[redacted]

03/08/90

PEER REVIEW FILES

007724

CHEMICAL NAME: Express
CASWELL NO.: 419S
CAS NO.: 101200-48-0
REVIEWER: Gardner

CURRENT AGENCY DECISION

C (HED); D (SAP).

TUMOR TYPE / SPECIES

Mammary gland adenocarcinomas;
Sprague-Dawley rats (F).

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. / /	2. 06/01/89	2. 07/14/89	2. C
1. 09/02/88	1. 12/14/88	1. 04/07/89	1. C; 4.46 x 10-2

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. 05/09/89	1. D (equivocal)

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. 04/19/89	1. / /
1. 08/23/88	

MISCELLANEOUS:

Miscellaneous: Reevaluation, 5/24/89; and Letter from Company 5/2/89 including "Ninety-day Feeding Study...". Stamped 2/5/90; #PR-007724; 125 p.; nha.

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007724

Peer Review Documents
(Memo dates)

7/14/89

007724

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

JUL 14 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Second Peer Review of Express® - Reevaluation Following the May 9, 1989 Science Advisory Panel Review

From: Roger Gardner, Toxicologist
Review Section 1 Roger Gardner 6-5-89
Toxicology Branch I (IRS)/HED (H7509C)

To: Richard Mountfort, Product Manager #23
Registration Division (H7507C)

The Peer Review Committee met on June 1, 1989 to examine the issues raised by the Science Advisory Panel (SAP) with respect to the classification of the carcinogenicity of Express®.

A. Individuals in Attendance:

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

Ed Budd	Edwin R Budd
Kerry Dearfield	Kerry Dearfield
Reto Engler	Reto Engler
Penelope Fenner-Crisp	Penelope A. Fenner-Crisp
George Ghali	G. Ghali
Richard Levy	Richard A Levy
Esther Rinde	Esther Rinde
William Sette	William Sette
Marcia Van Gemert	Marcia Van Gemert

2. Reviewers: (non-panel members responsible for data presentation signatures indicate technical accuracy of panel report).

Roger Gardner (Reviewer) Roger Gardner

Bernice Fisher Bernice Fisher

Robert Zendzian 6/6/89

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Diane Beal Karl P. Pfeiffer

Robert Beliles Robert Beliles

William Burnham

Marion Copley Marion Copley

Richard Hill —

Jack Quest Jack A. Quest

Lynnard Slaughter L. J. Slaughter

B. Material Reviewed:

The SAP response memorandum from the May 9, 1989 meeting was reviewed by the Committee.

C. Considerations:

The Science Advisory Panel (SAP) reviewed the weight-of-the-evidence considerations and classification of the oncogenic potential of Express®.

The Panel commented as follows:

> ..., Express® is most appropriately placed in Category D (equivocal). The only evidence for carcinogenicity was obtained with doses that greatly exceeded the MTD (Maximum Tolerated Dose). In addition, the mid dose also exceeded the MTD (26% wt loss). The middle and low doses did not produce any statistically significant evidence of carcinogenicity. It seems clear that only adenocarcinomas in the group treated with 1250 ppm were elevated, and that this result is from a data set that can not readily be interpreted due to excessive toxicity. It would be of interest to know whether prolactin or other hormones were altered under those conditions. However, this is of little relevance to low dose risk. The negative data obtained with male rats and mice and the lack of positive genetic toxicology also support Category D.

D. Conclusions

The Committee upheld its classification of Express® as a Category C oncogen because of a statistically significant and dose-related increase in the incidence of malignant tumors (mammary gland adenocarcinomas) in female rats. In addition, the increased incidence exceeded the historical control range, and there was evidence of a structure-activity relationship to Atrazine which also causes mammary tumors in rats.

The oncogenic response observed may be associated with a hormonal imbalance that may not occur at doses below an MTD. The Peer Review Committee concluded that a quantitative risk assessment for Express® is not appropriate because the increased incidence of mammary gland tumors was observed in female rats treated at dose levels exceeding the Maximum Tolerated Dose (MTD), there was no evidence of genetic toxicity shown in several studies, and structural analogs of Express® (other than Atrazine) were not associated with oncogenic responses in rats and mice.

5/24/89

007724

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

FILE COPY

MAY 24 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Reevaluation of Permethrin and Express Following SAP Review

FROM: Esther Rinde, Ph.D. E.R.
Manager, ONCO Peer Review
Health Effects Division (TS-769c)

TO: Addressees

On May 9, 1989, the SAP reviewed these two chemicals which were previously evaluated by the Peer Review Committee. A meeting to discuss the issues on Permethrin and Express is scheduled for Thursday, June 1, 1989, from 9:00 to 10:00 in Room 821.

Permethrin will be discussed from 9:00 to 9:30

Express will be discussed from 9:30 to 10:00

Copies of the Peer Reviews and SAP reports are attached.

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
K. Baetcke
E. Budd
M. Van Gemert
M. Copley
J. Quest
L. Slaughter
K. Dearfield
R. Levy
W. Sette
G. Ghali
B. Fisher
R. Gardner
P. Zendzian
J. Doherty

4/7/89

007724

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

FILE COPY

APR 7 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Express

FROM: Kerry L. Dearfield, Ph.D. 2-7-89
Executive Secretary, Peer Review Committee
Scientific Analysis and Coordination Branch
Health Effects Division (TS-769C)

TO: Richard Mountfort
Product Manager #23
Registration Division (TS-767C)

The Health Effects Division Peer Review Committee met on 12/14/88 to discuss and evaluate the weight-of-the-evidence on Express with particular reference to its oncogenic potential. The Committee unanimously classified Express as a Category C-Possible Human Carcinogen. Quantification of oncogenicity risk was not recommended at this time. The decision to quantitate risk is contingent upon further testing to examine what role possible Express-induced hormonal alterations may play in influencing the oncogenic process.

A. Individuals in Attendance:

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

William L. Burnam	
Reto Engler	
Judith Hauswirth	
Marcia Van Gemert	
John Quest	
Esther Rinde	
Kerry Dearfield	

Marion Copley

Robert Beliles

Richard Hill

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

Roger Gardner

Edwin Budd

Bernice Fisher

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

George Ghali

Richard Levy

William Sette

Lynnard Slaughter present

Diane Beal

4. Other Attendees: Sanford Bigelow (HED)

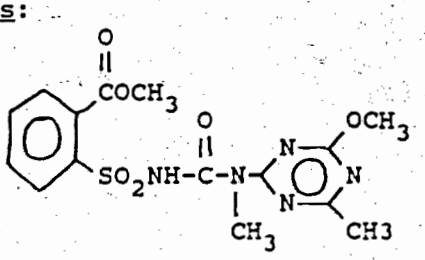
B. Material Reviewed:

The material available for review consisted of DER's for a rat oncogenicity study, a mouse oncogenicity study and five mutagenicity tests, and other data summaries prepared by Roger Gardner; tables and statistical analysis were prepared by Bernice Fisher. The material reviewed is attached to the file copy of this report.

C. Background Information: Express is a new chemical that is proposed for use as a herbicide on wheat and barley. The pesticide is also known as DPX-L5300. The registrant is E.I. DuPont de Nemours and Company. The chemical name is benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1,3,5-triazin-2-yl]-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester.

The Chemical Abstracts Number is 101200-48-0 and the Tox Chem Number is 419S.

Structure of Express:



D. Evaluation of Oncogenicity Evidence for Express:

1. Rat Oncogenicity Study

Reference: Tobia, A.J. March 10, 1987. Combined Chronic Toxicity/Oncogenicity Study with INL-5300: 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.

Express (technical, 96.8% a.i.) was administered in the diet to groups of 72 male and 72 female Charles River Crl:CD BR (Sprague Dawley) strain rats at levels of 0, 25, 250 and 1250 ppm for two years. At the end of 12 months, 10 animals of each sex/dose group were sacrificed.

An elevated incidence of mammary gland adenocarcinomas was observed in female rats treated with Express (Table 1). Male rats did not manifest any significant tumorigenicity responses.

a. Discussion of Tumor Data

Express was associated with a significant positive dose-related trend for female mammary gland i) adenocarcinomas and ii) combined adenomas and/or adenocarcinomas. In pairwise comparisons with control and the highest dose group, female mammary gland adenocarcinoma and combined adenoma and/or adenocarcinoma tumor incidences were found to have significant differences. Since there were no increases in the incidences of tumor types other than adenocarcinoma, the Peer Review Committee suggests concentrating only on the adenocarcinoma incidence, not the combined tumor types incidence. The increased incidence of adenocarcinoma seen at 1250 ppm of Express (i.e. 26/71 or 37%; Table 1) exceeded historical control incidences in twelve other studies performed in female rats of the same strain in the testing laboratory for the years 1980-1988 (range of 1.7% to 23.4%). 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

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Laboratory. There was no apparent decrease in the latency period for tumor occurrence. No compound-related increase in tumors was observed in male rats.

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Table 1. Express, Rat Study - Female mammary gland tumor rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Observation		Dose level (ppm) 0	25	250	1250
fibroadenoma		16/70 (23)	12/61 (20)	7/59 (20)	6/71 (13)[a]
	p=	0.0578	0.4103	0.4492	0.0862
adenoma		1/46 (2)	1/44 (2)	2/47 (4)[b]	2/47 (4)
	p=	0.2921	0.5054	0.3832	0.3832
adenocarcinoma		10/70 (14)	9/61 (15)	13/59 (22)	26/71 (37)[c]
	p=	0.0002**	0.6283	0.1802	0.0020**
adenoma and/or adenocarcinoma		11/70 (16)	10/61 (16)	15/59 (25)	28/71 (39)[c]
	p=	0.0002**	0.5513	0.1253	0.0014**

[+] Number of tumor bearing animals/number of animals at risk, excluding those that died before observation of the first tumor.
( ) Percent

[a] First adenoma at week 53.
[b] First adenoma at week 89.
[c] First adenocarcinoma at week 53.

Note: Significance of trend denoted at Control
Significance of pairwise comparison with control denoted * at Dose level.
* p<0.05 ** p<0.01

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b. Considerations of Adequate Dosing for Assessment of Oncogenic Potential

In this study, there was no significant effect on survival of treated animals. At both 13 weeks into the study and at the end of the study, there were significantly decreased body weight gains at the high dose in relation to controls: values at 13 weeks were 5.3 and 9.2% for 250 ppm and 21 and 34% for 1250 ppm for males and females, respectively; at the end of study, 10.8 and 26.6% for 250 ppm and 36.4 and 53.8% for 1250 ppm for males and females, respectively.

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. Mineralization of the aorta and stomach was 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.

The Peer Review Committee considered the large weight reduction and other toxic signs at the highest dose to indicate clear toxicity and that a highest adequate dose was exceeded. Also, the incidence of polyarteritis supports this consideration for male rats. There was also some weight loss in female rats at the mid-dose at the end of the study which indicated that an adequately high dose was slightly exceeded for females. The body weight gain changes seen in male rats at the mid-dose suggested that this dose was adequate for oncogenicity testing for males.

2. Mouse Oncogenicity Study

Reference: 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.

Express (technical, 96.8% a.i.) was administered in the diet to groups of 80 male and 80 female Charles River Crl:CD-1(ICR) BR strain mice at levels of 0, 20, 200 and 1500 ppm for 18 months.

Under the conditions of the study, Express did not produce evidence of compound-related increased incidences of any tumor type examined.

Considerations of Adequate Dosing for Assessment of Oncogenic Potential

The Peer Review Committee concurred that there was no evidence of oncogenicity due to Express seen in the CD-1 mouse; however, there was a question whether there was adequately high dosing for assessment of oncogenic potential in female mice. A ninety-day subchronic study (dose levels to 2500 ppm; reported in oncogenicity study) did not appear to support the adequacy of the dose levels used in the oncogenicity study; therefore, the toxic effects reported in the chronic study itself were considered for adequacy of dosing. Survival rates were not statistically different from control rates although there was a slight increase in mortality in the high dose male group compared to control (65% at 1500 ppm vs. 51% control). The most frequently identified probable cause of death was amyloidosis; the incidence was statistically significantly increased in male and female mice at the highest dose level ($p < 0.01$; Fisher's Exact Test). Also found in both sexes was a statistically significant increase in thyroid inflammation at the highest dose.

At 13 weeks, there was approximately a 10% decrease in body weight gain for the high dose group males while the female mice in that group gained the same amount of weight as control mice. By the end of the study, the highest dose groups had minimal effects on mean 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). Food consumption results and clinical signs observed suggested that the body weight decreases were not associated with other effects as diarrhea, anorexia, emaciation or poor palatability of test diets.

