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

004943

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

FROM Roger Gardner, Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Jane Harris, Ph. D., Head
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Experimental Use Permit (EPA Reg. No. 352-EUP-RGN) and Temporary Tolerances for Residues of DPX-L5300 in/on Wheat and Barley grain (0.05 ppm) and straw (0.1 ppm); Petition No. 5G3296. Tox. Chem. No. 419S. Tox. Proj. Nos. 407 and 408).

Actions Requested

1. Experimental Use Permit for DPX-L5300 Herbicide® (75% Dry Flowable) on wheat and barley.

2. Tolerance as described above (Petition No. 5G3203)

3. Review of reports listed in Section IV. below.

Recommendations and Conclusions

1. On the basis of available toxicological data (see points 2. through 8. below) the proposed temporary tolerances are supported.

2a. Technical grade DPX-L5300 is classified into Toxicity Category III for acute inhalation and dermal toxicity and Category IV for acute oral toxicity.

2b. Available irritation and sensitization data on the 75% DF formulation (see point 3. below), and the relatively small amounts of DPX-L5300 to be used (see Section I. A. below) suggest that irritation and sensitization hazards are not likely when conditions and precautions recommended on the label are followed.

Therefore, additional testing on primary eye and skin irritation and dermal sensitization studies are not needed to support the proposed Experimental Use Permit.

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

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

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

6. A supplementary reproduction study which was an extension of the subchronic study in rats (see point 4.) indicated that pup viability and pup weights were decreased at the 5000 ppm level. no final conclusions can be drawn from the reproduction experiment since there were only 3 to 6 dams with litters available for analysis and the experiment was carried out for only one generation.

7. Maternal toxicity reported in a rat teratology study (6) 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 and fetal deaths, and incomplete ossification were observed at the highest dose tested. These results indicate that the NOEL's and LOEL's for maternal toxicity and developmental toxicity are 20 and 125 mg/kg/day, respectively.

8a. No mutagenic activity was observed in Chinese Hamster Ovary cells <u>in vitro</u> (8); no cytogenetic effects were seen in bone marrow cells or treated rats (9); no induction of micronuclei were found in normochromatocytes from treated mice (10); and no unscheduled DNA sysnthesis was induced in primary hepatocytes from treated rats (11).

8b. A reverse mutation assay in Salmonella typhimurium is unacceptable because the report was not complete (missing page 4, (7)).

9. Using a NOEL of 100 ppm (5 mg/kg/day) established in a 90-day rat study (4) and a Safety Factor of 1000, a Provisional Acceptible Daily Intake (PADI) of 0.005 mg/kg/day is derived (see Section III. B. and Appendix III below).

10. The Theoretical Maximum Residue Contributions for the proposed temporary tolerances on wheat and barley grains (0.05 ppm) represent 2.6% of the PADI (see Section III. B. and Appendix III.).

I. Background

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

The formulation, which is called DPX L5300 Herbicide, is to be applied at rates of 1/6 to 2/3 oz. active ingredient per acre according to the label, and the application is to be made between the 2-leaf and boot stages of winter or spring wheat and spring barley. The herbicide is to be mixed with water, and the mixture is to be sprayed by air (1 gal/A) or ground (5 gal./A) equipment in the spring. The proposed experimental program is anticipated to use 75 lb active ingredient on 2500 acres during the first year and 150 lb on 5000 acres during the second and third years.

No previous tolerances have been requested for DPX-L5300. The proposed temporary tolerances are 0.05 ppm in/on wheat and barley grain and 0.1 ppm in/on wheat and barley straw.

According to a memorandum from the Residue Chemistry Branch (dated October 18, 1985. From: J. M. Worthington, Chemist. To: R. Mountfort, PM No. 23. Registration Division. Subject: PP #5G3296. DPX-L5300 on wheat and barley. Comments on the analytical method and residue data. Accession Numbers 073785 and 073786. RCB #1427 and 1428.), DPX-L5300 is the residue of primary concern, and there are adequate data to indicate that residues will not exceed the proposed temporary tolerances. Meat, milk, poultry, and egg tolerances have not been requested because of a restriction imposed against feeding treated crops or forage to livestock.

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II. Summary of Submitted Data

Appendix II. below contains Data Evaluation Records for all the toxicity studies submitted.

A. Technical grade DPX L5300

1. Acute toxicity

The results of acute toxicity studies on technical DPX L5300 are summarized in Table 1.

2. Subchronic toxicity

In a three-month feeding study (4), groups of rats were given diets containing 0, 100, 1750, or 5000 ppm DPX L5300. The 100 ppm dietary level had no effect (NOEL). The two higher

Table 1

Summary of acute toxicity data for technical grade DPX L5300 (references 1 - 3)

Route of administration	Species	Sex	LD_{50} or LC_{50}	Toxicity Category
Oral	Rat	Both	>5,000 mg/kg	IV
Inhalation	Rat	Both	>6.7 mg/L*	III
Dermal	Rabbit	Both	>2.000 mg/kg	III

*Four hour exposure.

levels caused significant dose-related decreases in food consumption, body weight gain, and lower food efficiency. There were also significant decreases in absolute weights for the heart, brain, liver, and kidneys at the two highest dietary levels. Relative organ weights for the heart, liver, kidneys, testes, and spleen were increased significantly because of the decreased body weights observed. Serum glucose, globulin, and cholesterol concentrations were also decreased in the mid and high dose groups, but there were no treatment-related histopathological effects. These effects were described by the investigators as indications of cachexia. The LOEL is 1750 ppm.

In another 90-day feeding study (5), DPX-L5300 was given to dogs in their diet at levels of 0, 50, 500, or 2500 ppm. The highest dose tested caused no clearly treatment related effects. These results suggest a NOEL >2500 ppm in dogs.

3. Reproductive effects

As part of the subchronic feeding study in rats (4), a one-generation reproduction experiment was conducted. Six male and 6 female rats from each group were mated to produce the litters. Decreased pup viability and pup weights were observed in the 5000 ppm dose group. These results were consistent with the decreased body weights and generally poor condition of the dams given the highest dose level in the subchronic experiment. However, no further conclusions can be drawn from the reproduction experiment since there were only 3 to 6 dams with litters available for analysis and the experiment was carried out for only one generation.

4. Developmental toxicity

Doses of 0, 20, 125, or 500 mg DPX-L5300 per kg body weight were administered to groups of pregnant rats on gestation days 6 through 16 (6). The mid and high doses were found to cause maternal toxicity in pregnant rats when administered by oral intubation on gestation days 6 through 15. Those doses decreased maternal body weight gain and food consumption, increased the liver-to-body weight ratios (at the highest dose), and caused excess salivation in some animals. Fetuses from dams given the two highest doses also had reduced body weights. The highest dose caused resorptions, fetal deaths, incomplete ossification. These results indicate that the NOEL's and LOEL's for maternal and developmental toxicity are also 20 and 125 mg/kg/day, respectively.

5. Mutagenicity

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 (7). 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 (8).

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 (9).

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 (10). 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 (11), DPX-L5300 did not induce UDS in rat primary hepatocytes at concentrations of 0 to 2500 uM.

B. Toxicity data on DPX-L5300 Herbicide (75% a. i.)

Acute toxicity results are summarized in Table 2 below. In the listing of toxicity studies provided with the submission, the Registrant stated that an acute inhalation toxicity study with the formulation was not conducted because <0.5% of the water-dispersible granules in the formulation are smaller than 105 um.

Table 2

Summary of acute toxicity data for DPX-L5300 Herbicide (references 12 and 13)

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

Most of the deaths observed in the acute oral toxicity study (12) occurred 2 to 3 days after treatment, and some were noted as long as 9 days after dosing. Gross lesions observed at necropsy were not organ-specific. Signs of toxicity noted during post-dosing observation includee staining of the face and perineum, chromodacryorrhea, and body weight loss.

An eye irritation study (14) indicated that mean irritation scores for unwashed eyes 24, 48, and 72 hours after treatment were 14, 6, and 6, respectively. At 4 and 7 days after instillation the mean scores were 4 and 0. The results were sufficient to classify DPX-L5300 Herbicide® into Toxicity Category III for eye irritation.

The Primary Irritation Score for dermal irritation was 0 (15) which places the formulation into Toxicity Category IV.

No dermal sensitization was observed in guinea pigs (16)

III. Discussion

A. Data Gaps

There are no primary eye irritation, skin irritation, or dermal sensitization studies available on the technical grade material. However, studies with a 75% Dry Flowable formulation (14, 15, and 16) indicated that DPX-L5300 is associated with no skin irritation (Toxicity Category IV) or sensitization and moderate eye irritation (Toxicity Category III). In addition, there were no signs of skin reactions noted in the acute dermal toxicity study on technical grade DPX-L5300 (2).

The label warns that DPX-L5300 is an eye irritant and recommends that skin contact with the herbicide should be avoided.

Available irritation and sensitization data and the relatively small amounts to be used in the three-year experimental program (see Section I. A. above) suggest that irritation and sensitization hazards are not likely when conditions and precautions recommended on the label are followed. Therefore, the three studies are not needed to support the proposed Experimental Use Permit.

