data from activated and nonactivated cultures were pooled for subsequent analysis against solvent controls.

Two separate experiments were performed.

Results:

In the preliminary toxicity test, MI was reduced (range, 2.2-3.3%) at 4 of the 5 dose levels of test article compared to controls (4.8%), but there was no consistent pattern or dose-relation; the largest reduction in MI was found in high-dose (125 ug/mL) cultures (1.4%), representing a 71 percent reduction in mean MI (Report Table 1). Therefore, 100 ug/mL was chosen as the HDT for the main assay, and two lower doses of 50 and 10 ug/mL were also selected. Nonactivated positive controls were treated with 2.5 ug/mL CB, and activated controls with 6.0 ug/mL CP.

In both main assays, nondose-related reductions in MI were again recorded in test cultures at all dose levels, group means of 7.0 percent being recorded at all three concentrations, compared to 9.0 percent in controls (Report Table 2). The authors offer no plausible explanation for this flat response in MI.

No significant differences were found in incidences of aberrations between nonactivated and S9-supplemented control or L5296 treatments (Report Tables 6 and 7, attached here). Hence, results from replicate treatments with or without S9 mix were pooled when further comparisons between test and solvent control groups were made. On the other hand, a marked difference in aberrations was produced in CP cultures by the presence of S9, 49% with S9 vs. 1 percent

without S9 when gaps were included ($p < 0.001$); 42% vs. 0.5 percent ($p < 0.001$), excluding gaps.

Compared to solvent control values for aberrant metaphases (ranging between 0 and 3 percent), L5296 cultures had comparable values (Report Table 4, attached here), whether gaps were included or excluded. Comparable to the cytogenetic damage in S9-supplemented CP-treated cultures, CB without S9 produced a significant increase in aberrant metaphases containing all types of chromosomal damage (Table 4).

The authors concluded that L5296 did not induce cytogenetic damage in cultured human lymphocytes.

TB Evaluation:

ACCEPTABLE. The study was conducted with adequate procedures and controls such that the negative results obtained (no induction of chromosome aberrations at levels into the toxic range, 100 ug/mL) are considered valid.

ttachments

Express science review

Page _____ is not included in this copy.

Pages 165 through 167 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.