Incidence of bilateral seminiferous degeneration (atrophy) and oligospermia were statistically significantly increased in the 200 and 1500 ppm male groups. The proportion of animals with seminiferous degeneration in each group having oligospermia increased with dose (58, 63, 78 and 80% for control, low, mid and high dose groups, respectively). The NOEL was 20 ppm (3 mg/kg/day) and the LEL was 200 ppm.

The combination of body weight alterations, slight increase in mortality at the high dose, the dose-related effects on the testes and the other age-related effects suggested adequate dosing by Express in male mice. Results from female mice at the high dose (specifically the minimal body weight alterations) were questioned as to whether adequate dosing was used for female mice. However, combined with thyroid inflammation (not a compromising toxic effect by itself), other age-related effects, and most importantly, use of analogue data (see Section E.5. below for fuller discussion) which provides little evidence for oncogenic effects in mice, this mouse study (both sexes) was

considered sufficient for assessment of oncogenic potential. Additionally, there was no evidence for genotoxic effects by Express (see Section E.3. below).

E. Additional Toxicology Data on:

1. Metabolism

A series of limited experiments (e.g. MRID No. 402455-16) 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 the value for female rats was 69 to 96 hours.

The major route of excretion in rats is 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.

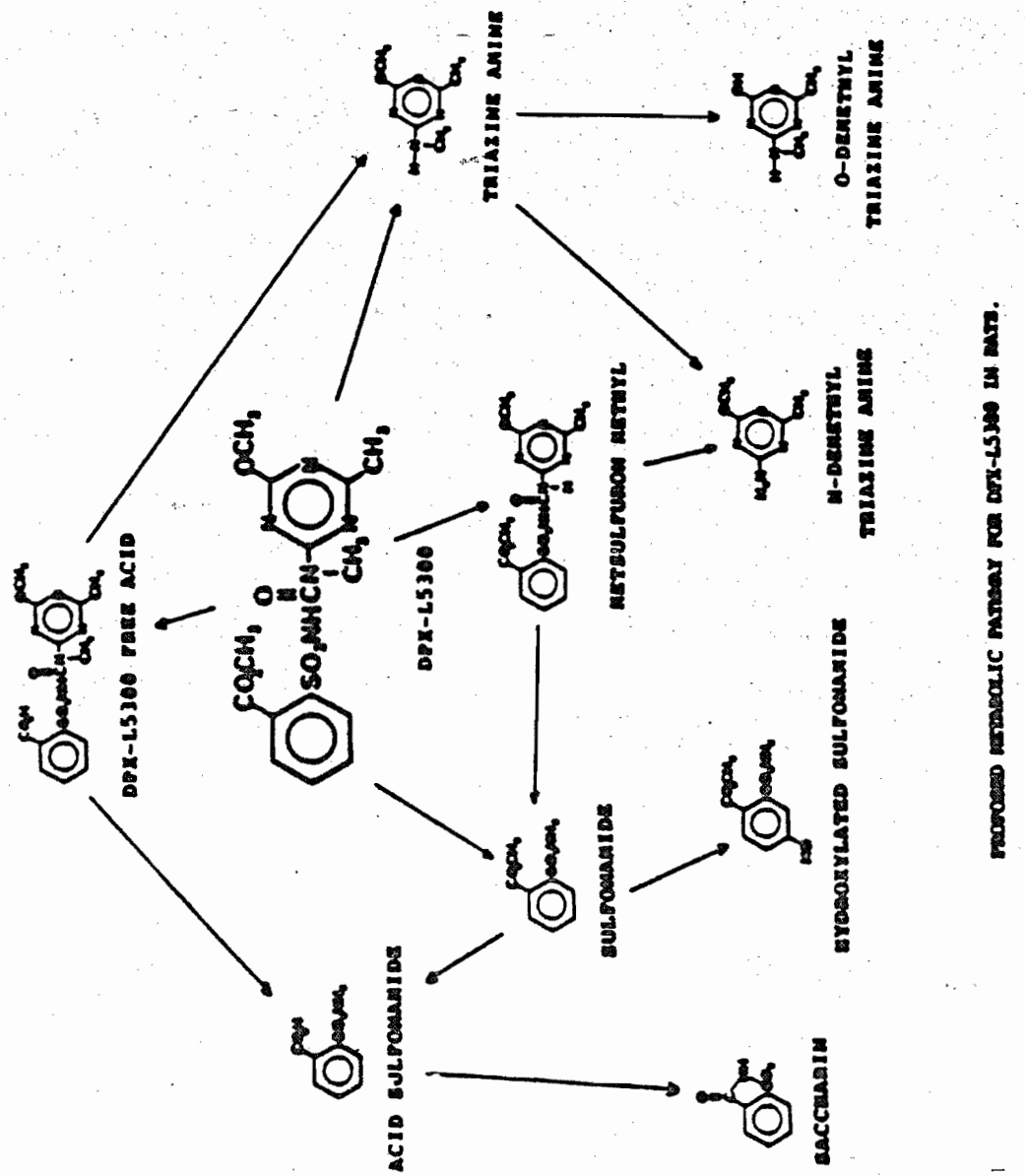
Major metabolites in the urine and feces included metsulfuron methyl, saccharin, and O-demethyl triazine amine. There was no evidence of glucuronide or sulfate conjugation. A diagram of the proposed metabolic pathways for Express in rats is found in Figure 1.

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.

FIGURE 1

Du Pont Report No. AMR-781-87

Du Pont HLR 31-87



PROPOSED METABOLIC PATHWAY FOR DPX-L5300 IN RATS.

2. Acute, Subchronic, Chronic Effects

The acute oral LD_{50} in rats is greater than 5000 mg/kg.

Toxic effects observed in a 90-day rat feeding study (EPA Acc. No. 073790) included decreases in food consumption, body weight gain, food efficiency and absolute organ weights for the heart, brain, liver and kidneys. Relative organ weights for the heart, liver, kidneys, testes and spleen were increased due to the decreased body weights observed. Serum glucose, globulin and cholesterol concentrations were also decreased, but no treatment-related histopathological effects were noted. The LOEL was 1750 ppm (highest dose tested) and the NOEL was 100 ppm (5 mg/kg/day).

In a one-year dog study (MRID No. 402455-12), the NOEL was established at 25 ppm (0.625 mg/kg/day) and the LEL was 250 ppm based on elevated blood levels of bilirubin and aspartate aminotransferase (AST), increased urinary volume and decreased body weight gain in males. Elevated bilirubin, AST, creatinine and globulin levels with decreased body weight gain were seen in females.

3. Mutagenicity

Five mutagenicity studies have been submitted with Express as the test substance. These studies are considered acceptable and indicate no genotoxic activity by Express. They satisfy the requirement for testing in the three categories of mutagenicity testing as follows:

Gene mutation assays:

a. Salmonella assay (EPA Acc. No. 073790): no increase in frequency of reverse mutations in strains TA97, TA98, TA100 and TA1535 when exposed up to 500 ug/plate without metabolic activation and up to 2000 ug/plate with metabolic activation.

b. Chinese hamster ovary (CHO) cells/HGPRT gene mutation assay (EPA Acc. No. 073790): no mutagenic activity at concentrations up to 5.0 mM with and without metabolic activation.

Structural chromosomal assays:

a. Rat bone marrow aberrations assay (EPA Acc. No. 073790): single oral doses up to 5000 mg/kg had no effect on the incidence of chromosomal aberrations or mitotic index of bone marrow in male and female rats.

b. Mouse micronucleus assay (EPA Acc. No. 073790): single oral doses up to 5000 mg/kg had no effect on the incidence of polychromatic erythrocytes with micronuclei in male and female

mice. A slight reduction of the ratio of polychromatic to normochromatic erythrocytes was noted.

Other genotoxic effects:

a. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (EPA Acc. No. 073790): UDS was not induced at concentrations up to 2500 uM.

4. Developmental and Reproductive Effects

In a rat teratology study (EPA Acc. No. 073790), doses of 125 and 500 mg/kg produced maternal toxicities of 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 indicated the NOEL for maternal and developmental toxicity was 20 mg/kg/day and the LOEL was 125 mg/kg/day in rats.

A developmental toxicity study in rabbits (MRID No. 402455-14) indicated 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 was 20 mg/kg/day. There were no fetal malformations or variations associated with administration of test substance to pregnant rabbits.

In a rat two-generation reproduction study (MRID No. 402455-15), no effects were seen on fertility, gestation or lactation at dietary levels up to 1250 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 (1.25 mg/kg/day) and the LEL was 250 ppm.

5. Structure-Activity Correlations

Express is structurally similar to other herbicides including Londax, Harmony, Beacon and Glean (see Figure 2). These compounds have moieties that are similar for a structure-activity relationship (SAR) comparison. Harmony and Glean both have a s-triazine component similar to Express, and Londax and Beacon have a similar substituted phenyl moiety (they also have pyrimidine rings versus a s-triazine ring). Londax, Harmony and Glean have been tested for oncogenicity in mice and rats and were negative. These compounds appear to be relatively non-toxic as substantial doses were used in these studies (e.g. for Londax, 7500 ppm in rats and 5000 ppm in mice), but maximum adequate

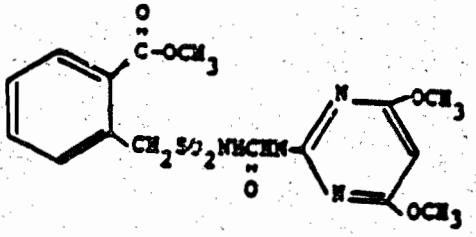
dosing for assessing oncogenic effects may not have been attained. Beacon was reported negative for oncogenicity in rats, but was associated with an increased incidence of masses in the liver. These compounds do not provide substantial support for an oncogenicity concern for Express based on SAR.

On the other hand, another analogue, Atrazine, provides support to the increased incidence of adenocarcinomas seen in rats after administration of Express. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a s-triazine analogue of both the s-triazine moiety of Express and the triazine amine metabolite of Express (Atrazine itself is not a metabolite of Express). Atrazine produces the same mammary tumor type as Express. In the same strain of female rats, atrazine induced a significant dose-related increase in adenocarcinomas (including two rats with carcinosarcomas at the highest dose tested (HDT)) at doses of 70, 500 and 1000 (HDT) ppm. In addition, atrazine increased the incidence of fibroadenomas at the high dose and this increase was associated with a significant dose-related trend. As an additional note, a statistically significant increase in testicular interstitial cell tumors was seen in male rats at the highest dose, but this increase was within historical control values. This however is consistent with some of the testicular effects induced by Express.

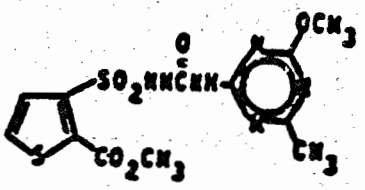
Atrazine was classified by the Peer Review Committee as a Category C oncogen. A quantitative risk characterization was not recommended contingent upon generation of additional data to elucidate the most appropriate method of risk characterization, i.e. data to support a hormonal mechanism that may be involved in the production of the mammary tumors in Sprague-Dawley female rats. This hormonal hypothesis may be appropriate for the induction of mammary tumors by Express. In addition to the mammary tumors, the testicular effects of Express suggests a possible hormonal influence.

Two of the major metabolites of Express present additional concerns for oncogenic potential. A large proportion of Express is excreted in the urine as saccharin, which is implicated as a bladder carcinogen. While there may be potential for bladder oncogenic effects (indeed there were kidney effects noted with Express administration), there were no unusual effects that would be considered oncogenic in the bladder reported. Another metabolite, sulfonamide, has been associated with thyroid problems. Again, while there was some thyroid inflammation in mice, there did not appear to be effects considered oncogenic in these studies due to sulfonamide. A third metabolite, metsulfuron methyl, was tested for oncogenicity in rats and mice and found negative. The metsulfuron methyl studies have not been rigorously reviewed and may have maximum adequate dosing problems associated with them.