B. Tolerance assessment

Using a safety factor of 1000 and the NOEL of 100 ppm (5 mg/kg/day) established by the 90-day rat feeding study (4), the Provisional Acceptable Daily Intake (PADI) is calculated as follows:

$$\frac{5.0 \text{ mg/kg/day}}{1000} = 0.005 \text{ mg/kg/day}$$

The Theoretical Maximum Residue Contribution (TMRC) which would result from the proposed tolerance of 0.05 ppm in/on barley is based on a food factor of 0.03% and a total of 1.5 kg diet consumed daily by a 60 kg person. The TMRC is calculated as follows:

$$1.5 \text{ kg diet/day} \times 0.0003 \times 0.05 \text{ ppm} = 0.00002 \text{ mg/day}$$

For a 60 kg person this result would represent a daily intake of

$$\frac{0.00002 \text{ mg/day}}{60 \text{ kg body weight}} = 3.3 \times 10^{-7} \text{ mg/kg/day}$$

The percentage of the PADI represented by the propcsed tolerance would be

$$\frac{3.3 \times 10^{-7} \text{ mg/kg/day}}{0.005 \text{ mg/kg/day}} \times 100 = 0.66\%$$

Similar calculations with the proposed temporary tolerances on wheat indicates that TMRC accounts for approximately 1.99% of the PADI (see APPENDIX III below).

IV. Bibliography

1. Wylie, C. N., J. W. Sarver, D. B. Warheit, and N. C. Chromey. May 5, 1985. Median lethal dose (LD_{50}) of INL-5300-20 in rats. Unpublished report no. 167-85 prepared by Haskell Laboraty. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

2. Cunningham, B.,J. W. Sarver, and D. B. Warheit. May 30, 1985. Median lethal dose (LD_{50}) of INL-5300-25 in rats. Unpublished report no. 280-85 prepared by Haskell Laboraty. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

3. Gargus, J. L., N. G. Phipps, and S. D. Webber. December 24, 1984. Skin absorption single dose study in rabbits: Haskell No. 15,527: Final Report. Unpublished report no. HLO-21-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

4. Brock, W. J., R. W. Rickard, D. F. Krahn, and C. Barba. June 6, 1985. Ninety-day feeding and one-generation reproduction study in rats with benzoic acid, 2-[[[[N-(methoxy-6-methyl-1,3,5-triazin-2-yl)-Nmethylamino]carbonyl]amino]sulfonyl]-, methyl ester (INL-5300). Unpublished Report No. 413-83 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

5. Daly, I. @., and A. L. Knezevich. August 14, 1985. A Three-Month Feeding Study in Dogs with H-15527. Final Report. Unpublished Report No. HLO-514-85 prepared by Bio/dynamics Inc. for Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. Nos. 073788 and 073789.

6. Christian, M. S., and A. M. Hoberman. August 16, 1985. Developmental toxicity study of L-5300 in the rat. Unpublished Report No. HLO-513-85 prepared by Argus Research Laboratories, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073790.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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.

12. Gargus, J. L., P. Burlew, and R. J. Dean. April 11, 1985. Skin absorption LD_{50} study in rabbits. Unpublished report no. HLO-234-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

13. Hutt, C. W., L. A. Kinney, and N. C. Chromey. August 13, 1985. Inhalation Median Lethal Concentration (LC50) INL-5300-20 by EPA Protocol. Unpublished report no. 431-85 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

14. Gargus, J. L., P. L. Burlew, and. J. A. Ralph. May 24, 1985. Primary eye irritation study: Haskell No. 15,734. Unpublished report no. HLO-305-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

15. Gargus, J. L., P. L. Burlew, and L. D. Williams. April 11, 1985. EPA Skin irritation test. Haskell No. 15,734. Final Report. Unpublished report no. HLO 233-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073 .

16. Gargus, J. L., P. L. Burlew, and S. L. Brozena. May 23, 1985. Dermal sensitization in guinea pigs: Haskell No. 15,734. Unpublished report no. HLO 29585 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

40 CFR Number [redacted] DPX-L5300 [redacted] 4/85

Unverified Printout

ACCEPTABLE DAILY INTAKE DATA DRAFT 004943

Adult,Older NOEL		S.F.	ADI	TPI
mg/kg	ppm		mg/kg/day	mg/day(60kg)
5.000	100.00	1000	0.0050	0.3000

NOEL not recorded as

Current Action 5G3236

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Barley(6)	0.050	0.03	0.00002
Wheat(170)	0.050	10.36	0.00777

TPI	TMRC	% ADI
0.3000 mg/day(60kg)	0.0078 mg/day(1.5kg)	2.60

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APPENDIX I

Inert ingredient clearance

Express science review

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Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

X Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data

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

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

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

APPENDIX II

Data Evaluation Records for New Studies on DPX-L5300

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).

3. STUDY/ACTION TYPE: Acute oral toxicity - rats (limit test)

4. STUDY IDENTIFICATION: Wylie, C. N., J. W. Sarver, D. B. Warheit, and N. C. Chromey. May 5, 1985. Median lethal dose (LD_{50}) of INL-5300-20 in rats. Unpublished report no. 167-85 prepared by Haskell Laboraty. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787 (Tab 10).

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 C Harris
Date: 2/12/86

7. CONCLUSIONS: The results of the study indicated that the acute oral LD_{50} is greater than 5000 mg/kg, and DPX-L5300 should be classified into Toxicity Category IV.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Eight-week old male and female Sprague-Dawley (Crl: COBS CD (SD) BR) rats were used.

Experimental procedure: A group of 5 male and 5 female rats was given a single oral dose of 5000 mg test substance per kg body weight. The test substance was suspended in corn oil and administered by gavage. The rats were fasted for approximately 24 hours before treatment, and they were observed for mortality and appearance of toxicological and pharmacological signs twice daily for the 14 days that followed dosing. Surviving animals were sacrificed at the end of the observation period and necropsied. Postmortem examinations were limited to gross observations.

9. REPORTED RESULTS

There were no clinical signs of toxicity observed in the test animals during the 14-day period following dosing, and no deaths occurred.

There were no specific gross observations at necropsy that could be associated with the test substance according to the report.

The reported LD_{50} for both sexes was greater than 5000 mg/kg.

10. DISCUSSION

There were adequate data to support the reported conclution.

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).

3. STUDY/ACTION TYPE: Acute dermal toxicity - rabbits (Limit test)

4. STUDY IDENTIFICATION: Gargus, J. L., N. G. Phipps, and S. D. Webber. December 24, 1984. Skin absorption single dose study in rabbits: Haskell No. 15,527: Final Report. Unpublished report no. HLO-21-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787 (Tab 11).

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: J E Harris
Date: 2/12/86

7. CONCLUSIONS: The results of the study indicated that the acute dermal LD_{50} is greater than 2000 mg/kg, and DPX-L5300 should be classified into Toxicity Category III.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Male and female New Zealand White strain rabbits weighing 2139 to 2390 g (males) or 2086 to 2153 g (females) were used

Experimental procedure: Twenty-four hours before the beginning of the study, the rabbits were prepared by clipping their backs free of hair. The report stated that 5 animals of each sex were used, and the clipped skin of each was abraded.

The test substance was moistened to form a paste, and 2000 mg was applied per kg body weight to the prepared skin of each test animal.

8. MATERIALS AND METHODS (continued)

After the application of test substance, the trunks of the test animals were wrapped with nonabsorbant rubber damming, and the animals were restrained with plastic collars. At the end of the 24-hour exposure period the dressings were removed, and the test sites were gently rinsed and wiped clean.

All animals were observed twice daily for the next 14 days for the appearance of toxic signs and mortality. The rabbits were weighed on the day of dosing and on days 7 and 14 of the observation period. Surviving rabbits were sacrificed at the end of the 14-day observation period, but gross postmortem examinations were not conducted.

9. REPORTED RESULTS

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

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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).

3. STUDY/ACTION TYPE: Acute inhalation toxicity - rats

4. STUDY IDENTIFICATION: Hutt, C. W., L. A. Kinney, and N. C. Chromey. August 13, 1985. Inhalation Median Lethal Concentration (LC50) INL-5300-20 by EPA Protocol. Unpublished report no. 431-85 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787 (Tab 12).

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: J E Harris
Date: 2/12/86

7. CONCLUSIONS: The LC_{50} for male and female rats in a 4-hour exposure was greater than 6.7 mg/L. The results of the study indicate that DPX-L5300 should be classified into Toxicity Category III with respect to acute inhalation toxicity.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Male and female Crl:CD®(SD)BR strain rats were used.

Exposure conditions: Test atmospheres were generated by a K-Tron® Bin Feeder with a K-Tron® Volumetric Feed Controller. They were discharged into a 38 liter test chamber. The report stated that different atmospheric concentrations were generated by changing the flow rate of air through the feeder mechanism. Test substance concentrations were determined gravimetrically at 30 minute intervals during the 4-hour exposure period. A cascade impactor was used to measure the mass median aerodynamic diameter and per cent respirable particles during the exposure.

8. MATERIALS AND METHODS (continued)

Chamber temperature, oxygen content, and relative humidity were measured also. The test animals were restrained in perforated stainless steel cylinders designed for nose only exposure.

Experimental procedure: Two groups of 10 male and 10 female rats were exposed nose only to concentrations of the test substance, and two concurrent control groups were exposed to air containing no test substance. Exposure was 4 hours in duration. After exposure, the animals were observed twice a day for the appearance of toxic signs and mortalities during the next 14 days after exposure. The rats were also weighed each day during the experiment. Three rats per sex from each group were sacrificed at the end of the observation period and necropsied. Gross observations were noted.