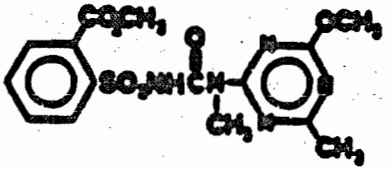
Londax (DPX-F5384)



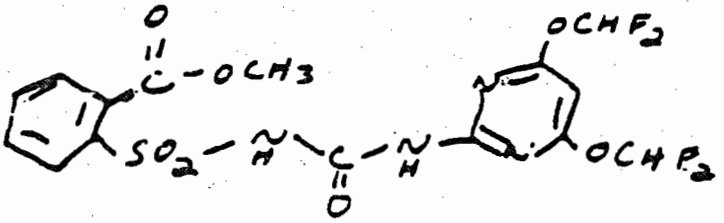
Harmony (DPX-M6316)



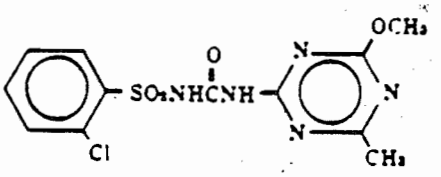
Express (DPX-L5300)



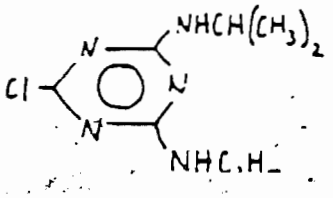
Beacon (CGA-136872)



Glean (DPX-W4189)



Atrazine



F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Express to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Express, when administered in the diet to female Charles River Crl:CD BR (Sprague-Dawley) strain rats at dietary doses of 0, 25, 250 and 1250 ppm for two years, was associated with significant positive dose-related trends for female mammary gland tumors (malignant and benign/malignant combined). The incidences of adenocarcinoma and combined adenoma/adenocarcinoma tumors at the highest dose tested (1250 ppm) were also significantly elevated above concurrent control values when tested by the Fisher Exact Test. This significance was due entirely to the increase in malignant tumors at the highest dose tested.

2. The increased incidence of adenocarcinoma seen at 1250 ppm exceeded historical control incidences in twelve other studies performed in female rats of the same strain in the testing laboratory during the same time period as the Express study.

3. There was no evidence for a reduction in the latency period for the time to mammary gland tumor appearance in female rats.

4. There was no compound-related increase in tumors observed in male rats or in male and female mice.

5. For the rat study, the highest dose tested apparently exceeded the highest adequate dosing for assessment of oncogenic potential. The mid-dose had some toxic effects which indicated that an adequately high dose was approached in males, but slightly exceeded in females. The incidence of adenocarcinomas in female rats was increased at the mid-dose, but this increase was not significant. The dosing in the mouse study was considered sufficient for assessment of oncogenic potential.

6. Express did not have a mutagenicity concern with negative results found in five mutagenicity studies (Salmonella assay, cultured CHO mammalian cells mutation assay, rat bone marrow aberrations assay, mouse micronucleus assay and an unscheduled DNA synthesis assay).

7. Express did not evoke adverse reproductive effects in rats and was not teratogenic in rats or rabbits.

8. Express is structurally related to other herbicides/chemicals. Londax, Harmony, Glean and metsulfuron methyl did not provide evidence of oncogenic effects. Beacon was associated with increased liver masses, but this was not seen in the Express studies. Atrazine, however, had a similar tumor profile as Express and the induction of tumors by a hormonal mechanism may

be similar for both compounds.

9. Major metabolites of Express, specifically saccharin and sulfonamide, were of concern as they are suspected carcinogens. However, no tumors in their respective target organs were seen after administration of Express.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Peer Review Committee concluded that the data available for Express provided evidence to classify Express as a Category C oncogen ("Possible Human Carcinogen"). This was based on the significant increase in mammary gland adenocarcinomas found in female Sprague-Dawley rats (by NTP criteria, this finding would be considered "clear evidence of carcinogenicity in female rats"). There was no evidence of carcinogenicity in male rats or in male or female mice. There was no evidence of genotoxic activity.

The Committee considered the appropriateness of risk quantification for Express. Factors supporting a quantification included the clearly malignant response at the high dose, the dose-related trend for the malignant tumors and the similarity of tumor profile to other s-triazines, specifically atrazine. However, factors supporting no quantification at this time included: the tumor was observed in one sex and one species, it was significantly increased only at the highest dose tested at which the compound was clearly toxic and exceeded a maximum adequately high dose to assess oncogenic potential, other analogues besides the s-triazines had little evidence of oncogenic potential, and the Committee had not found quantification appropriate for the s-triazine analogues.

The Committee concluded that quantification of oncogenic risk by Express was not appropriate at this time. However, this is an interim decision contingent upon required testing to examine the possible association between Express-induced tumors and a hormonal influence. Possible hormone/receptor effects by Express should be examined including measurement of serum estrogen and other endocrine levels (including prolactin if possible). This information is required for the Committee to determine the most appropriate method of risk determination.

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FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Express® as a Class C Oncogen

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of a set of scientific issues being considered by the Environmental Protection Agency in connection with the peer review classification of Express®. The review was conducted in an open meeting held in Arlington, Virginia, on May 9, 1989. All Panel members, except Dr. Robert Anthony and Dr. James Swenberg, were present for the review.

Public notice of the meeting was published in the Federal Register on April 17, 1989.

Oral statements were received from staff of the Environmental Protection Agency and from Dr. Fredrick O'Neal (DuPont).

Written comments were received from Dr. James Swenberg (FIFRA SAP) and read into the record for consideration by the Panel.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Express®

Issue

The Agency requests any specific comments the Panel may have on the weight of the evidence and classification of Express with respect to Agency Guidelines for Carcinogen Risk Assessment.

Panel Comments on Express®

After reviewing the data submitted, the Scientific Advisory Panel believes that Express® is most appropriately placed in Category D (equivocal). The only evidence for carcinogenicity was obtained with doses that greatly exceeded the MTD. In addition, the mid dose also exceeded the MTD (26% wt loss). The middle and low doses did not produce any statistically significant evidence of carcinogenicity. It seems clear that only adenocarcinomas in

the group treated with 1250 ppm were elevated, and that this result is from a data set that cannot readily be interpreted due to excessive toxicity. It would be of interest to know whether prolactin or other hormones were altered under those conditions. However, this is of little relevance to low dose risk. The negative data obtained with male rats and mice and the lack of positive genetic toxicology also support Category D.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Robert B. Jaeger
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 5/16/89

007724

SAP Executive Summary; Meeting Date(s)

007724

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

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FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Robert B. Jaeger
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 5/16/89

Qualitative/Quantitative Risk Assessment

4/19/89

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007146

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

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Express (INL-5300) - Quantitative Risk Assessment,
Two Year Chronic/Oncogennicity Sprague-Dawley Rat Study

caswell no. 419S

From: Bernice Fisher, Biostatistician Bernice Fisher 4/19/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

To: Roger Gardner, Toxicologist
Review Section I
Toxicology Branch I - Insecticide/Rodenticide Support
Health Effects Division (H7509C)

Thru: John A. Quest, Ph.D., Section Head John A. Quest 4/19/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Summary

The unit risk, Q_1^* of express is 4.46×10^{-2} $(mg/kg/day)^{-1}$ in human equivalents. This estimate of Q_1^* is based upon female mammary gland tumors (adenoma and/or adenocarcinoma) in Sprague-Dawley rats with dose levels of 0, 25, 250 and 1250 ppm.

Survival in the female rats was not significantly dose related. There was a significant dose related trend in female mammary gland tumor (adenoma and/or adenocarcinoma) rates. See the memorandum on "Express (INL-5300) - Qualitative Risk Assessment, B.Fisher -8/23/88" for further details in the selection of female mammary gland tumors for use in the quantitative risk assessment.

Dose-Responce Analysis

The most sensitive reaction to express occurred in female rats, in terms of a statistically significant dose related trend in mammary gland tumor (adenoma and/or adenocarcinoma) rates and also in its significant difference in the pair-wise comparison of controls and the highest dose group. Since there was no statistical evidence of significant dose related mortality in the female rats, the estimate of unit risk, Q_1^* of express based upon mammary tumor data, was calculated by the use of the Global86 (Multi-Stage process) computer program program of K.Crump. The unit risk calculated from the female data in ppm doses was coverted to rat mg/kg/day by the use of Lehman's Tables and then to human equivalents by the use of interspecies surface area adjustments as recommended by EPA Cancer Giudelines (1986).

The resultant estimate of Q_1^* is as follows:

	Rat, Q_1^* $(mg/kg/day)^{-1}$	In Human Equivalents
female mammary gland tumor(adenoma &/or adenocarcinoma)	8.41×10^{-3}	4.46×10^{-2}

It is to be noted that Q_1^* is an estimate of the upper (95%) bound on risk and that (as stated in the EPA Guidelines) the "true value of the risk is unknown and that the lower limit of the risk may be as low as zero".

References

Howe, R.B., Crump, K.S. and Van Landingham, C. (1986)
A Computer Program to Extrapolate Quantal Animal
Toxicity Data to Low Doses (unpublished report), 25 pgs.

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007146

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Express (INL-5300) - Qualitative Risk Assessment,
Two Year Chronic/Oncogenicity Rat Study

caswell no. 419 S

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch, HED (TS-769C)

Bernice Fisher 8/23/88

TO: Roger Gardner, Toxicologist
Section VI
Toxicology Branch, HED (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostistics Team
Scientific Mission Support Staff
Toxicology Branch, HED (TS-769C)

R. Levy
8-23-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch, HED (TS-769C)

SUMMARY:

The qualitative risk assessment of the two year dietary express (96.8 % purity) study in Sprague Dawley rats indicated that there were no significant survival disparities in females with dose levels of 0, 25., 250., and 1250. ppm.

Female mammary gland adenocarcinoma, adenoma and/or adenocarcinoma tumor rates exhibited significant trends with dose increments of express.

In the pairwise comparisons with control and the highest dose group, female mammary gland adenocarcinoma, and adenoma and/or adenocarcinoma tumor rates were found to have significant differences.

Male rats did not manifest any siginificant tumorgenicity responces thus no further evaluation was attempted in this report.

30

BACKGROUND

A 24 month 96.8 % pure express (INL-5300) dietary study in Sprague Dawley rats was conducted by Haskell Laboratories (study 61-87) for E.I. DuPont and reported in March, 1987.

The study design allocated groups of 72 males and 72 females, each to a dietary regimen of 0, 25., 250., or 1250. ppm of DPX-L5300 for two years. At the end of 12 months, 10 animals of each sex/dose group were sacrificed.

Table 1. Express (96.8 % pure) - Experimental Design of the Rat Study

Dose	No. of Rats		Week of Interim sacrifice (12 months)	
(ppm)	Male	Female	Male	Female
0	72	72	10	10
25.	72	72	10	10
250.	72	72	10	10
1250.	72	72	10	10

Survival Analysis

No significant increases in female mortality with dose increments of express was found by using a statistical survival analysis based upon the Thomas, Breslow, and Gart computer program. See Table 2. for details.

Tumor Analysis

In the absence of survival disparities among the dose levels of express in female rats, the evaluation of tumor rat trends with dose increments were made by the use of the Cochran-Armitage Trend test (one-sided) and the pairwise comparisons of controls and each dose level by the Fisher Exact test.

The only evidence of substantial tumorgenicty occurred in the female mammmary glands with dose increments of express. Statistical evaluation of their tumor rates indicated a significant ($p<.01$) trend in mammary gland adenocarcinomas, and in mammary gland adenomas and/or adenocarcinomas with dose increments of express.

Only the pairwise comparison of controls with the highest (1250. ppm) dose level of express resulted in significant ($p<.01$) differences in tumor rates of female mammary gland adenocarcinomas and of adenomas and/or adenocarcinomas. See Table.3 for details.

Table 2. Express, Rat Study - Female Mortality Rates+ and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[a]	53-78	79-104	105[a]	Total
0	0/72	2/72	10/10	10/60	22/50	28/28	34/62 (55)
25.	0/72	2/72	10/10	9/60	25/51	26/26	36/62 (58)
250.	2/72	2/70	10/10	7/58	15/51	36/36	26/62 (45)
1250.	0/72	1/72	10/10	6/61	19/55	36/36	26/62 (45)

+ Number of animals that died/Number of animals alive at the beginning of the interval.

( ) percent

a Interim Sacrifice at 53 weeks and Final Sacrifice at 105 weeks.

Note: Above time intervals were selected for display purposes only.

Significance of trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

** $p<.01$ and * $p<.05$

Table 3. Express, Rat Study - Female Mammary Gland Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dose (ppm) 0	25.	250.	1250.
A. Fibroadenomas				
	16/70	12/61	7/59	6/71
	(23)	(20)	(20)	(13)[a]
p =	0.0578	0.4103	0.4492	0.0862
B. Adenomas and Adenocarcinomas				
Adenomas	1/46	1/44	2/47	2/47
	(2)	(2)	(4)[b]	(4)
p =	0.2921	0.5054	0.3832	0.3832
Adenocarcinomas	10/70	9/61	13/59	26/71
	(14)	(15)	(22)	(37)[c]
p =	0.0002**	0.6283	0.1802	0.0020**
Adenomas and/or Adenocarcinomas	11/70	10/61	15/59	28/71
	(16)	(16)	(25)	(39)[c]
p =	0.0002**	0.5513	0.1253	0.0014**

+ Number of tumor bearing animals/number of animals at risk, excluding those that died before observation of the first tumor.

( ) percent

a first fibroadenoma at week 53.

b first adenoma at week 89.
c first adenocarcinoma at week 53.

Note: Significance of trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.
** $p < .01$ and * $p < .05$

References

Armitage, P. (1955) Tests for Linear Trends in Proportions, Biometrics 11, 375-386.

Cochran, W.G. (1954) Some Methods for Strengthening the Common X^2 Test, Biometrics 10, 417-451.

Cox, D.R. (1972) Regression Models and Life Tables (with discussion) J. Royal Stat. Soc. Ser. B. 34, 187-220.

Thomas, D.G., Breslow, N., and Gart, J.J. (1977) Trend and Homogenity Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, 373-381.

8/23/88

007724

FILE COPY

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Express (INL-5300) - Qualitative Risk Assessment,
Two Year Chronic/Oncogenicity Rat Study
caswell no. 419 S

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch, HED (TS-769C)
Bernice Fisher 8/23/88

TO: Roger Gardner, Toxicologist
Section VI
Toxicology Branch, HED (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostistics Team
Scientific Mission Support Staff
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R. Levy
8-23-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch, HED (TS-769C)

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The qualitative risk assessment of the two year dietary express (96.8 % purity) study in Sprague Dawley rats indicated that there were no significant survival disparities in females with dose levels of 0, 25., 250., and 1250. ppm.

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+ Number of animals that died/Number of animals alive at the beginning of the interval.

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a Interim Sacrifice at 53 weeks and Final Sacrifice at 105 weeks.

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Significance of trend denoted at Control.
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The only evidence of substantial tumorgenicty occurred in the female mammmary glands with dose increments of express. Statistical evaluation of their tumor rates indicated a significant ($p<.01$) trend in mammary gland adenocarcinomas, and in mammary gland adenomas and/or adenocarcinomas with dose increments of express.

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a first fibroadenoma at week 53.

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037724

Reviewer's Peer Review Package for 1st Meeting

2/8/88

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

FILE COPY

DEC 8 1988

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Express

FROM: Esther Rinde, Ph.D. ER
Manager, ONCO Peer Review
Health Effects Division (TS-769c)

TO: Addressees

Attached for your review is a package on EXPRESS,
prepared by Mr. Roger Gardner.

A meeting to consider the classification of Express
is scheduled for 12/14/88 at 2:00 in Room 821, CM2.

Addressees

W. Burnam
R. Engler
R. Hill
B. Beliles
D. Beal
J. Hauswirth
M. Van Gemert
M. Copley
J. Quest
L. Slaughter
K. Dearfield
R. Levy
W. Sette
G. Ghali

9/2/88

C.7724

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Reto Engler, Ph. D., Chief
Science Analysis and Coordination Branch
Health Effects Division

FROM: Roger Gardner, Toxicologist 9-2-88
Insecticides and Rodenticides Branch
Health Effects Division

THRU: Judith W. Hauswirth, Ph. D., Acting Chief
Insecticides and Rodenticides Branch
Health Effects Division
9/2/88

SUBJECT: Peer Review of Express®

Attached please find the following information for consideration by the Peer Review Committee:

1. Background information
2. DER on a rat feeding/oncogenicity study
3. DER on a mouse oncogenicity study
4. DER's on five mutagenicity studies

42

11/23/88

SACB 9/2

ATTACHMENT 1

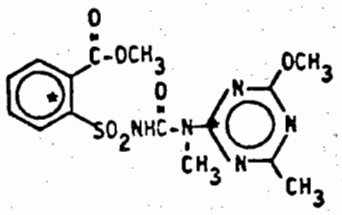
Background Information for Peer Review of
the Oncogenic Potential of Express®

037724

Background Information for Peer Review of Express

I. Background

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



benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester

A. Metabolism of Express

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 is 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 or 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.

A diagram of the proposed metabolic pathways for Express® in rats is included in Addendum A. below.

B. 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). Structures for these chemicals are included in Addendum B. below.

C. Non-Oncogenic Effects

The acute oral LD_{50} in rats is greater than 5000 mg/kg.

Toxic effects observed in a 90-day rat feeding study included decreases in food consumption, body weight gain, food efficiency, and absolute organ 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 mg/kg/day).

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

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 in rats.

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.

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.

II. Data Relevant to Oncogenicity

A. Long-Term Studies

1. 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.

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). The respective decreases in the mid dose group for male and female rats were approximately 5.3 and 9.2%. 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. Mineralization of the aorta and stomach 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.

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).

Oncogenicity: 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.

2. 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.

Toxicity: 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 mean 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 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.

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

3. 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.

B. Short Term Studies - 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.

ADDENDUM A

Diagram of the Proposed Metabolic Pathways
for Express (DPX-L5300)

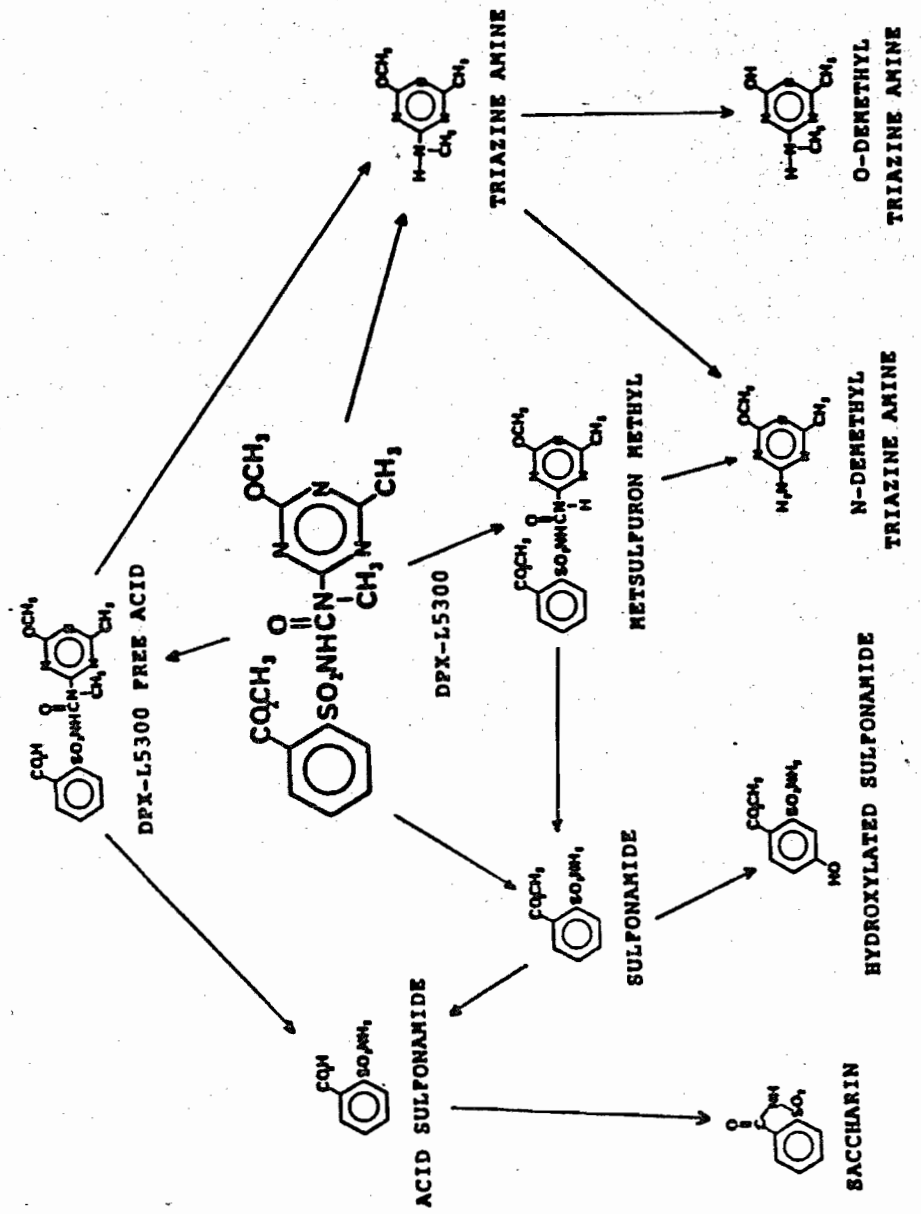


FIGURE 39.

PROPOSED METABOLIC PATHWAY FOR DPX-L5300 IN RATS.

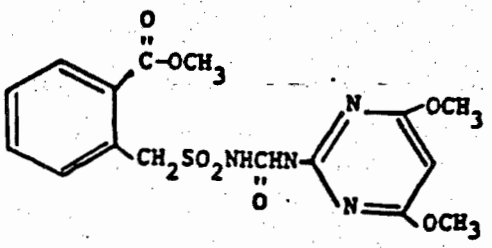
ADDENDUM B

Structures of Compounds Related to Express

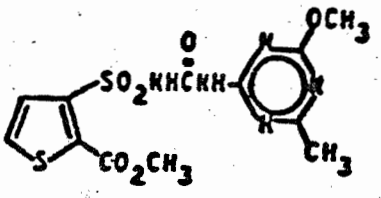
COMPARISON OF LONDAX AND ITS ANALOGS

Structures:

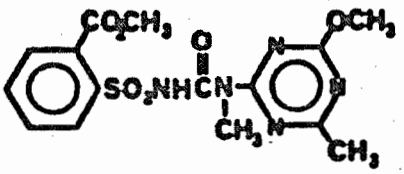
Londax (DPX-F5384)



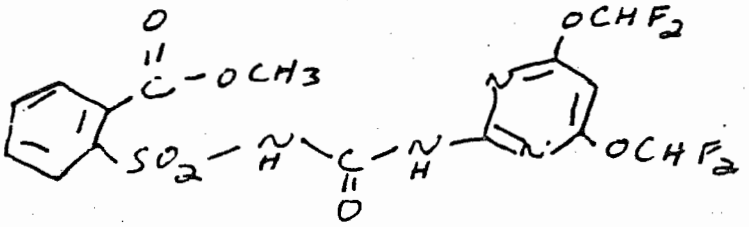
Harmony (DPX-M6316)



Express (DPX-L5300)



Beacon (CGA-136872)



ATTACHMENT 2

Data Evaluation Record for the Rat Feeding Study

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

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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 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

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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	At 12 months
	Survivors	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.

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 amino-transferase (AST)
- X Alanine amino-transferase (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

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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.

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	Mortalities* during days					
	Males			Females		
(ppm)	0-365	366-546	547-730	0-365	366-546	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

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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.

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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*	.739	0.804*
Termination	2.907	3.818*	.716	0.884*

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

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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.

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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).

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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

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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:

	Dose level (ppm)			
Location	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:

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

he body weight and body weight gain decreases observed in male and female ats 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).

ATTACHMENT 3

Data Evaluation Record for the Mouse Oncogenicity Study

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

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Reviewed by: Roger Gardner Roger Gardner 8-19-85
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: 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 Medicine

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 dose group males, and the female mice in that group gained the same amount of 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.

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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 groups		Dose	Animals per sex	
No.	Designation	(ppm)	Main study*	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. 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, and 18 months.
Necropsy	Animals found dead or moribund	When found.
	10	At 12 months
	Survivors	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. 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.

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	

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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.

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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:

	Mortalities during days					
Dose	Males			Females		
(ppm)	0-365	366-553	Termination	0-365	366-546	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.

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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:

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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.

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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.

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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.

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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	46/78 **
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).

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
Harderian 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

. Authors' Conclusions

he 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.

The 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:

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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	29.3
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

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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 stated conditions of the study.

ATTACHMENT 4

Data Evaluation Studies on Mutagenicity Studies

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DATA EVALUATION ASSAY

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE; Benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester (The composition was reported on page 4 of the original report which was not included in the copy reviewed herein.)

3. STUDY/ACTION TYPE: Mutagenicity - Ames assay

4. STUDY IDENTIFICATION: Haskell Laboratory for Toxicology and Industrial Medicine. May 25, 1985. Mutagenicity evaluation in Salmonella typhimurium. Unpublished Report No. 245-83 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 2/11/86

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 2/12/86

7. DISCUSSION AND CONCLUSIONS: No increase in the incidence 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.

Core classification: Unacceptable because the report is incomplete as described in the previous paragraph.

8. MATERIALS AND METHODS

Reference mutagens: 2-Aminoanthracine, 9-aminoacridine, N-methyl-N'-nitro-N-nitrosoguanidine, and 2-nitroflourene were used as positive controls.