9. REPORTED RESULTS

The reported temperature and humidity for the control group animals were 26° C and 24-32%, respectively. Those respective values for the treated group were 24-25° C and 29-34%. The reported concentration of test substance in the first atmosphere ranged from 0.88 to 1.9 mg/l with an average for the 4-hour exposure period of 1.3 mg/l. The second test atmosphere contained from 3.4 to 13 mg/L with an average of 6.7 for the exposure period. The mass median aerodynamic diameters for the first and second test atmospheres were 2.2 and 3.5 um, respectively. Respecitve percentage respirable particles were 96 and 86.

There were no mortalities observed during the study.

The authors noted that the test animals' faces became coated with the test substance during exposure, and when removed from the restraints, they exhibited oral and nasal discharges, wet perineum, and feces stained with test substance. The signs were not seen after the third day following exposure. Rats which were not exposed to the test substance also had nasal discharges according to the report.

Group mean body weights were comparable at days 7 and 14 of the observation period, and the animals gained weight during that time.

The only necropsy findings included a soft testis in one control group rat and a female in the treated group with red foci in the lungs.

10. DISCUSSION

There were adequate data to support the conclusions of the investigators.

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: Subchronic feeding/one-generation reproduction study - rats

4. STUDY IDENTIFICATION: Brock, W. J., R. W. Rickard, D. F. Krahn, and C. Barba. June 6, 1985. Ninety-day feeding and one-generation reproduction study in rats with benzoic acid, 2-[[[[N-(methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl]amino]sulfonyl]-, methyl ester (INL-5300). Unpublished Report No. 413-83 prepared by Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787 (Tab 18).

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: ~~Geneticist~~ Sec. Head Signature: J E Harris
Organization: Review Section 6 Date: 2/12/86
Toxicology Branch

7. CONCLUSION: A level of 100 ppm DPX-L5300 in the diet of rats for 90 days had no effect (NOEL). Two higher levels (1750 and 5000 ppm) caused significant dose related decreases in food consumption, body weight gain, and lower food efficiency. There were also significant decreases in absolute weights for the heart, liver, and kidneys at the two highest dietary levels. Relative organ weights for the brain, heart, liver, kidneys, testes, and spleen were increased significantly because of the decreased body weights observed. Serum glucose and globulin concentrations were also decreased in the mid and high dose groups, and cholesterol was increased in high dose group females. There were no treatment-related histopathological effects. These effects were described by the investigators as indications of cachexia. The LOEL is 1750 ppm.

Core classification: Minimum for the subchronic portion of the experiment.

7. CONCLUSION (continued) Decreased viability and pup weights observed in the 5000 ppm dose group during the reproduction phase of the experiment were consistent with the decreased body weights and generally poor condition of the dams given the highest dose level in the subchronic experiment. However, no further conclusions can be drawn from the reproduction experiment since there were only 3 to 6 dams with litters available for analysis and the experiment was carried out for only one generation.

Core classification: Supplementary for the reasons described above.

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.

Experimental procedure (subchronic phase): Groups containing 16 male and 16 female rats were given test diets containing 0, 100, 1750, or 5000 ppm DPX-L5300 for at least 90 days. Ten animals of each sex from each group in the experiment were designated for sacrifice after 90 days on test diets. The 6 remaining rats of each sex in each group were maintained on test diets during their mating and until weaning of their offspring (approximately 146 days after initiation of the study).

Test diets were analyzed for stability and homogenity of mixture. The report also stated that all rats were weighed and food consumption was estimated weekly during the first 90 days of the study. These results were used to determine food efficiency and test substance intake for the animals.

All animals in the study were observed twice each day for the occurrence of mortality, abnormal behavior, and changes in appearance. They were also individually handled and examined for changes in appearance at least once a week. These observations were made throughout the experiment.

The 10 animals of each sex in each group which were designated for sacrifice after 90 days were placed in metabolism cages for 24 hours and subsequently fasted for 16 hours prior to collection of blood and urine samples. These samples were collected after one, two, and three months' feeding of test diets.

Blood samples were drawn from the tail vein of the animals. Hematological observations included hemoglobin, hematocrit, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential white cell counts, and platelet count.

8. MATERIALS AND METHODS (continued)

Clinical chemistry observations of blood samples included urea nitrogen, creatinine, calcium, potassium, sodium, glucose, triglycerides, albumin and total protein (globulin was calculated from these two results), cholesterol, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. These tests followed the same schedule as that of the hematology.

Urine was collected, and the following observations were made: volume, pH, osmolality, occult blood, glucose, ketones, protein, bilirubin, and urobilinogen. Color and transparency were also noted, and sediment was examined microscopically.

According to the report, the animals designated for the 90-day subchronic phase of the study were sacrificed after 91 or 92 days on test diets. These animals were subjected to necropsies, and the investigators noted that one animal that died during the 90-day feeding period was also necropsied.

The report noted that the following organs were weighed: brain, heart, liver, kidneys, spleen, and testes. Organ-to-body weight ratios were calculated and expressed as a percentage of the final body weight.

The following tissues from the control and high dose groups were processed for histological examination:

Adrenals	Ileum	Spleen
Aorta	Jejunum	Stomach
Bone	Kidneys	Thymus
Brain	Liver	Thyroid with parathyroid
Cecum	Lungs	
Colon	Lymph nodes	Trachea
Duodenum	Pancreas	Urinary bladder
Epididymides	Pituitary	Uterus
Esophagus	Prostate	Vagina
Eyes	Salivary gland	All macroscopic abnormalities
Gonads	Skeletal muscle	
Heart		

The heart, liver, kidneys and all gross lesions from the low and mid dose groups were prepared and examined microscopically also.

The report also stated that samples of brain, heart, liver, kidney, spleen, muscle, testis, fat and blood were collected at necropsy for residue analysis. Samples from each animal within a group were pooled for the analysis. Fecal and urine samples were also collected during the feeding period for similar residue analyses. According to the report, results from these tests were reported elswhere.

8. MATERIALS AND METHODS (continued)

Experimental procedure (reproduction phase): Six rats of each sex from each group were continued on test diets as part of a one-generation one-litter reproduction study.

After 97 days on test diets, one F_0 male was cohabited with a female from the same test group for 15 days. Each day after pairing the investigators examined the females for the presence of a copulatory plug indicative of mating. At the end of the 15-day mating period, the females were transferred individually to cages that contained nesting material. Six days after transfer, each female was checked twice a day for birth of a litter. The offspring were designated F_{1A} generation animals.

According to the report, live and dead pups were counted as soon as the last pup of each litter was born. Counts of live and dead pups were again made one, four, 12, and 21 days post partum. On the fourth day after birth each litter was culled to 10 pups with an effort to maintain equal numbers of each sex whenever possible.

Litters were weighed 24 hours and 4 days after birth (before and after culling), and pups were individually weighed 21 days post partum. The weanlings were also sexed, sacrificed, and discarded without further examination on day 21 after birth. The weight of the dam was also noted.

All of the F_0 animals were then sacrificed and discarded without further examination according to the report.

Reproduction and lactation indices were calculated as follows:

$$\text{Fertility} = \frac{\text{total number of litters delivered}}{\text{total number of females mated}} \times 100$$

$$\text{Gestation} = \frac{\text{total number of litters with live pups}}{\text{total number of litters delivered}} \times 100$$

$$\%\ \text{pups born alive} = \frac{\text{number born alive}}{\text{number born}} \times 100$$

$$\text{Viability (days 0-4)} = \frac{\text{number of live pups at day 4}}{\text{number born alive}} \times 100$$

$$\text{Viability (days 1-4)} = \frac{\text{number of live pups at day 4}}{\text{number alive on day 1}} \times 100$$

$$\text{Lactation} = \frac{\text{number of pups alive at weaning}}{\text{number alive after culling (day 4)}} \times 100$$

$$\text{Litter survival} = \frac{\text{total number of litters at weaning}}{\text{total number of litters delivered}} \times 100$$

8. MATERIALS AND METHODS (continued)

The report stated that these indices were calculated for each litter containing one or more live pups on a "per litter" basis. The percentages for each female in a group were averaged, and those results were reported.

Statistical analyses: Body weight, body weight gain, organ weight (relative and absolute), and clinical laboratory parameters were analyzed by one-way analysis of variance.

Pairwise comparisons were made between treated groups and the control group if statistically significant differences among group means were observed (F-test). The pairwise tests were as follows:

Test	Parameter
Least significant difference (LSD)	Body weight and body weight gain
LSD and Dunnett's	Organ weights

Bartlett's test for homogeneity of variances was used with clinical laboratory results, and the reproduction and lactation indices were analyzed with Mann-Whitney U, Kruskall-Wallis, and Jonckheere tests.

Incidence of clinical signs was analyzed by Fisher's Exact tests.

The level of significance for differences was $p<0.05$.

9. REPORTED RESULTS

Subchronic phase: After correction of a diet mixing problem during the first week of the study, the investigators noted that test diets were within 9% of the nominal concentrations chosen for the experiment. The mean concentrations (± standard deviation for concentration, stability, and homogeneity measurements) were reported as follows:

Anticipated test concentration (ppm)	Concentration measured on test day 6	84	139
100	98.9 (20.0)	98.1 (14.8)	92.1 (5.7)
1750	1758.4 (111.9)	1658.4 (75.9)	1717.1 (112.4)
5000	5095.7 (74.8)	4997.1 (189.7)	4567.9 (201.1)

The investigators stated that mean body weights and body weight gains for the mid and high dose group males and females were 20 to 50% less than those values for the control groups

9. REPORTED RESULTS (continued)

(see Table 1 below) they described the results as dose-related. There were also 15 to 25% decreases in food consumption and food efficiency noted in the mid and high dose group male and female rats (see Table 1 below).

The group mean daily intake of the test substance for males was reported to be 7, 118, and 335 mg/kg for the low, mid, and high dose groups, respectively. The respective daily intakes for low, mid, and high dose group females were 8, 135, and 386 mg/kg.

The only clinical sign observed to occur in a dose-related manner was colored nasal discharge in male rats. The incidences reported for the 16 control, low, mid, or high dose group males were 1, 0, 5, and 9, respectively. These signs were noted after approximately 5 weeks in the control group and after 1 week in the high dose group. The discharges were noted in the mid dose group approximately 3 weeks after the study began.

During the third week of the experiment, the investigators stated that a control group female was switched with a low dose group female. These animals remained on the wrong test diets for a week according to the report, and the control group rat (designated for the reproduction study) was removed from the study without further evaluation. The low dose group animal was placed back on the appropriate diet.

A female rat in the 5000 ppm dose group was found dead on day 28 of the feeding period, and after histological examination of the tissues, the investigators concluded that cachexia was probably the cause of death.

No other mortalities were reported during the course of the study.

Tables 2 and 3 below summarizes hematology and clinical chemistry results that showed statistically significant changes. The authors noted that only the statistically significant decreases in serum glucose, total protein, and globulin at the two and three-month observations in male rats were treatment-related. The decreased total protein was further associated with the observed decrease in globulins. The remaining differences between control and treated groups were described as being within the normal ranges of variation for the parameters in male rats. The only statistically significantly different observations in treated groups that were considered to be toxicologically significant in female rats were the increases in serum cholesterol and decreases in glucose (see Table 3 below) at one and three months.

Table 1

Group mean body weight, weight gain, food consumption, and food efficiency results (summarized from Tables 2-9 of the original report)

Parameter	Dietary concentration (ppm) 0	100	1750	5000
	Males			
Body weight (g) (Week 13)	537.8	537.7	428.1*	373.9*
Weight gain (g) (weeks 0-13)	408.1	408.1	297.7*	243.7*
Food consumption (weeks 0-13; g/rat/day)	25.1	25.2	21.3	18.9
Food efficiency (weeks 0-13; g wt gain/ g food consumed)	0.179	0.178	0.154	0.142
	Females			
Body weight (g) (Week 13)	272.0	262.8	210.3*	197.3*
Weight gain (g) (weeks 0-13)	154.9	144.7	93.9*	80.3*
Food consumption (weeks 0-13; g/rat/day)	17.1	17.0	14.2	13.2
Food efficiency (weeks 0-13; g wt gain/ g food consumed)	0.099	0.093	0.073	0.067

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

Table 2

Summary of hematology and clinical chemistry results that exhibited statistically significant changes ($p<0.05$) during the 90-day feeding period for male rats

Parameter	Dietary concentration (ppm) 0	100	1750	5000
One-month observations				
Hematology				
Mean corpuscular volume volume (fL)	59	---	---	56
Lymphocytes (WBCx%)	11078	---	---	15270
Platelets (10^6/mm^3)	1026	---	1211	---
Blood chemistry				
Globulin (g/dL)	1.8	---	---	1.6
Sodium (mM/L)	143	---	145	145
Two-month observations				
Hematology				
Platelets (10^6/mm^3)	962	---	---	1144
Leucocytes (10^6/mm^3)	17.0	12.8	---	---
Blood chemistry				
Glucose (mg/dL)	119	---	100	95
Total protein (g/dL)	6.8	---	6.4	6.4
Potassium (mM/L)	5.3	---	---	5.7
Globulin (g/dL)	2.9	---	---	2.5
Sodium (mM/L)	143	145*	145*	145
Alkaline phosphatase (U/L)	72	104	---	---
Three-month observations				
Hematology				
Neutrophils (WBCx%)	1603	---	936	749
Blood chemistry				
Glucose (mg/dL)	116	---	98	92
Total protein (g/dL)	6.9	---	6.4	6.5
Globulin (g/dL)	2.9	---	2.5	2.4
Sodium (mM/L)	144	---	---	146
Potassium (mM/L)	5.5	---	---	5.8
Cholesterol (		---	---	---
Alkaline phosphatase (U/L)	53	76	---	---
Urine analysis				
Osmolality (mOs)	1015	---	---	588

*Not statistically significantly different from controls.

9. REPORTED RESULTS (continued)

Table 3

Summary of hematology and clinical chemistry results that exhibited statistically significant changes ($p<0.05$) during the 90-day feeding period for female rats

Parameter	Dietary concentration (ppm) 0	100	1750	5000
One-month observations				
Hematology				
Platelets (10^6/mm^3)	986	---	1238	1368
Blood chemistry				
Glucose (mg/dL)	105	---	89	90
Cholesterol (mg/dL)	73	---	---	105
Potassium (mM/L)	5.2	---	---	5.8
Two-month observations				
Hematology				
Lymphocytes (WBCx%)	6942	---	---	10682
Blood chemistry				
Total protein (g/dL)	6.9	---	6.5	6.4
Globulin (g/dL)	2.5	2.3	2.3*	2.3*
Three-month observations				
Hematology				
Platelets (10^6/mm^3)	1054	---	1323	1260
Blood chemistry				
Glucose (mg/dL)	113	---	89	96
Cholesterol (mg/dL)	79	---	---	105

*Not statistically significantly different from controls.

Table 4 presents selected organ weight results as they were shown in the report cited above.

The investigators described the gross and microscopic observations at necropsy as nonspecific. The incidence of lesions did not suggest dose-related effects.

Table 4

Summary of absolute organ weights (g) and organ to body weight ratios (% body weight) for those showing statistically significant differences from controls

Weight	Dietary concentration (ppm) 0	100	1750	5000
	Males			
Final body weight	539.2	516.7	415.2*	353.3*
Brain weight	2.232	2.162	2.161	2.076
Weight ratio	0.416	0.422	0.525*	0.596*
Heart	1.429	1.410	1.255*	1.111*
Weight ratio	0.265	0.274	0.303*	0.316*
Liver	18.155	18.773	13.707*	13.866*
Weight ratio	3.367	3.627	3.288	3.923*
Kidneys	3.323	3.407	2.931*	2.862*
Weight ratio	0.616	0.661	0.705*	0.762*
Spleen	0.806	0.716	0.714	0.724
Weight ratio	0.149	0.139	0.170	0.205*
Testes	3.382	3.461	3.496	3.335
Weight ratio	0.630	0.675	0.850*	0.954*
	Females			
Final body weight	274.3	260.7	209.4*	199.1*
Brain weight	1.870	1.902	1.856	1.878
Weight ratio	0.689	0.736	0.891*	0.945*
Heart	0.905	0.855	0.756*	0.738*
Weight ratio	0.330	0.330	0.361*	0.371*
Liver	9.436	8.949	7.919*	8.040*
Weight ratio	3.431	3.446	3.772*	4.036*
Kidneys	2.012	1.864	1.708*	1.647*
Weight ratio	0.736	0.719	0.814*	0.827*
Spleen	0.471	0.546*	0.454	0.468
Weight ratio	0.173	0.209*	0.218*	0.235*

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

Table 5

Incidence of microscopic lesions observed
in rats given DPX-L5300 in the diet for 90 days

Tissue and lesion	Dietary concentration (ppm) 0	100	1750	5000
Males				
Number examined	10	0*	0*	10
Heart				
Focal myocarditis	3	5*	1*	2
Kidney				
Rodent nephropathy	6	4*	4*	4
Liver				
Inflammatory cell infiltration	1	1*	-*	1
Lung				
Interstitial pneumonitis	4	1**	2†	5
Pancreas (exocrine)				
Periductular lymphoid aggregates	4	-	-	4
Thymus				
Multifocal hemorrhage	-	1**	3††	-
Thyroid				
Folicular atrophy	-	-	-	2
Urinary bladder				
Proteinaceous cast	3	-	-	1
Females				
Kidneys				
Bilateral hydronephrosis	-	-*	2*	-
Rodent nephropathy	3	2*	1*	-
Lungs				
Interstitial pneumonitis	6	-	2**	5
Pancreas (exocrine)				
Periductular lymphoid aggregates	2	-*	-*	-

*Only the heart, liver, and kidneys of all 10 animals of each sex from these groups were examined microscopically.
**All gross lesions examined microscopically showed the diagnosis.
†Two of the three gross lesions examined showed the diagnosis indicated.
††Three of the four gross lesions examined showed the diagnosis indicated.

9. REPORTED RESULTS (continued)

One-generation reproduction experiment: The fertility indices reported for the control, low, mid, and high dose groups were 80, 83, 100, and 83%, respectively. The gestation index was 100% for all groups in the experiment, and the percentage of pups born alive per litter was 100% for the control and low dose groups and 98.2 and 98.6% for the mid and high dose groups, respectively. The reported lactation indices were 100, 78, 100, and 98% for the control, low, mid, and high dose groups, respectively. With the exception of the low dose group, the litter survival was 100%. In the low dose group that value was 80%.

The investigators noted a significant decrease in the viability indices for the high dose group (80.8% for the day 0-4 and 85.7% for the day 1-4 index compared with 100% for the control indices). They attributed the decrease in viability indices to the loss of 9 pups in one litter during the first 4 days post partum. There were also statistically significantly decreased pup weights which were consistent with the decreases in viability (see Table 6 below).

Group mean body weights for the dams at weaning of their F_{1A} offspring were also reduced in the mid and high dose groups. The reported group means for the control, low, mid, and high dose groups were 306.4, 311.7, 260.1, and 234.4 g, respectively. The group mean weights for the mid and high dose groups were statistically significantly less than that of the control group ($p<0.05$).