Vehicle: Dimethyl sulfoxide (DMSO) was used as the vehicle for the test substance and reference mutagens.

8. MATERIALS AND METHODS (continued)

Bacterial culture media: Top agar for selection of histidine revertants. This minimal agar medium contained 0.6% agar and 0.6% NaCl. Immediately before use of the selective top agar 0.05 mM L-histidine and 0.05 mM D-biotin was added to the medium.

Minimal bottom agar. Davis Minimum agar was used as the bottom agar.

Microsomal enzyme (S-9) preparation: Liver microsomal preparations were obtained from Aroclor 1254 induced Charles River CD® rats. To each 0.3 ml sample of the S-9 was added 0.7 ml of the following: 8uM $MgCl_2$, 33 uM KCl, 4 uM NADP, 100 uM sodium phosphate buffer (pH 7.4), and 5 uM glucose-6-phosphate

Toxicity testing and dose-selection procedures: Up to 10 mg test substance per plate were tested with cultures of the TA1535 strain on minimal selective agar plates with and without metabolic activation. The stated criteria for selection of the highest dose level to be used in the mutagenicity assays was slight toxicity.

Mutagenicity assay procedure: The report stated that the test substance was serially diluted and 5 or 6 doses were tested in all 4 strains with and without metabolic activation. The test substance was solubilized in DMSO. Each dose and vehicle control was tested in duplicate. For tests without metabolic activation, 100 ul of each tester strain (10^8 cells) and 100 ul test or control solution were added to 20 ml selective minimal top agar. In tests with metabolic activation, 100 ul of the test strain, 500 ul of test solution, and 0.5 ml of the S-9 mixture were added to 2.0 ml of the selective minimal top agar. These solutions were overlaid on minimal bottom agar, and the plates were then incubated at 37° C for 48 hours. After incubation the revertant colonies on each plate were counted. 2-Aminoanthracine (2AA) was used in all test strains as the positive control for assays with metabolic activation; for assays without metabolic activation, 2-nitrofluorene (2-NF) was used in strains TA98 and TA100, 9-aminoacridine (9AA) was used in strain TA1535, and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was used as the positive control in strain TA97.

9. REPORTED RESULTS

Concentrations of the test substance >500 ug/plate without metabolic activation were described as toxic. On plates with the S-9 mix, concentrations >1000 ug/plate were described as toxic.

9. REPORTED RESULTS (continued)

On the basis of the preliminary results, the report noted that maximum dose levels of 2000 and 500 ug were selected for the mutagenicity assays with and without metabolic activation, respectively.

Mutagenicity assays: The investigators noted that the test substance did not cause a positive response in any strain tested.

The mean numbers of revertants/plate (calculated from the duplicate plates in each trial independent of the original report) are as follows:

Dose (ug per plate)	TA1535	TA97	TA98	TA100
	Without activation			
0	23	98	16	100
5	23	109	16	87
10	26	106	15	98
50	21	167	15	101
100	17	110	19	96
500	16	106	13	79
2NF	--	--	1683	3753
MNNG	3200	--	--	--
9AA	--	826	--	--
	With activation			
0	16	133	25	98
10	17	139	25	101
50	14	124	23	88
100	14	133	27	95
500	11	130	20	87
1000	8	123	18	90
2000	7	120	15	40
2AA	146	968	1405	618

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DATA EVALUATION ASSAY

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE: Benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester (96.8% active ingredient).

3. STUDY/ACTION TYPE: Mutagenicity - Point mutation assay in Chinese Hamster Ovary cells in vitro.

4. STUDY IDENTIFICATION: Richard, L. B., D. V. Ullman, W. N. Choy, and A. M. Sarriff. May 30, 1985. Mutagenicity evaluation of INL 5300-20 in the CHO/HGPRT assay. Unpublished Report No. 58-85 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
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Signature: Roger Gardner
Date: 2/11/86

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E. Harris
Date: 2/12/86

7. CONCLUSIONS: No mutagenic activity was observed in CHO cells exposed to 0.5 to 5.0 mM DPX-L5300 with and without activation.

Core classification: Acceptable

8. MATERIALS AND METHODS

Test species: The BH4 clone of the Chinese Hamster Ovary (CHO) K1 cell line was used. They were routinely maintained in HaM's F12 medium without hypoxanthine and contained 5% dialyzed heat-inactivated fetal bovine serum (DHIFBS) without antibiotics. Cultures were incubated at 37° C in 5% CO_2 and 90% relative humidity. Cells were removed from these cultures for subculturing with 0.05% trypsin.

Positive control substances: 9,10-Dimethyl-1,2-benzanthracene (DMBA) and methanesulfonic acid, ethyl ester (EMS) were used.

Solvent: Dimethylsulfoxide (DMSO) was used.

8. MATERIALS AND METHODS (continued)

Metabolic activation (S-9) mixture: Ten male Crl:CD® rats were induced with Aroclor 1254. Subsequently, the animals were sacrificed. The livers were removed and homogenized in cold (4° C) phosphate buffered saline. The homogenate was centrifuged at 9000 X g, and the supernatant (S-9 fraction) was decanted and stored at -70° C until needed. According to the report, the protein concentration was determined, and then the Cytochrome P_{450} concentration was determined (3.3 mmoles/mg protein).

Media: Treatment medium for cultures in assays without metabolic activation was the same as that for culture maintenance mentioned above with addition of penicillin (50 units/ml) and streptomycin (50 ug/ml). The medium (pH 7.2) was buffered with HEPES (2.5×10^{-2}M). Test substance was added in the solvent at an appropriate concentration with the total volume of 60 ul added to 3 ml of the culture medium.

The same treatment medium was modified for assays with metabolic activation. For these assays the S-9 fraction (1 mg protein/ml), magnesium chloride (5.6×10^{-3}M), glucose-6-phosphate (5×10^{-3}M), and nicotinamide adenine dinucleotide phosphate (1.5×10^{-3}M).

The medium used to allow the cells to express mutations contained 6-thioguanine, but the composition of that medium was not described in the report.

Cytotoxicity studies: The report stated that preliminary cytotoxicity studies with and without metabolic activation were conducted to provide a basis for dose selection in the mutagenicity assy. The criteria for selection of test concentrations were described as follows:

> Ideally, the highest concentration of test chemical used should give about 10% survival as compared to the control. In cases where sufficient toxicity could not be demonstrated, the test compound was tested up to and slightly beyond the limit of solubility in the treatment medium.

Experimental procedure: The report stated that approximately 5×10^5 cells were plated in a 25 cm^2 culture flask with 5 ml of the culture medium (described above). These flasks were incubated until the next day when the culture medium was removed and the treatment medium was added to the cells. The cultures were then incubated for 16 to 20 hours (without the S-9 fraction) or for 5 hours (with the S-9 fraction). Subsequently, treatment medium was removed, and the cells were

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8. MATERIALS AND METHODS (continued)

washed with culture medium. Those cells treated without the S-9 fraction were immediately subcultured in the expression medium (described above), while those treated with the S-9 fraction were placed in culture medium and incubated for 21 to 25 hours before subculturing in the expression medium. Incubation conditions were the same as those described above for routine culture maintenance.

Data analysis: A two variable (dose and experiment) Analysis of Variance (ANOVA) model allowing for unequal sample sizes and numbers of doses for each trial was used according to the report. Mutation frequencies had to be transformed before such a statistical model could be usd because of the complex nature of the experimental errors and the data characteristics required by the assumptions associated with the analytical model. The investigators indicated that the frequencies were subjected to a power transformation ($Y = [\text{mutation freaquency} + 1]^{0.15}$).

Each set of results for a given test substance concentration was compared with the solvent control by a t test to determine statistically significant increases in mutation frequency. ANOVA was used to test for statistically significant dose-response relationships. The report stated that linear, quadratic or higher order effects were analyzed by an F test.

Historical control data from 20 assays without S-9 fraction and 20 with the fraction were used to establish the following criteria of acceptability for each trial:

1. A cloning efficiency between 42 and 93%.
2. A spontaneous mutation frequency between 0 and 45 per million cells

According to the report, a positive result meets the following criteria:

1. The mutation frequency at one or more test substance concentrations is significantly greater ($p<0.01$) than that of the solvent control.

2. The correlation between mutant frequency and test substance concentration is significantly ($p<0.01$) greater than 0.

A test substance is considered to be negative if:

1. The mutation frequency at none of the test substance concentrations is significantly greater ($p<0.01$) than that of the solvent control.

9. REPORTED RESULTS

2. The correlation between mutant frequency and test substance concentration is not significantly ($p<0.01$) greater than 0.

solubility: The report noted that the maximum attainable concentration of the test substance in DMSO was 250 mM.

Test substance cytotoxicity: In preliminary cytotoxicity studies with and without activation (S-9 fraction), respective concentrations up to 5.0 mM or 2.5 mM did not cause toxicity. The authors noted that concentrations higher than 5.0 mM would require cytotoxic concentrations of DMSO, and therefore, higher concentrations were not used. (The report noted that the 5 mM concentration was achieved by adding 60 ul of the 250 mM stock solution to 3 ul of the culture medium.)

On the basis of the number of cells per flask, the investigators concluded that concentrations of 2.5 mM and higher were cytotoxic. Results reported for those concentrations and the control group are summarized in Table 1.

Table 1

Cytotoxicity of the test substance to CHO/HPRT cells (prior to inoculation of the expression medium)

Concentration (mM)	Cells per flask (x 10^6) Without activation Trial 1	Without activation Trial 2	With activation Trial 1	With activation Trial 2
0	2.70	3.04	2.11	3.21
	2.55	3.07	2.03	2.87
2.5	2.39	2.82	1.81	2.35
	2.49	2.56	1.69	2.81
3.75	2.22	2.39	1.23	2.42
	2.25	2.85	1.36	2.22
5.0	2.03	2.61	1.43	2.45
	2.13	2.55	1.63	2.11

Table 2 summarizes the reported mutant frequencies. The only statistically significant increased mutant frequency results were those of the positive control groups when compared with results from the solvent controls. The dose-response correlation was determined to be insignificantly different from 0 ($p=0.1342$ for the assay without activation and $p=0.0607$ in the activated assay).

9. REPORTED RESULTS (continued)

Table 2

Mutant frequency (per 10^6 surviving cells)

Concentration (mM)	Without activation		With activation	
	Trial 1	Trial 2	Trial 1	Trial 2
0	0	4.4	14.0	10.0
	1.2	3.6	6.9	6.2
0.5	2.4	0	0	0
	8.5	21.7	5.6	0
1.0	13.0	16.5	7.6	20.1
	9.9	5.9	9.5	24.9
2.5	0	16.5	0	31.8
	8.1	1.3	0	4.3
3.75	0	0	0	0
	3.1	7.5	6.7	1.9
5.0	10.5	1.5	4.4	3.1
	14.4	10.3	0	5.9
0.5**	141.3	191.2	---	---
	167.0	134.4	---	---
0.015**	---	---	134.0	162.4
	---	---	132.1	108.5

*Positive control; EMS
**Positive control; DMBA

10. DISCUSSION

There were adequate data presented by the authors to support their conclusion that DPX-L5300 is not mutagenic in CHO cells under the conditions of the experiment.

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DATA EVALUATION RECORD

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE; Benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester (96.8% active ingredient) was used.

3. STUDY/ACTION TYPE: Cytogenetics - rats

4. STUDY IDENTIFICATION: Ullmann, D. V., and A. M. Sarriff. June 14, 1985. In vivo assay of INL-5300-20 for chromosomal aberrations in rat bone marrow cells. Unpublished Report No. 286-85 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist Signature: Roger Gardner
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6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: ~~Geneticist~~ Head Signature: Jane E Harris
Organization: Review Section 6 Date: 2/12/86
Toxicology Branch

7. CONCLUSION: 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.

Core classification: Acceptable

8. MATERIALS AND METHODS

Test species: Eight-week old male and female Sprague-Dawley Crl:CD® (SD)BR strain rats were used. The males weighed from 213 to 271 g, and the females weighed from 171 to 213 g.

Positive control substance: Cyclophosphamide was used as the reference mutagen in this study.

Experimental procedures: Three groups containing 15 male and 15 female rats were given single oral doses of 50, 500, or 5000 mg test substance per kg body weight. The test substance was administered in corn oil by gavage. One group of 15 males and 15 females was given corn oil without the test substance, and a second group of 5 animals per sex was given 20 mg cyclophosphamide per kg in distilled water.