10. DISCUSSION

The report contained sufficient information to support the authors' conclusions.

A level of 100 ppm DPX-L5300 in the diet of rats for 90 days had no effect on body weight, body weight gain, or food consumption, but the two higher doses used in the experiment (1750 and 5000 ppm) caused significant decreases in those parameters (see Table 1 above). Along with these decreases, lower food efficiencies were noted in animals given the mid and high dose levels.

There were also significant decreases in absolute weights for the heart, liver, and kidneys in the two highest dosed groups (see Table 4 above). Relative organ weights for the brain, heart, liver, kidneys, testes, and spleen were increased significantly because of the decreased body weights observed.

Table 6

Summary of group mean litter data as provided in Table 19 of the original report

Parameter	Dietary concentration (ppm) 0	100	1750	5000
Live pups per litter				
At birth	10.7	11.4	13.0	12.0
At 24 hours	11.3*	11.4	12.7	11.4
At 4 days**	11.3	11.4	12.7	9.6
At 4 dayst	8.8	8.2	9.7	8.8
At weaning	8.8	7.8	9.6	8.6
Males	4.5	3.6	5.0	5.6
Females	4.3	4.2	4.7	3.0
Mean pup weight				
At 24 hours	7.2	6.8	6.1	5.1††
At 4 days**	10.8	9.4	8.7††	7.1††
At 4 dayst	10.8	9.5	8.7	7.1††
At weaning				
Males	51.2	50.2	42.0	32.8††
Females	48.0	47.9	39.0††	30.9††

*Individual animal data indicated that one litter was eliminated from calculations of means because of a discrepancy in pup counts.
**Before litters were culled.
†After culling.
††Group mean is statistically significantly different from that of the control ($p<0.05$).

10. DISCUSSION (continued)

The above mentioned body weight and food consumption effects, the lower serum glucose, total protein, and cholesterol concentrations, and the lack of specific dose-related histopathological findings support the authors' conclusion that the test substance has a generalized effect on several organ systems. The investigators further characterize these effects as cachexia which, as they point out, was the apparent cause of death for a female rat in the 5000 ppm dosed group.

On the basis of these results the lowest-observed-effect level (LOEL) is 1750 ppm. Since there were no consistent differences between the low dose group and control group with respect to any observations made in the study, the 100 ppm dietary level is a no-observed-effect level (NOEL) under the above described test conditions.

10. DISCUSSION (continued)

Decreased viability and pup weights (see Table 6 above) observed in the 5000 ppm dose group during the reproduction phase of the experiment were consistent with the decreased body weights and generally poor condition of the dams given the highest dose level. However, no further conclusions can be drawn from the reproduction experiment since there were only 3 to 6 dams with litters available for analysis and the experiment was carried out for only one generation.

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: Subchronic feeding - dogs

4. STUDY IDENTIFICATION: Daly, I. @., and A. L. Knezevich. August 14, 1985. A Three-Month Feeding Study in Dogs with H-15527. Final Report. Unpublished Report No. HLO-514-85 prepared by Bio/dynamics Inc. for Haskell Laboratory. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. Nos. 073788 and 073789.

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: ~~Scientist~~ Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane P. Harris
Date: 2/12/86

7. CONCLUSION: Levels as high as 2500 ppm DPX-L5300 (highest dose tested) in the diet of dogs for 90 days caused no clearly treatment related effects. These results suggest that a NOEL is likely to be >2500 ppm in dogs under the test conditions.

Core classification: Minimum.

8. MATERIALS AND METHODS

Test species: Male and female beagle dogs from 4-1/2 to 5 months of age were used. The body weights of the males were from 7.9 to 10.2 kg, and that for females was from 6.6 to 8.2 kg.

Experimental procedure: Four groups containing 4 male and 4 female dogs were given diets containing 0, 50, 500, or 2500 ppm test substance for 3 months.

8. MATERIALS AND METHODS (continued)

All test animals were observed twice each day during the study, and the appearance of toxicological signs or mortality were noted. Body weights were measured once each week starting two weeks prior to initiation and during the course of the study. The amount of food eaten by each animal was noted daily, and the amount of test substance consumed by each animal was calculated in terms of mg/kg body weight.

The test animals were given a physical examination to evaluate the central and autonomic nervous systems: cardiovascular, gastrointestinal, and genitourinary systems; general condition; and eyes, mucous membranes, skin, and fur condition. These examinations were conducted prior to the start and at weekly intervals until the end of the study.

Blood and urine samples were taken prior to the start (at two and one weeks prior to start of the study) and at 1, 2, and 3 months in the experiment. The dogs were fasted overnight before blood samples were collected.

Hematological observations included hematocrit, hemoglobin, differential and total white cell count, red cell count, and platelet count. From these observations mean corpuscular volume, hemoglobin concentration, and hemoglobin were calculated. Erythrocyte morphology was also observed.

Blood biochemistry observations included fasting glucose, total protein, albumin, globulin, urea nitrogen, creatinine, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, cholesterol, uric acid, total bilirubin, and electrolytes.

Urinalysis included gross appearance, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, protein, occult blood, and microscopy of sediment.

At the end of the 3-month feeding period the animals were sacrificed and subjected to gross necropsy. Lesions were noted, and organ weights were determined for the adrenals, brain, heart, kidneys, liver, pituitary, spleen, gonads, and thyroids/parathyroid.

Samples of the following tissues were processed for microscopic examination: adrenals, aorta, bone, bone marrow, brain, cervix, epididymis, esophagus, eys with optic nerve, gall bladder, heart, intestines, kidneys, liver, lungs, lymph nodes, mammary gland, nerve (sciatic), ovaries, pancreas, pituitary, prostate, rectum, salivary gland, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thyroid/parathyroid, trachea, urinary bladder, uterus, vagina, and gross lesions

8. MATERIALS AND METHODS (continued)

Samples of blood, urine, and feces were collected prior to sacrifice of the animals and sent to the sponsor for residue analysis. Samples of brain, kidney, liver, muscle, spleen, testis, and fat were collected from each animal at necropsy, pooled by group, frozen, and sent to the sponsor for possible residue analysis also.

Statistical procedures are described below as appropriate.

9. REPORTED RESULTS

None of the animals died during the study, and the investigators noted no physical or clinical signs that could be attributed to the test substance.

There were no statistically significant differences noted between the control and treated groups with respect to group mean body weight. However, the authors stated that the mean body weight for the high-dose group males was lower than that for the controls throughout the test period. The decrement in mean body weight for the high dose group was described as progressive, and the difference between that group and the control group was a 6% decrease by the end of the study. The progressive nature of the weight difference was considered by the authors as suggestive of a test substance effect. Table 1 summarizes the reported group mean body weights for the control and high dose group males.

Table 1

Summary of selected group mean (and standard deviation) body weights (kg) for control and high dose group male dogs.

	Dose group	
Week of study	Control	2500 ppm
0	9.5 (0.5)	9.5 (1.1)
4	10.5 (0.8)	10.2 (1.3)
8	11.2 (0.9)	10.8 (1.1)
13	11.8 (0.9)	11.1 (0.9)

The report stated that there were no statistically significant or remarkable group differences for food consumption or food efficiency (g body weight gained/g food consumed).

The daily intake of test substance during the feeding period (mg/kg/day) was reported to be 1.5, 14.9, and 73.3 mg/kg/day for low, mid, and high dose group males, respectively. Those

9. REPORTED RESULTS (continued)

respective values calculated for females were 1.6, 15.1, and 78.0 mg/kg/day. The authors described these values as within 80 to 100% of nominal values.

The only statistically significantly different hematological values observed included slightly decreased mean corpuscular hemoglobin concentration (MCHC) and slight increases in mean platelet and total leukocyte counts. These differences were noted in the high dose group males and at one of the observation times. The difference in MCHC values were observed at 2 months (36.3 g/dL in the control group and 34.6 g/dL in the high dose group; $p<0.05$). Differences in platelet and total leukocyte counts were significant at the month 3 observation ($p<0.05$). Platelet counts for the control and high dose group males averaged 2.53 X 10^5 and 3.54 X 10^5 cells/mm^3, respectively. Respective leukocyte counts reported for the control and high dose groups were 10.2 and 13.3 (X 10^3 cells/mm^3).

According to the report, there were no consistent patterns in the clinical chemistry or urinalysis results that could be attributed to the test substance.

The report stated that the thyroid/parathyroid weights were the only organ weights possibly affected by the test substance. The only group that appeared to be significantly affected was the high dose group. Table 2 summarizes group mean weights and weight ratios for the thyroid/parathyroid in the control and high dose group males and females.

Table 2

Summary of group mean organ weight and organ-to-body weight ratios for the thyroid/parathyroid

Dose group	Body wt (g)*	Organ wt (g)	Weight ratio (X 100.000)
		Males	
Control	1190	0.839	7.13
High	1110	1.087	9.85
		Females	
Control	810	0.607	7.56
High	870	0.898**	10.28

*Converted from weights reported in kg.
**Statistically significantly different from control, $p<0.05$.

9. REPORTED RESULTS (continued)

The report concluded that macroscopic and histopathologic findings occurred in both treated and control animals, or they were noted sporadically. None were associated with administration of the test substance. Histopathology data are reproduced in Appendix A below.

10. DISCUSSION

The investigators concluded that there were no treatment-related effects on survival, food consumption, food efficiency, clinical signs, clinical chemistry, urinalysis, or post mortem observations. A no-observed-effect level (NOEL) of 500 ppm was selected by the investigators on the basis of hematological effects and organ weight changes for the thyroid/parathyroid seen in the high dose group.

Differences noted in platelet and total leukocyte counts were described as slight, and they were reported at one observation time during the study. The increased thyroid/parathyroid weights also appeared to be slight, and in the case of the females, were probably associated with the increased body weights observed for the high dose group. These effects are unlikely to be toxicologically significant, especially when considered along with the absence of other dose related effects described above.

APPENDIX A

Summary of histopathology in dogs treated with DPX-L5300 (reproduced from the original report cited above)

Express science review

Page _____ is not included in this copy.

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The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

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_X_ FIFRA registration data

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

004943

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 (94.2% active ingredient) was used.

3. STUDY/ACTION TYPE: Teratology - rat

4. STUDY IDENTIFICATION: Christian, M. S., and A. M. Hoberman. August 16, 1985. Developmental toxicity study of L-5300 in the rat. Unpublished Report No. HLO-513-85 prepared by Argus Research Laboratories, Inc. 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. CONCLUSION: Doses of 125 and 500 mg/kg/day were found to cause maternal toxicity in pregnant rats when administered by oral intubation on gestation days 6 through 15. Those doses decreased maternal body weight gain and food consumption, increased the liver-to-body weight ratios (at the highest dose), and caused excess salivation in some animals. Fetuses from dams given the two doses also had reduced body weights. The highest dose caused resorptions, fetal deaths, and incomplete ossification. These results indicate that the NOEL's and LOEL's are 20 and 125 mg/kg/day for maternal and developmental toxicity, respectively.

Core Classification: Guideline

8. MATERIALS AND METHODS

Test species: Seven- to ten-week-old male and female Crl: COBS® CD® (SD)BR strain rats were used. Each female was cohabited with a male for up to five days and was examined daily for a vaginal plug or the presence of sperm in vaginal smears. The day either were found was designated Day 0 of

gestation, and the animal was assigned to a test group.

8. MATERIALS AND METHODS (continued)

Experimental procedures: The test substance was suspended in 0.5% aqueous (w/v) Methocel® and administered by gavage on days 6 through 15 of gestation. Doses of 0, 20, 125, or 500 mg test substance per kg body weight were given to groups of 25 mated dams.

Each dam was observed at least once daily for occurrence of toxic signs and mortality during the treatment period. Body weight determinations were made on day 0 and on days 6 through 20 of gestation. Feed consumption was also measured on days 0, 6, 10, 16, and 20.

The rats were sacrificed on day 20 of gestation. Gravid uteri, maternal livers, and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and resorptions were noted.

The report described the examination of fetuses as follows:

> ...All live fetuses were examined for gross external alterations. Dead fetuses were examined for these parameters to the extent possible.
>
> Gross external alterations observed in fetuses found dead were not included in data summarization or statistical analyses.
>
> ...Approximately one-half of the fetuses in each litter, excluding stunted fetuses (fetuses that weighed less than 1.25 grams), were examined for visceral alterations using Staples' technique. These fetuses were decapitated, and the heads were fixed in Bouin's solution for subsequent free hand sectioning and examination. Approximately one-half of all remaining fetuses were eviscerated. One-half of all stunted fetuses were examined for soft tissue alterations.
>
> All fetuses were examined for possible skeletal alterations.

Statistical procedures are discussed below as appropriate. The report noted that animals dying during gestation, aborted, or not pregnant were not included in the litter analysis of results.

9. REPORTED RESULTS

Maternal observations: One death was reported in the study. It was noted in the low dose group after the sixth dose was given, and the authors attributed it to intubation injury.

9. REPORTED RESULTS (continued)

The only sign associated with the test substance was excess salivation which occurred in 0, 0, 2, and 11 animals from the control, low, mid, and high dose groups, respectively.

The authors reported statistically significant decreases in mean body weight for the mid and high dose groups compared with the control group at Day 9 of gestation. The differences persisted in the high dose group until termination of the study, but the difference between control and mid dose animals remained statistically significant through the Day 16 observation. Body weight gain for the mid and high dose groups was statistically significantly decreased during the dosing period, especially during the first three days of dosing. After the dosing period, body weight gain for the mid dose group was statistically significantly greater than that for the control group. A similar "rebound effect" was not observed in the high dose group according to the report. Table 1 summarizes reported maternal body weight results that reflect these patterns.

Table 1

Summary of group mean maternal weight and food consumption results

Observation	Dose (mg/kg/day) 0	20	125	500
Body weight (g) at				
Day 0	262.5	263.6	263.5	262.0
Day 6	282.0	280.5	283.1	278.2
Day 9	290.9	285.2	275.4**	265.3**
Day 12	304.7	300.3	284.5**	271.9**
Day 16	329.4	327.0	306.5**	282.1**
Day 20	393.3	394.7	377.9	323.3**
Day 20*	319.6	315.2	303.2	263.4**
Body weight gain				
Days 6-9	8.9	4.7	-7.7††	-12.9††
Days 16-20	63.9	67.7	71.4†	41.2††
Days 0-20	130.8	131.0	114.4††	61.3††
Days 0-20*	57.2	51.5	39.7††	1.3††

*Day 20 weight and weight gain are based on body weight corrected for weight of the gravid uterus.
**Statistically significantly different from controls as determined by Dunnett's test ($p<0.01$)
††Statistically significantly different from controls as determined by Mann-Whitney U test ($p<0.01$)

9. REPORTED RESULTS (continued)

Feed consumption for dams in the mid and high dose groups were also statistically significantly decreased from that of the control group. Reported group mean daily consumption values during the dosing period for the control, low, mid, and high dose groups were 77.2, 77.1, 66.7, and 50.7, respectively (the latter two means were significantly less than the control group mean, $p<0.01$ by Mann-Whitney U test).

Although the authors stated that there were no statistically significant differences between treated groups and the control group with respect to group mean liver weight, they noted that the liver weight was decreased at the high dose (15.55 g compared with 16.05 g for the control group). The liver to body weight ratio (%) for the high dose group dams was statistically significantly increased over that for the control group (4.38 vs. 4.08, $p<0.01$ by Mann-Whitney U test).

Group mean uterine weights were reported to be 73.66, 79.51, 74.75, and 59.94 g for the control, low, mid, and high dose groups, respectively.

Litter observations: Table 2 summarizes the litter observations as they were reported. The authors noted that 15 of the 16 fetuses in one litter from the high dose group were found dead, but there were no totally resorbed or aborted litters in any of the test groups.

Fetal observations: The most frequently observed fetal alterations are summarized in Table 3. The authors stated that the number of litters containing one or more altered fetuses was statistically significantly increased in the mid and high dose groups above that for the control group. These increases were associated with increased incidences of incompletely ossified or unossified sites (see Table 3), and in the high dose group, an increased incidence of edematous fetuses. The authors described the dose-related alterations as follows:

> Dose-dependent increases in the litter incidences of incomplete ossification of the thoracic and caudal vertebrae, sternebrae, xiphoid and pubes occurred in the middle and high dosage groups. These increases were significant ($p<0.05$ to $p<0.01$) for the high dosage group litters, and bifid thoracic vertebral centra and unossified caudal vertebrae were significantly increased ($p<0.05$) for middle dosage group litters, as compared with control values. Although not significantly increased ($p>0.05$), the observations of edema,

9. REPORTED RESULTS (continued)

enlarged fontanelle, unossified supraoccipital, altered ossification of lumbar and sacral vertebrae and unossified metacarpals and metatarsals in high dosage group fetuses were also considered related to administration of this dosage of the test substance to the dams.

The authors also noted that there were no incidences of other fetal alterations occurring in a dose-related manner.

Table 2

Summary of litter observations

Observation	Dose (mg/kg/day) 0	20	125	500
No. presumed pregnant	25	25	25	25
No. pregnant	22	20	23	22
Deaths	0	1	0	0
No. litters with live fetuses	22	19†	23	22
Corpora lutea/dam	17.9	18.6	18.0	18.5
Implantations/dam	14.8	15.8	15.7	15.8
Resorptions/litter	1.0	1.0	0.9	1.5
Mean % resorptions or dead conceptuses/litter	5.7	6.2	5.1	13.9
Live fetuses/litter	13.8	14.8	14.9	13.6
Dead fetuses	0	0	0	15
Uterine weight (g)	73.66	79.51	74.75	59.94*
Mean live fetal weight (g)	3.22	3.20	2.98**	2.38**

†Excluding the litter of the dam that died.
*Statistically significantly different from controls ($p<0.01$) by Dunnett's test.
**Statistically significantly different from controls ($p<0.01$) by Jonckeere's test.

9. REPORTED RESULTS (continued)

Table 3

Summary of frequently reported fetal alterations

Observation	Dose (mg/kg/day) 0	20	125	500
No. fetuses examined	304	282	342	299
External alterations				
Edematous	0	0	0	4
Skeletal alterations				
Skull				
Enlarged fontanelle	0	0	0	1
Supraoccipital unossified	0	0	0	1
Hyoid unossified	26	15	24	21
Vertebrai				
Thoracic centra				
Unossified	0	0	1	6
Unossified (unilat.)	1	0	0	2
Bifid	0	1	6	11
Lumbar centra				
Unossified	0	0	0	4
Unossified (unilat.)	0	0	0	2
Bifid	0	0	0	1
Sacral - unossified	0	0	0	2
Caudal - unossified	0	1	5	17
Sternebrae				
Manubrium (total with altered ossification)	1	1	3	3
Centra (total with altered ossification)	7	4	11	77
Xiphoid unossified	9	4	17	132
Pelvis (total with altered ossification)	6	3	7	44
Metacarpals - unossified	0	0	0	1
Metatarsals - unossified	0	0	0	2

*Statistically significantly different from controls, $p<0.05$.
**Statistically significantly different from controls, $p<0.01$.