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8. MATERIALS AND METHODS (continued)

Five animals of each sex from each of the three groups given the test substance and from the vehicle control group were sacrificed 6, 24, and 48 hours after dosing. The 5 rats of each sex from the positive control group were also sacrificed 24 hours after dosing. Two hours before the animals were sacrificed, each was given an intraperitoneal injection of 1 mg colchicine per kg body weight to arrest dividing cells in metaphase.

The report stated that bone marrow cells were then harvested from both femurs by aspiration with Hank's Balanced Salt solution. Cells were treated with a hypotonic KCl solution (0.075 M) and fixed with glacial acetic acid:methanol (1:3). Slides were made from these preparations and flame dried. They were stained with Giemsa stain and mounted in Permount® for microscopic observation.

Cytotoxicity was determined by the mitotic index for each sample.

According to the report, there were 50 metaphase cells evaluated on each slide. The number and type of chromosomal aberrations were noted along with the position in the optical field scanned of cells with abnormal metaphases. Only those cells with 40 to 43 chromosomes were considered adequate for scoring.

The authors stated that chromosomal aberrations were classified as follows:

> Chromatid type aberrations including chromatid breaks, isochromatid breaks, fragments, triradials, quadriradials, intrachanges, and cells with 10 or more aberrations.
>
> Chromosome type aberrations including acentric fragments, double minutes, ringed chromosomes, translocations, dicentric chromosomes, pulverized chromosomes, and pulverized cells.
>
> Chromatid and isochromatid gaps which were noted but not considered as aberrations.

Statistical analyses: The individual animal was considered as the experimental unit with the percentage of cells with one or more aberrations, percentage of abnormal cells with more than one aberration, and the number of aberrations per cell were subjected to statistical analyses. The analyses included the Mann-Whitney U test, Fisher's Exact test, and the Jonckheere test for trends. Results from both sexes for each of the three observation times were pooled before statistical procedures were conducted.

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8. MATERIALS AND METHODS

Mitotic indices and body weight data were analyzed by two-way analysis of variance.

9. REPORTED RESULTS

A red discharge from the eyes, nose, and mouth was reported in 2, 1, and 8 females from the low, mid, and high dose groups, respectively. Five males in the high dose group also exhibited the discharges.

Other signs which occurred sporadically included wheezing, lethargy, hunched back, sensitivity to touch, and one closed eye. There were one or two animals with one of these signs.

The only other clinical sign noted by the investigators was soft feces or diarrhea which was associated with the use of corn oil as the vehicle in the experiment.

A statistically significantly decreased body weight was reported at the highest dose level for males and females and mid-dose group females 24 and 48 hours after dosing (see Table 1).

Table 1

Group mean body weight (g) results

Parameter	Dose (mg/kg) 0	50	500	5000
	Males			
Body wt at 24 hr	249.4	240.2	251.2	235.6
Body wt gain	9.6	4.6	2.0*	-8.0***
Body wt at 48 hr	265.6	252.6	254.0	226.4
Body wt gain	14.2	14.8	3.8**	-8.4***
	Females			
Body wt at 24 hr	197.4	189.2	196.6	182.2
Body wt gain	1.6	1.4	4.6	-13.4***
Body wt at 48 hr	202.4	195.6	190.0	189.6
Body wt gain	7.6	4.2	-1.4*	-11.6***

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

9. REPORTED RESULTS (continued)

There were no compound related effects on the mitotic index, percentage of cells with aberrations, percentage of abnormal cells with more than one aberration, or number of aberrations per cell (See Appendix below for reported group means).

10. DISCUSSION

There were adequate data presented in the report to support the conclusions of the authors (see Section 7. CONCLUSIONS above).

APPENDIX

Summary of group mean mitotic indices and chromosomal aberration data as presented in the original report cited in Section 4. above

TABLE 2A

SUMMARY OF ABERRATION DATA - 6 HR SACRIFICE

INL-5300-20 IN VIVO ASSAY FOR CHROMOSOME ABERRATIONS IN RAT BONE MARROW CELLS

Treatment	Sex	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Percent Abnormal Cells Per Group[1]	Percent Cells Per Group With >1 Aberration[1]	Average Number of Aberrations Per Cell[1]	Average Number of Mitoses Per 500 Cells (S.E.)
Corn Oil	M	5	250	0.0	0.0	0.000	15.6 (1.7)
	F	5	250	0.4	0.0	0.004	13.6 (2.7)
	Combined:	10	500	0.2	0.0	0.002	14.6 (1.6)
50 mg/kg INL-5300-20	M	5	250	0.0	0.0	0.000	17.0 (1.5)
	F	5	250	0.0	0.0	0.000	16.2 (1.7)
	Combined:	10	500	0.0	0.0	0.000	16.6 (1.1)
500 mg/kg INL-5300-20	M	5	250	0.0	0.0	0.000	16.4 (1.6)
	F	5	250	0.4	0.0	0.004	16.0 (2.6)
	Combined:	10	500	0.2	0.0	0.002	16.2 (1.4)
5000 mg/kg INL-5300-20	M	5	250	0.4	0.0	0.004	16.8 (1.6)
	F	5	250	0.0	0.0	0.000	16.8 (2.5)
	Combined:	10	500	0.2	0.0	0.002	16.8 (1.4)

INL-5300-20 = H-15,527
MR 4581-222

[1] Data for males and females were combined for analysis.

TABLE 2B

SUMMARY OF ABERRATION DATA - 24-HR SACRIFICE

INL-5300-20 IN VIVO ASSAY FOR CHROMOSOME ABERRATIONS IN RAT BONE MARROW CELLS

Treatment	Sex	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Percent Abnormal Cells Per Group[1]	Percent Cells Per Group With >1 Aberration[1]	Average Number of Aberrations Per Cell[1]	Average Number of Mitoses Per 500 Cells (S.E.)
Corn Oil	M	5	250	0.4	0.0	0.004	10.2 (1.0)
	F	5	250	0.0	0.0	0.000	11.6 (1.5)
Combined:		10	500	0.2	0.0	0.002	10.9 (0.9)
50 mg/kg INL-5300-20	M	5	250	0.8	0.0	0.008	14.6 (1.8)
	F	5	250	0.4	0.4	0.008	11.2 (1.4)
Combined:		10	500	0.6	0.2	0.008	12.9 (1.2)
500 mg/kg INL-5300-20	M	5	250	0.4	0.0	0.004	11.6 (1.9)
	F	5	250	0.0	0.0	0.000	10.4 (1.4)
Combined:		10	500	0.2	0.0	0.002	11.0 (1.1)
5000 mg/kg INL-5300-20	M	5	250	0.0	0.0	0.000	8.8 (1.9)
	F	5	250	0.4	0.0	0.004	12.8 (3.3)
Combined:		10	500	0.2	0.0	0.002	10.8 (1.9)
Cyclophosphamide (20 mg/kg)	M	5	250	19.2	16.0	1.136	5.2 (0.9)
	F	5	250	28.0	22.8	1.596	8.0 (1.4)
Combined:		10	500	23.6***	19.4***	1.368***	6.6* (0.9)

INL-5300-20 = II-15,527
MR 4581-222

[1] Data for males and females were combined for analysis.

* $p < 0.05$
*** $p < 0.001$

TABLE 2C

SUMMARY OF ABERRATION DATA - 48-HR SACRIFICE

INL-5300-20 IN VIVO ASSAY FOR CHROMOSOME ABERRATIONS IN RAT BONE MARROW CELLS

Treatment	Sex	Number of Animals Per Group	Number of Metaphases Analyzed Per Group	Percent Abnormal Cells Per Group[1]	Percent Cells Per Group With >1 Aberration[1]	Average Number of Aberrations Per Cell[1]	Average Number of Mitoses Per 500 Cells (S.E.)
Corn Oil	M	5	250	0.8	0.0	0.008	18.8 (2.6)
	F	5	250	0.0	0.0	0.000	26.0 (2.6)
	Combined:	10	500	0.4	0.0	0.004	22.4 (2.1)
50 mg/kg INL-5300-20	M	5	250	0.0	0.0	0.000	14.6 (2.7)
	F	5	250	0.8	0.0	0.008	17.0 (3.2)
	Combined:	10	500	0.4	0.0	0.004	15.8 (2.0)
500 mg/kg INL-5300-20	M	5	250	0.0	0.0	0.000	16.0 (1.0)
	F	5	250	0.4	0.0	0.004	23.0 (1.7)
	Combined:	10	500	0.2	0.0	0.002	19.5 (1.5)
5000 mg/kg INL-5300-20	M	5	250	0.0	0.0	0.000	15.4 (2.9)
	F	5	250	0.4	0.0	0.004	20.6 (4.2)
	Combined:	10	500	0.2	0.0	0.002	18.0 (2.6)

INL-5300-20 = H-15,527
MR 4581-222
[1]Data for males and females were combined for analysis.

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DATA EVALUATION RECORD

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE; Benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester (96.8% active ingredient) was used.

3. STUDY/ACTION TYPE: Micronucleus assay - rats

4. STUDY IDENTIFICATION: Ullmann, D. V., and A. M. Sarrif. July 22, 1985. Mouse bone marrow micronucleus assay of INL-5300-20. Unpublished Report No. 420-85 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

5. REVIEWED BY:

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6. APPROVED BY:

Name: Jane Harris Ph. D.
Title: ~~Section Head~~
Organization: Review Section 6
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Signature: Jane E Harris
Date: 2/11/86

7. DISCUSSION AND CONCLUSION: The report contained adequate information to support the authors' conclusions. 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. The 5000 mg/kg dose did not increase the incidence of polychromatic erythrocytes with micronuclei in treated mice.

8. MATERIALS AND METHODS

Test species: Seven-week-old male and female Crl:CD®-1 (ICR)BR strain mice were used. The males weighed from 30.2 to 34.2 g, and the females weighed from 22.5 to 27.0 g.

Positive control substance: Cyclophosphamide was used as the positve control.

Preliminary considerations The investigators stated that information was available on the acute oral toxicity for rats which suggested an approximate LD_{50} of >11,000 mg test substance per kg body weight. According to the report, a test dose

8. MATERIALS AND METHODS

of 5000 mg/kg was selected because it is equivalent to a limit dose for acute oral toxicity studies.

Experimental procedure: A group containing 18 male and 18 female mice was given a single dose of 5000 mg/kg by oral intubation, and a second group containing 15 animals of each sex was given the corn oil vehicle without test substance. A third group that contained 5 male and 5 female animals was given a single oral dose of cyclophosphamide in distilled water. Four hours after dosing, the animals were observed for the appearance of clinical signs. They were also weighed and observed daily for toxic signs thereafter.

Subgroups of 6 animals of each sex given the 5000 mg/kg dose and 5 of each sex from the vehicle control group were subsequently sacrificed 24, 48, and 72 hours after dosing. The 5 male and female mice in the positive control group were sacrificed 24 hours after treatment.

The bone marrow was asparated from both femurs of each mouse, and the cells were suspended in fetal bovine serum and centrifuged for 5 min at 1000 X g. One or two drops of fetal bovine serum were added to each button, and the suspension was smeared on a microscope slide. Four slides were prepared for each animal, and they were dried at 56° C and fixed with methanol. Slides were then stained with Giemsa stain, cleared in xylene, and coverslipped with Permount®.

Scoring of the slides was described as follows:

> Only cells showing good morphology and staining were selected for scoring. PCEs (polychromatic erythrocytes) were identified by their characteristic blue-purple-gray staining; NCEs (normochromatic erythrocytes) appeared reddish-orange. One-thousand PCEs per animal were scored for the presence of micronuclei,...Inclusions which were irregularly shaped or stained, or not in the focal plane of the cell were judged to be artifacts and were not scored. Cells containing more than one micronucleus were counted as having s single micronucleus; the unit of scoring was the micronucleated PCE, not the micronucleus. The number of micronucleated NCEs seen in the optic field scored for PCEs was also recorded.

The report further stated that the ratio of the number of PCEs to NCEs encountered during scoring of PCEs was determined. A ratio less than one was defined as an indicator of bone marrow toxicity.

8. MATERIALS AND METHODS (continued)

Statistical analyses: Each animal was considered the experimental unit, and the report-stated that an arcsine transformation of the proportion of PCEs and the PCE:NCE ratios was done. The transformed data were then subjected to analysis of variance on the basis of a 3-factor model (treatment, sex, and time of observation). Two factor considerations were also included in the model according to the report. Body weight changes were analyzed by a two-way (treatment and sex) ANOVA. If dose related effects were noted, pairwise comparisons were made using the Student's t test. Differences were considered to be statistically significant if $p \leq 0.05$.

9. REPORTED RESULTS

According to the report, there were no clinical signs observed in the negative control group, but 6 hours after dosing, one treated male exhibited tremors, hypersensitivity, and hyperactivity. These signs were observed in more male mice on the day after treatment, but only 1 to 4 animals were reported with these signs. One male and one female from the treated group were observed in moribund condition on the day after dosing and were found dead on the second day after treatment. One female given the test substance was found dead on the day after dosing also. One male from the positive control group showed decreased activity the day after dosing. At the 72-hour observation none of the surviving animals exhibited clinical signs according to the report.

The investigators concluded that there was no statistically significant effect on body weight gain during the study (see Appendix A below). There was a statistically significant decrease in the PCE:NCE ratio (Appendix B below) which was described as an indication that the test substance is cytotoxic under the test conditions.

There was no significant effect noted on the proportion of PCEs with micronuclei in the INL-5300-20 treated mice (see Appendix B below).

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APPENDIX A

Body weight results form mice treated with
INL-5300-20

TABLE 1: BODY WEIGHT DATA

INL-5300-20 MOUSE MICRONUCLEUS ASSAY

Treatment	Sacrifice Time (hrs)	Sex	Animals Per Group	Initial Body Weight (g) x ± S.E.	Terminal Body Weight (g) x ± S.E.	Change in Body Weight (g) x ± S.E.
Corn Oil	24	M	5	32.8 ± 0.4	32.4 ± 0.4	- 0.4 ± 0.1
		F	5	24.5 ± 0.3	24.6 ± 0.5	0.1 ± 0.2
		Combined:	10	28.7 ± 1.4	28.5 ± 1.3	- 0.2 ± 0.1
INL-5300-20 5000 mg/kg	24	M	6	32.2 ± 0.4	32.4 ± 0.6	0.2 ± 0.6
		F	6	24.7 ± 0.4	25.7 ± 0.5	1.0 ± 0.2
		Combined:	12	28.4 ± 1.2	29.0 ± 1.1	0.6 ± 0.3
Cyclophos-phamide 40 mg/kg	24	M	5	32.1 ± 0.2	31.6 ± 0.8	- 0.5 ± 0.9
		F	5	24.3 ± 0.3	23.8 ± 0.4	- 0.5 ± 0.1
		Combined:	10	28.2 ± 1.3	27.7 ± 1.4	- 0.5 ± 0.4
Corn Oil	48	M	5	32.3 ± 0.4	32.4 ± 0.6	0.1 ± 0.3
		F	5	24.5 ± 0.5	24.7 ± 0.4	0.3 ± 0.6
		Combined:	10	28.4 ± 1.3	28.6 ± 1.3	0.2 ± 0.3
INL-5300-20 5000 mg/kg	48	M	6	32.3 ± 0.3	32.3 ± 0.7	- 0.1 ± 0.6
		F	6	24.6 ± 0.5	25.6 ± 0.3	1.1 ± 0.4
		Combined:	12	28.1 ± 1.2	28.6 ± 1.1	0.5 ± 0.4
Corn Oil	72	M	5	32.7 ± 0.4	33.1 ± 0.6	0.4 ± 0.2
		F	5	24.9 ± 0.7	24.3 ± 0.4	- 0.5 ± 0.6
		Combined:	10	28.8 ± 1.4	28.7 ± 1.5	- 0.1 ± 0.3
INL-5300-20 5000 mg/kg	72	M	6	32.3 ± 0.6	31.9 ± 0.6	- 0.4 ± 0.5
		F	4	25.7 ± 0.6	26.0 ± 0.4	0.3 ± 0.5
		Combined:	10	29.6 ± 1.2	29.5 ± 1.1	- 0.1 ± 0.3

INL-5300-20 = H-15,527
MR 4581-232

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APPENDIX B

Summary of micronucleus results
for mice treated
with INL-5300-20

TABLE 2: MICRONUCLEUS DATA SUMMARY

INL-5300-20 MOUSE MICRONUCLEUS ASSAY

Treatment	Kill Time (hr)	Mean % Micronucleated PCEs ± S.E. (1000 PCEs Scored)					Mean Ratio PCE:NCE ± S.E.		
		N	Males	N	Females	Combined	Males	Females	Combined
Corn Oil 16 mL/kg	24	5	0.28 ± 0.05	5	0.06 ± 0.04	0.17 ± 0.05	1.02 ± 0.07	0.98 ± 0.14	1.00 ± 0.07
	48	5	0.16 ± 0.10	5	0.18 ± 0.09	0.17 ± 0.06	1.27 ± 0.17	1.43 ± 0.09	1.35 ± 0.09
	72	5	0.10 ± 0.05	5	0.12 ± 0.04	0.11 ± 0.03	1.13 ± 0.19	1.01 ± 0.11	1.07 ± 0.11
	Total	15	0.18 ± 0.04	15	0.12 ± 0.03	0.15 ± 0.03	1.14 ± 0.09	1.14 ± 0.08	1.14 ± 0.06
INL-5300-20 5000 mg/kg	24	6	0.18 ± 0.04	6	0.13 ± 0.05	0.16 ± 0.03	0.76 ± 0.14	1.00 ± 0.21	0.88 ± 0.13
	48	5[a]	0.20 ± 0.05	6	0.12 ± 0.05	0.16 ± 0.03	0.85 ± 0.14	1.01 ± 0.13	*0.94 ± 0.09
	72	5[b]	0.14 ± 0.04	3[b,c]	0.23 ± 0.03	0.18 ± 0.03	0.91 ± 0.13	0.95 ± 0.16	0.92 ± 0.09
	Total	16	0.18 ± 0.02	15	0.15 ± 0.03	0.16 ± 0.02	0.84 ± 0.08	0.99 ± 0.10	*0.91 ± 0.06
Cyclophosphamide 40 mg/kg	24	5	1.22 ± 0.26	5	1.18 ± 0.18	**1.20 ± 0.15	0.92 ± 0.11	0.91 ± 0.19	0.92 ± 0.10

INL-5300-20 = H-15,527
MR 4581-232

[a] One animal found dead before scheduled sacrifice time.
[b] Bone marrow suspensions from 1 male and 1 female were combined by technical error; these animals were excluded from the analysis.
[c] Two animals found dead before scheduled sacrifice time.
* $p < 0.01$
** $p < 0.001$

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DATA EVALUATION RECORD

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE; Benzoic acid, 2-[[[[N-4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)-N-methylamino]carbonyl]amino]-sulfonyl]-, methyl ester (96.8% active ingredient) was used.

3. STUDY/ACTION TYPE: Unscheduled DNA synthesis assay

4. STUDY IDENTIFICATION: Vincent, D. R., G. T. Arce, and A. M. Sarriff. July 18, 1985. Assessment of INL-5300-20 in the in vitro unscheduled DNA synthesis assay in primary rat hepatocytes. Unpublished Report No. 565-84 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist Signature: Roger Gardner
Organization: Review Section 6 Date: 2/11/86
Toxicology Branch

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: ~~Section Head~~ Signature: Jane E Harris
Organization: Review Section 6 Date: 2/12/86
Toxicology Branch

7. DISCUSSION AND CONCLUSION: There was adequate information presented in the report to support the conclusion of the investigators. Under the conditions of the experiment, DPX-L5300 did not induce unscheduled DNA synthesis in rat primary hepatocytes at concentrations of 0 to 2500 uM.

Core classification: Acceptable

8. MATERIALS AND METHODS

Test species and cell cultures: Eight-week-old male Crl:CD® (SD)BR strain rats were anesthetized. Abdomens of the animals were then opened and the livers were perfused with Hanks Buffered Salt Solution (pH 7.35). The livers were then perfused with William's Medium E containing L-glutamine (292 mg/l), gentamicin (50 ug/ml), and collagenase (Type IV, 100 units/ml) and buffered to pH 7.3. The perfused livers were then excised, placed in sterile dis[illegible] the collagenase solution, and the hepatocytes were [illegible] from the organ and

8. MATERIALS AND METHODS (continued)

collected by centrifugation. The cells were resuspended in the William's medium with gentamicin, L-glutamine, and bovine fetal serum, and the suspension was filtered to remove debris and break up clumps of cells.

Viability and cell density of the suspensions was checked by adding trypan blue dye and counting the stained and unstained cells in a hemacytometer. According to the report, the unstained cells were viable.

The report stated that culture plates (35 mm i. d. wells, 6 wells per plate) were inoculated with 5×10^5 cells/well. Each well contained 2 ml William's Medium E and was covered with a 25 mm diameter coverslip. Cells were allowed to attach to the coverslips in an incubator (37° C; 5% CO_2; 90% relative humidity) for 2 hours.

Positive control and vehicle: The reference substance used in this assay was dimethylbenzanthracene (DMBA), and the vehicle for the test substance was dimethylsulfoxide (DMSO).

Treatment media: The treatment consisted of William's Medium E with L-glutamine (292 mg/l), gentamicin (50 ug/ml), and 5 uCi/ml [methyl-^{3}H]-thymidine.

Experimental procedure: The culture medium (described under "Test species and cell cultures" above) was removed, and the cultures were washed with William's Medium E. Two ml of the treatment medium described above were added to each washed culture along with 20 ul of stock solutions or dilutions of the test substance and positive control substance in DMSO. The cultures were then incubated for 18 hours.

According to the report, the treatment medium removed after the 18-hour incubation was assayed for lactate dehydrogenase activity as an indicator of cytotoxicity.

The cultures were washed with William's Medium E, and the adhering cells were treated with 1% sodium citrate to swell the nuclei. They were then fixed with ethanol:glacial acetic acid (3:1), dipped in distilled water, and air dried. The coverslips with these treated cells adhering to them were then attached with the cell surface up onto labelled glass slides.

The slides were dipped into nuclear track emulsion and dried for two hours. After 3 days of storage in dessicated slide boxes kept at 4° C, the slides were developed and stained with methyl-green pyronin Y.

The report stated that 4 slides were examined for each test concentration in each trial. Cells were selected for

8. MATERIALS AND METHODS (continued)

examination according to the following criteria:

Those without morphologically altered nuclei.

Cells with apparent cytoplasm as indicated by tritium labelling or the pink counter stain.

Cells free of debris and staining artifacts.

Cells with one nucleus.

All four criteria were required before a cell was evaluated, and 25 cells were scored on each slide.

The report described the scoring procedure as follows:

The areas of the grains over the nucleus and several nuclear-sized regions over the cytoplasm adjacent to the nucleus were measured. The areas were converted to grains, and the net nuclear grains value (nuclear grains minus cytoplasmic grains) was calculated for each of the 25 cells. The highest cytoplasmic value was used in these calculations.

Statistical analysis: A two-variable (dose and trial) analysis of variance was conducted to evaluate differences between the treated and negative control groups and between trials. The relationship between concentration and response was evaluated by linear or higher order F-tests.

The criteria used to identify a positive result were described as follows:

An average increase of 5 or more net grains at one or more test concentrations, and the increase is statistically significant ($p<0.01$) when compared with the negative control.

The probability is <0>01 that there is not a positive correlation between the average net grains and increasing concentrations of the test compound.

Both of these criteria must be satisfied according to the report.

A test substance is considered negative if one of the following criteria are met:

An average increase of 5 or more net grains is not seen at any test concentration, or the increase is

8. MATERIALS AND METHODS (continued)

not statistically significant ($p>0.01$) when compared to the negative control response.

The probability is greater than 0>01 that there is not a positive correlation between the average net grains and increasing concentrations of the test compound.

9. REPORTED RESULTS

There was no cytotoxicity reported (see Appendix A below).

The investigators noted that three trials were attempted, but the second was rejected because the criteria for acceptability (see Section 8. MATERIALS AND METHODS, above) were not met by the control groups. On that basis only results for the first and third trials were reported.

No group, except that treated with DMBA, was reported to have a net grain value of 5 or more, and the probabilities of dose response for each trial were reported to be greater than 0.5 (See Appendix B below).

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Cytotoxicity results from primary hepatocyte cultures treated with INL-5300-20

HLR# 565-84

TABLE I

CYTOTOXICITY IN PRIMARY HEPATOCYTES

Compound	Concentration (uM) in Medium	Lactate Dehydrogenase Activity (U/L)* Trial 1	Avg.	Trial 3	Avg.
H-15,527	0	86	163	64	71
		88		59	
		120		71	
		186		89	
		302			
		195			
	0.1	95	94	78	78
		79		73	
		109		76	
		94		85	
	1	122	147	69	68
		149		61	
		123		67	
		193		74	
	3.3	175	222	50	57
		236		79	
		230		45	
		248		53	
	10	250	230	76	64
		304		40	
		174		86	
		192		53	
	33	117	215	90	81
		248		78	
		261		83	
		233		71	

*One International Unit (U) of enzyme activity will transform one (1) micromol of substrate per minute.