9. REPORTED RESULTS (ocntinued)

Table 3 (continued)

Observation	Dose (mg/kg/day) 0	20	125	500
No. litters examined	22	19	23	22
External alterations				
Edematous	0	0	0	2
Skeletal alterations				
Skull				
Enlarged fontanelle	0	0	0	1
Supraoccipital unossified	0	0	0	1
Hyoid unossified	12	8	12	11
Vertebrai				
Thoracic centra				
Unossified	0	0	1	5*
Unossified (unilat.)	1	0	0	2
Bifid	0	1	5*	7**
Lumbar centra				
Unossified	0	0	0	4
Unossified (unilat.)	0	0	0	1
Bifid	0	0	0	1
Sacral - unossified	0	0	0	2
Caudal - unossified	0	1	5*	8**
Sternebrae				
Manubrium (total with altered ossification)	1	1	3	3
Centra (total with altered ossification)	6	3	9	18**
Xiphoid unossified	6	3	12	19**
Pelvis (total with altered ossification)	4	3	7	11*
Metacarpals - unossified	0	0	0	1
Metatarsals - unoossified	0	0	0	2

*Statistically significantly different from controls, $p<0.05$.
**Statistically significantly different from controls, $p<0.01$.

10. DISCUSSION

The report concluded that the 125 and 500 mg/kg/day dose levels caused maternal toxicity as indicated by decreases in body weight gain and food consumption, excess salivation, and at the highest dose tested increased liver-to-body-weight ratios. The data presented in the report indicated that the adult toxicity NOEL and LOEL were 20 and 125 mg/kg/day, respectively.

The investigators further concluded that the test substance was associated with a decrease in fetal weight at the maternally toxic doses of 125 and 500 mg/kg/day. The highest dose also increased the incidences of resorptions, fetal deaths, and fetuses with incomplete ossification. There were adequate data presented to indicate that the NOEL and LOEL for developmental toxicity are also 20 and 125 mg/kg/day, respectively.

004943

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 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 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

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
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. 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

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 Trial 1	Without activation Trial 2	With activation Trial 1	With activation 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.

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

6. APPROVED BY:

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

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.

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 (1.5)
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***	1.366***	6.6* (0.8)

INL-5300-20 = M-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.

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 - rat

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:

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: 2/11/86

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).

APPENDIX A

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

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Pages 74 through 76 are not included in this copy.

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___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

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

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
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 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 dishes with the collagenase solution, and the hepatocytes were combed 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 X 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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Cytctoxicity results from primary hepatocyte cultures treated with INL-5300-20

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___ Description of the product manufacturing process

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___ Identity of the source of product ingredients

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

DATA EVALUATION RECORD

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE: DPX-L5300 Herbicide™ or Benzoic acid, 2-[[[[N-4-methoxy-6-methyl1, 3, 5-triazin-2-yl)-N-methyl-amino]carbonyl]amino]-sulfonyl]-, methyl ester (75% active ingredient).

3. STUDY/ACTION TYPE: Acute oral toxicity - rats

4. STUDY IDENTIFICATION: Cunningham, B.,J. W. Sarver, and D. B. Warheit. May 30, 1985. Median lethal dose (LD_{50}) of INL-5300-25 in rats. Unpublished report no. 280-85 prepared by Haskell Laboraty. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

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: 2/11/86
Toxicology Branch

7. CONCLUSIONS: The results of the study indicated that the acute oral LD_{50} is 5200 mg/kg for both sexes, 5700 mg/kg for male rats, and 3800 mg/kg for female rats. These results indicate that DPX-L5300 Herbicide should be classified into Toxicity Categories IV and III for males and femeles, respectively.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Eight-week old male and female Sprague-Dawley (Crl: COBS CD (SD) BR) rats were used.

Experimental procedure: Groups of 10 male rats were given single oral doses of 5000, 6000, or 7000 mg test substance per kg body weight. Groups of 10 female rats were given single oral doses of 3000, 5000, 6000, or

8. MATERIALS AND METHODS (continued)

The test substance was suspended in corn oil and administered by gavage. The rats were fasted for approximately 24 hours before treatment, and they were observed for mortality and appearance of toxicological signs twice daily for the 14 days that followed dosing. Necropsies were done (where possible) on 3 animals from each group that died during the observation period and 3 from each group that survived and were sacrificed 14 days after dosing.

The report stated that a probit analysis was used to calculate an LD_{50} and 95% confidence limits.

9. REPORTED RESULTS

Signs of toxicity observed by the authors included red nasal and oral discharges, wet perineum, limpness, and slight to severe weight loss.

Two of 10 males given the 5000 mg/kg dose died, and there was one death in the 10 females given 3000 mg/kg. All rats given the 7000 mg/kg dose died. Most of the deaths observed during the study occurred from 2 to 3 days after treatment to as long as 9 days after dosing.

The authors reported that the gross lesions observed at necropsy of animals were not organ-specific. Those observations reported were consistent with clinical observations made during the post-dosing period (e. g., staining of the face and perineum, chromodacryorrhea, etc).

The reported LD_{50} for both sexes was 5200 mg/kg with undefined 95% confidence limits. The calculated LD_{50} for males alone was reported to be 5700 mg/kg with 95% confidence limits of 5200 to 6200 mg/kg, and those values for females were reported to be 4800 and 3800 to 5700 mg/kg, respectively.

10. DISCUSSION

There was adequate information presented in the report to support the conclusions of the investigators (see Section 7., above).

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DATA EVALUATION RECORD

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE; DPX-L5300 Herbicide® or benzoic acid, 2-[[[[N-4-methoxy-6-methyl1, 3, 5-triazin-2-yl)-N-methyl-amino]carbonyl]amino]-sulfonyl]-, methyl ester (75% active ingredient).

3. STUDY/ACTION TYPE: Acute dermal toxicity - rabbits (Limit test)

4. STUDY IDENTIFICATION: Gargus, J. L., P. Burlew, and R. J. Dean. April 11, 1985. Skin absorption LD_{50} study in rabbits. Unpublished report no. HLO-234-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

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. CONCLUSIONS: The results of the study indicated that the acute dermal LD_{50} is greater than 2000 mg/kg, and DPX-L5300 Herbicide® should be classified into Toxicity Category III.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Male and female New Zealand White strain rabbits weighing 2139 to 2390 g (males) or 2086 to 2153 g (females) were used

Experimental procedure: Twenty-four hours before the beginning of the study, the rabbits were prepared by clipping their backs free of hair The report stated that 5 animals of each sex were used, and the clipped skin of each was abraded.

The test substance was moistened to form a paste, and 2000 mg was applied per kg body weight to the prepared skin.

8. MATERIALS AND METHODS (continued)

After the application of test substance, the trunks of the test animals were wrapped with nonabsorbant rubber damming, and the animals were restrained with plastic collars. At the end of the 24-hour exposure period the dressings were removed, and the test sites were gently rinsed and wiped clean.

All animals were observed twice daily for the next 14 days for the appearance of toxic signs and mortality. The rabbits were weighed on the day of dosing and on days 7 and 14 of the observation period. Surviving rabbits were sacrificed at the end of the 14-day observation period, but gross postmortem examinations were not conducted.

9. REPORTED RESULTS

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

DATA EVALUATION RECORD

1. CHEMICAL: DPX-L5300

2. TEST SUBSTANCE; DPX-L5300 Herbicide® or benzoic acid, 2-[[[[N-4-methoxy-6-methyl1, 3, 5-triazin-2-yl)-N-methyl-amino]carbonyl]amino]-sulfonyl]-, methyl ester (75% active ingredient).

3. STUDY/ACTION TYPE: Primary eye irritation - rabbits

4. STUDY IDENTIFICATION: Gargus, J. L., P. L. Burlew, and J. A. Ralph. May 24, 1985. Primary eye irritation study: Haskell No. 15,734. Unpublished report no. HLO-305-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

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. CONCLUSIONS: The results of the study indicate that DPX-L5300 Herbicide® should be classified into Toxicity Category III with respect to eye irritation.

Core classification: Guideline

8. MATERIALS AND METHODS

Test species: Male and female New Zealand White strain rabbits were used.

Experimental procedure: Nine rabbits previously examined and found without signs of corneal damage were used in the experiment. Thirty-seven mg of the test substance was instilled into the left eye of each rabbit, and the eyelids were gently held together for one second. Thirty seconds after the instillation, the treated eyes of 3 rabbits were washed for one minute with warm water. The eyes of the 6 remaining rabbits were not washed.

All eyes were examined 24, 48, and 72 hours after instillation of the test substance and 4 and 7 days after treatment. (ccu-

8. MATERIALS AND METHODS (continued)

lar reactions were scored according to the following scales:

Corneal opacity

Degree of density

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

Area of cornea involved

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

score = score for degree x score for extent x 5
maximum = 80

Iris

1 - folds above normal, congestion, swelling, circumcorneal injection (any one or a combination of these), iris still reacting to light (sluggish reaction is positive)
2 - no reaction to light, hemorrhage, gross destruction (any one or all of these)

score = score for iris x 5
maximum score = 10

Conjunctivae

Redness

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

Chemosis

1 - any swelling above normal (including

8. MATERIALS AND METHODS (continued)

nictitation membrane
2 - obvious swelling with parital eversion of the lids
3 - swelling of lids about half closed
4 - swelling of lids about half to completely closed

Discharge .

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

Score = sum of values for redness, chemosis, and discharge multiplied by 2. Maximum = 20

9. REPORTED RESULTS

According to the report, opacities covering 75% of the cornea were observed in 5 of 6 of the unwashed eyes 24 hours after treatment, and conjunctival redness was observed (grades 1 to 2) in all of the unwashed eyes. The redness persisted through the 72 hour examination and was observed in two unwashed eyes at 4 days. Chemosis was seen in 4 of the 6 unwashed eyes at 24 hours. A discharge was noted in 5 of the unwashed eyes at 24 hours and in 2 at the 72 hour observation.

Opacities covering 25% of the cornea were reported in 2 of the 3 washed eyes at the 24-hour examination, and iritis was noted in 1 of the three eyes at that time. Conjunctival redness was observed at the 24, 48, and 72-hour examinations; chemosis and discharges were seen in one eye 24 hours after treatment.

Mean irritation scores for unwashed eyes at the 24, 48, and 72-hour examinations were 14, 6, and 6, respectively. At 4 and 7 days after instillation the mean scores were 4 and 0.

10. DISCUSSION

The report included adequate information to support the conclusions of the investigators.

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 (75% active ingredient) was used.

3. STUDY/ACTION TYPE: Dermal irritation study - rabbits

4. STUDY IDENTIFICATION: Gargus, J. L., P. L. Burlew, and L. D. Williams. April 11, 1985. EPA Skin irritation test: Haskell No. 15,734. Final Report. Unpublished report no. HLO 233-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073787.

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. CONCLUSIONS: The results of the study indicate that DPX-L5300 Herbicide® should be classified into Toxicity Category IV with respect to dermal irritation (primary irritation score = 0).

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: New Zealand White strain rabbits were used.

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

Five-hundred mg test substance was placed under one-inch square gauze patches at four sites on each animal. Two sites were left intact and two were abraded on each rabbit. The gauze patches were secured with transparent adhesive tape, and the trunk of each animal was wrapped with rubber damming.

8. MATERIALS AND METHODS (continued)

Twenty-four hours after application of the test substance the dressings were removed, and the test sites were rinsed and gently wiped clean. The test sites were scored for edema and erythema at that time, and they were scored again 48, and 72 hours after removal of the dressings.

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

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

9. REPORTED RESULTS

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

10. DISCUSSION

The report included adequate information to support the conclusion that DPX-L5300 Herbicide® is practically non-irritating.

004943

DATA EVALUATION RECORD

1. CHEMICAL: DPX L5300

2. TEST SUBSTANCE; DPX-L5300 Herbicide® or benzoic acid, 2-[[[[N-4-methoxy-6-methyl1, 3, 5-triazin-2-yl)-N-methyl-amino]carbonyl]amino]-sulfonyl]-, methyl ester (75% active ingredient) was used.

3. STUDY/ACTION TYPE: Dermal sensitization study - guinea pigs

4. STUDY IDENTIFICATION: Gargus, J. L., P. L. Burlew, and S. L. Brozena. May 23, 1985. Dermal sensitization in guinea pigs: Haskell No. 15,734. Unpublished report no. HLO 295-85 prepared by Hazleton Laboratories America, Inc. Submitted by E. I. DuPont de Nemours and Co. EPA Acc. No. 073 .

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 C. Harris
Date: 2/12/86

7. CONCLUSIONS: DPX L-5300 Herbicide® was shown to cause no dermal sensitization in male guinea pigs under the test conditions described below.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: According to the report, young male Hartley albino guinea pigs were used.

Experimental procedure: In a preliminary range-finding experiment, the hair was clipped from the backs of three test animals 24 hours prior to the treatment, and the test substance was applied to the prepared skin in 0.05 ml aliquots. Three concentrations of the test material in dimethyl phthalate were used (25, 35, and 50% w/v), and each was applied to a 25 mm test site on the prepared skin. Treated sites were scored 24 and 48 hours after application according to the following scale:

8. MATERIALS AND METHODS (continued)

0 = no reaction
1 = scattered mild redness
2 = moderate and diffuse redness
3 = intense redness
4 = intense redness and swelling (edema)
5 = necrosis

B = blanching
R = raw areas

Results from the preliminary study were the basis for chosing 5 and 50% concentrations for the primary phase of the main study.

In the primary irritation phase of the study, two groups of 10 animals were prepared in the same manner as the test animals in the preliminary experiment. Each test concentration was applied to a test site on each animal in the first group while the second group received only the dimethyl phthalate vehicle without the test substance. Each animal received 0.05 ml aliquots of the appropriate solutions. The report stated that irritation scores for these sites were compared to those recorded after the challenge applications at the end of the sensitization period.

After the last primary irritation scoring, the same groups of animals were used in the induction phase of the sensitization experiment. The hair was shaved from the sacral/hip area, and a 0.05 ml aliquot of a 35% suspension was topically applied to the prepared skin. These applications were repeated on alternate sides of the sacral/hip area three times a week for three consecutive weeks.

Two weeks after the last induction application, the backs of all 20 animals were shaved in preparation for challenge applications. and 0.05 ml of 2.5 and 25% suspensions of the test substance were each applied to test sites on the treated and control animals.

One week after the first challenge, a second challenge dose was applied in the same manner as the first.

After the range-finding, primary irritation, and challenge applications, test sites were scored according to the scale described above. These observations were made at 24 and 48 hours after treatment. Test sites were scored 24 hours after each induction phase application.

All animals were observed for mortality and morbidity throughout the study.

9. REPORTED RESULTS

According to the report, there were no mortalities during the study.

The only skin reaction noted in the study was mild scattered redness at some treated sites (a score of 1). The authors summarized the responses as follows:

> During the induction phase, the dermal response noted was scattered mild redness in the test animals at eight of the nine treatment sites. Scattered mild redness was noted in most of the test and control animals following the challenge dose. Scattered mild redness was noted in most of the test and control animals following the rechallenge dose.

The investigators concluded that the test substance was not a skin sensitizer under the conditions of the experiment described above.

10. DISCUSSION

There was enough information presented in the report to support the conclusion that DPX-L5300 Herbicide® is not a skin sensitizer.

APPENDIX III

Acceptable Daily Intake
(ADI) Printout for
DPX-L5300

004943

5G3 296

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WALKER'S MILL, BARLEY MILL PLAZA
WILMINGTON, DELAWARE 19898

AGRICULTURAL CHEMICALS DEPARTMENT

August 22, 1985

REGISTERED MAIL
RETURN RECEIPT REQUESTED

Mr. Richard Mountfort, PM 23
U. S. Environmental Protection Agency
Registration Division (TS-767-C)
401 M Street, S. W.
Washington, DC 20460

Dear Sir:

The undersigned, E. I. du Pont de Nemours and Company, submits this petition pursuant to Section 408 (d) (1) of the Federal Food, Drug, and Cosmetic Act, as amended, and regulations thereunder with respect to the herbicidal chemical methyl 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl]amino]sulfonyl] benzoate(DPX-L5300).

Specifically, request is made that temporary tolerances for residues of DPX-L5300 be established as follows:

Commodity	ppm
Barlsy, grain	0.05
Barley, straw	0.1
Wheat, grain	0.05
Wheat, straw	0.1

Attached hereto in duplicate, and constituting a part of this petition, are the following data in the form directed by Section 180.7 (b) of the regulations:

A - Name, Chemical Identity, and Composition
B - Amount, Frequency, and Time of Application
C - Investigations Made With Respect to Safety
D - Results of Tests on the Amount of Residue Remaining
E - Practicable Methods for Removing Residue that Exceeds any Proposed Temporary Tolerance
F - Proposed Temporary Tolerances

We enclose herewith six copies of an application for an Experimental Use Permit for Du Pont DPX L5300 Herbicide in wheat and barley, dated August 22, 1985. In the application, the proposed period of shipment/use is February 1, 1986, through October 1, 1988. We request that the temporary tolerances be effective for a time period consistent with that proposed in the application for Experimental Use Permit.

Enclosed is a check, certified by the Wilmington Trust Company, in the Sum of $6,000, payable to the Environmental Protection Agency as required by Section 180.33 (c).

We would like to be notified in advance if the Agency determines that they must disclose this petition for temporary tolerance on EUP application in the Federal Register prior to its acceptance.

It is requested that all communications in reference to this petition be addressed to:

E. I. du Pont de Nemours & Co., Inc.
Attn: Mrs. Billie Lynn Rash
Agricultural Chemicals Department
Walker's Mill Building
Barley Mill Plaza
Wilmington, DE 19898

If any questions arise, we would appreciate that Mrs. Rash be advised by phone (collect) at (302) 992-6207.

This submission contains confidential Du Pont trade secret formulation or commercial information, and protection from public disclosure is claimed to the extent permitted by Section 301 (j) of the Federal Food, Drug, and Cosmetic Act; Section 552 (b)(3) and (4) of the Freedom of Information Act, 5 U.S.C. 552.

Respectfully submitted,
E. I. DU PONT DE NEMOURS & CO., INC.

BY: Billie Lynn Rash
Billie Lynn Rash
Registration Coordinator

BLR:kmm
Attachments
04

Express science review

Page _____ is not included in this copy.

Pages 105 through 115 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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

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

Express science review

Page 116 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

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___ FIFRA registration data

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Express science review

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