H-15,527 = INL-5300-20
MR-4581-222

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HLR# 565-84

TABLE I (CONT'D)

CYTOTOXICITY IN PRIMARY HEPATOCYTES

Compound	Concentration (uM) in Medium	Lactate Dehydrogenase Activity (U/L)* Trial 1	Avg.	Trial 3	Avg.
H-15,527	100	232	249	74	81
		212		68	
		248		75	
		306		108	
"	330	263	263	44	51
		316		47	
		144		62	
		328		50	
"	1000	177	258	43	59
		301		40	
		347		87	
		207		66	
"	2500	350	327	82	77
		334		56	
		239		94	
		386		74	
DMBA	100	403	437	136	132
		495		132	
		370		131	
		479		129	
"	500	416	442	140	212
		405		255	
		512		225	
		435		227	
"	1000	421	392	276	262
		413		244	
		252		263	
		483		265	

*One International Unit (U) of enzyme activity will transform one (1) micromol of substrate per minute.

H-15,527 = INL-5300-20
MR-4581-222

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APPENDIX B

Results of the unscheduled DNA synthesis assay (as reported) with INL-5300-20

HLR# 565-84

TABLE II

UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF RAT HEPATOCYTES

Compound	Concentration (μM) in Medium	Average Net Grains Per Nucleus ± SD		Pooled Avg. ± S.E.M.
		Trial 1	Trial 3	
H-15,527	0	1.3 ± 6.7	-3.9 ± 4.5	-1.8 ± 1.2
		-3.5 ± 6.4	-4.2 ± 5.5	
		3.7 ± 7.6	-5.4 ± 4.5	
		1.6 ± 5.4	-4.0 ± 3.0	
"	0.1	-2.1 ± 5.8	-4.4 ± 3.5	-2.5 ± 1.2
		-2.8 ± 5.6	-5.3 ± 5.0	
		-2.2 ± 6.0	-2.4 ± 4.0	
		4.9 ± 7.5	-5.3 ± 4.5	
"	1	1.5 ± 4.5	-5.3 ± 5.5	-2.0 ± 1.5
		5.3 ± 7.4	-5.1 ± 4.0	
		-4.7 ± 4.7	-5.9 ± 6.5	
		2.0 ± 4.6	-3.7 ± 4.5	
"	10	1.3 ± 4.2	-4.0 ± 4.5	-2.2 ± 1.4
		-1.1 ± 6.9	-5.0 ± 4.0	
		**	-6.8 ± 5.0	
		3.8 ± 3.7	-3.3 ± 3.5	
"	100	3.1 ± 5.7	-7.0 ± 4.5	-2.7 ± 1.2
		-2.6 ± 4.3	-4.4 ± 4.0	
		0.8 ± 4.8	-3.8 ± 4.5	
		-1.3 ± 6.5	-6.7 ± 6.5	

** This slide was not available for analysis.

H-15,527 = INL-5300-20
MR-4581-222

HLR# 565-84

TABLE II (CONT'D)

UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF RAT HEPATOCYTES

Compound	Concentration (μM) in Medium	Average Net Grains Per Nucleus ± SD		Pooled Avg. ± S.E.M.
		Trial 1	Trial 3	
H-15,527	1000	0.5 ± 4.1	-4.8 ± 5.5	-2.0 ± 1.6
		3.2 ± 7.3	-3.7 ± 6.5	
		1.3 ± 3.7	-3.8 ± 6.5	
		2.0 ± 6.0	-10.8 ± 7.0	
	2500	3.1 ± 4.5	-4.4 ± 5.5	-1.7 ± 1.5
		0.9 ± 5.0	-6.5 ± 5.5	
		1.7 ± 7.5	-4.2 ± 4.0	
		2.7 ± 4.5	-7.0 ± 6.0	
DMBA	100	31.7 ± 17.6	24.8 ± 11.0	32.7 ± 3.7
		55.2 ± 21.0	20.7 ± 10.0	
		37.0 ± 12.1	33.5 ± 15.5	
		28.1 ± 9.7	30.7 ± 14.5	

H-15,527 = INL-5300-20
MR-4581-222

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HLR# 565-84

TABLE III

STATISTICAL ANALYSIS OF UDS RESULTS
FOR H-15,527

A. COMPARISON OF MUTANT FREQUENCY AT EACH CONCENTRATION TO THE NEGATIVE CONTROL BY STUDENT'S T TEST

CONCENTRATION (μM)	NO. OF RESULTS	AVERAGE	T	PROB	
0	8	-1.8			
0.1	8	-2.4	-0.57	0.5710	
1	8	-2.0	-0.16	0.8700	
10	7	-1.7	0.06	0.9541	
100	8	-2.7	-0.82	0.4147	
1000	8	-2.0	-0.19	0.8528	
2500	8	-1.7	0.08	0.9391	
		POSITIVE CONTROL DMBA			TRIAL
100	4	38.0	13.12	0.0000	1
100	4	27.4	11.21	0.0000	3

B. DOSE RESPONSE ANALYSIS OF VARIANCE

	DEGREES OF FREEDOM	F RATIO	PROB
TOTAL	54		
TRIAL	1		
DOSE	6		
LINEAR	1	0.22	0.6396
QUADRATIC	1	0.02	0.8765
HIGHER ORDER	4	0.29	0.8861
DOSE X TRIAL	6/41	0.66	0.6792
RESIDUAL	47	(MEAN SQUARE= 5.1922)	

H-15,527 = INL-5300-20
MR-4581-222

Miscellaneous

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EXPRESS 037724

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

FILE COPY

May 2, 1989

Dr. Bruce Jaeger, Section Head
Special Analysis and Outreach Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)
Office of Pesticide Programs
Document Processing Desk
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202

Subject: SAP Meeting May 9, 1989
Du Pont Express® Herbicide (DPX-L5300)
Abstract of 90-Day Feeding Study to
Investigate Effects of DPX-L5300 on
Estrous Cycle

Dear Dr. Jaeger:

Per your May 2, 1989 telephone conversation with Dr. Fred. O'Neal, Du Pont, enclosed please find an abstract of the recently completed 90-day feeding study conducted to determine the affects of DPX-L5300 on the rat estrous cycle. This abstract is for your use in preparation for the upcoming May 9, 1989 SAP meeting. Dr. O'Neal is planning on making some general comments on this study from the floor during the May 9th meeting.

Copies of the completed study entitled "Ninety-Day Feeding Study with IN-L5300-20: Effect on Estrous Cycle" (HLR-112-89) by J. C. Cook have been submitted through the Product Manager's office for review.

If you have any questions, please contact me at:

E. I. du Pont de Nemours & Co. (Inc.)
Attn: Diane M. Stanley
Agricultural Products Department
Barley Mill Plaza, Walker's Mill 6-174
Wilmington, DE 19880-0038

Sincerely,

Diane M. Stanley

Diane M. Stanley
Registration Specialist

DMS/ckz
Enclosure
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CC: Mr. Larry J. Schnaubelt (PM-23)
Office of Pesticide Programs
Registration Division (H7505C)
Document Processing Desk (APPL)
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Mr. Frank Sanders, Section Head
Office of Pesticide Program
Registration Division (H7505C)
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Arlington, VA 22202

Du Pont EXPRESS® Herbicide
[INL-5300]
Report No. HLR-112-89

NINETY-DAY FEEDING STUDY WITH INL-5300: EFFECT ON ESTRUS CYCLE

ABSTRACT

In a previous two-year feeding study, an increased incidence of mammary adenocarcinomas was observed in female Crl:CD®BR rats dosed with INL-5300 above the MTD. Since this product is non-genotoxic, a study was conducted to clarify any secondary mechanisms associated with this tumorigenic effect. Published reports have demonstrated a role of the endocrine system in mammary gland tumorigenesis in rodents. For example, chronic treatment of rodents with estrogens such as 17-beta-estradiol and estrone, increases the frequency of spontaneous mammary tumors.

This study was designed to investigate the estrogenic activity and determine if there are endocrine system effects of INL-5300 in female rats that would support a non-genotoxic mechanism for the increased mammary adenocarcinoma incidence. Effects on the endocrine system were assessed by monitoring the estrous cycle, measuring serum hormone levels, characterizing the estrogen and progesterone receptors from the uterus and mammary gland, and determining the effects on reproductive organ weights. Cell proliferation in the mammary gland and uterus were also monitored.

Twenty female Crl:CD®BR rats per group were fed either 0 or 5,000 ppm INL-5300 in the diet for approximately three months. The rats were weighed weekly and food consumption was monitored throughout the study. The estrous cycle was monitored daily during pre-test and throughout the study via vaginal smears. After 80 days treatment, three rats/group were implanted with osmotic mini-pumps that contained tritiated thymidine. At seven days post-implantation, these rats were sacrificed and inguinal mammary gland and uteri were collected for measurements of cell proliferation.

After 81 days of treatment, the rats not selected for the cell proliferation studies were sacrificed and the body, liver, uterus, ovaries, and mammary glands were weighed. The number of corpora lutea and follicles per ovary were also determined. The uterus and mammary glands were also prepared for analysis of estrogen and progesterone receptor levels. A cytosolic fraction was isolated from these tissues and *in vitro* receptor binding studies were conducted with INL-5300 and several of its metabolites.

Female rats fed diets that contained 5,000 ppm INL-5300 had decreased mean body weight (26% lower) and decreased mean body weight gain (40% lower) when compared to the controls. There were no other compound-related clinical observations in this study. An association between INL-5300 dietary administration and endocrine system effects in female rats was demonstrated by the following:

1) Increased mean relative uterine weight (25 to 31%) and increased uterine cell proliferation.
2) Increased mean relative ovarian weight (23 to 29%).
3) Increased incidence of prolonged estrus, measured as both the number of rats with prolonged estrus and number of prolonged cycles among control and treated rats.
4) Two-fold increase in serum prolactin from rats sacrificed in estrus.
5) Two- to three-fold decrease in uterus and mammary gland estrogen receptor affinity.
6) Two-fold increase in mammary progesterone receptor number.

The above are consistent with the results of the in vitro competitive binding studies with estrogen receptors from the uterus. In these studies competition between estradiol binding and that of seven metabolites of INL-5300 was demonstrated. It is known that estrogens increase uterine weight and produce a persistent estrus, which is consistent with the increased mean relative uterine weight and prolonged estrus observed in the INL-5300 treated rats. Compounds with estrogenic activity are also known to increase serum prolactin levels. The lower affinity estrogen receptor in the uterus and mammary tissue could occur from the occupation of the high affinity receptor sites by INL-5300 metabolites since they appear to be agonists for the estrogen receptor. Estrogens are known to increase the number of cytoplasmic progesterone receptors which is consistent with the increased numbers of mammary progesterone receptors.

When considered together, these data strongly suggest that some of the INL-5300 metabolites possess estrogenic activity. These data also support the hypothesis that the INL-5300-induced mammary adenocarcinoma is hormonally mediated.

[redacted]

DUPONT
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E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

C27724

May 2, 1989

Mr. Larry J. Schnaubelt (PM-23)
Office of Pesticide Program
Registration Division (H7505C)
Document Processing Desk (APPL)
U.S. Environmental Protection Agency
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202

Subject: Response to Peer Review for Express®,
Dated April 7, 1989

Dear Mr. Schnaubelt:

This submission is in response to the request in the April 7, 1989 Peer Review for Express® for "examination of the possible association between Express®-induced tumors and a hormonal influence". We are submitting herein three (3) copies of the recently completed study entitled "Ninety-Day Feeding Study with IN L5300-20: Effect on Estrous Cycle" (HLR-112-89) by J. C. Cook to address this question.

This study contains strong evidence in support of the hypothesis that the Express (DPX-L5300)-induced mammary adenocarcinoma observed in the 2-year rat study (MRID 40245511) was hormonally mediated. In this study, DPX-L5300 altered the endocrine system of female rats fed 5000 ppm of the test substance in the diet for about 3 months as evidenced by increased relative uterine weight and cell proliferation, increased relative ovarian weight, prolonged estrous, increased serum prolactin and mammary progesterone receptor number, and decreased uterus and mammary estrogen receptor affinity. In vitro studies also confirmed that metabolites of DPX-L5300 were able to successfully compete for binding with the estrogen receptor as estrogenic agonists.

We believe these findings address the questions raised by the Peer Review Committee. Please forward this study to the appropriate individuals for review in conjunction with the Express® peer review and risk assessment. We appreciate your expeditious handling of this matter. Do not hesitate to call me at (302) 992-6260 if you have any questions.

Sincerely,

Diane M. Stanley

Diane M. Stanley
Registration Specialist

DMS/ckz
Enclosure

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