BB-590
TXR-3786

Toxicology Branch/HED Review

003786

Caswell No(s).: 193 B

/ Walters

Registration No(s).:

Petition No(s).: 352-EUP-RRG, 3G2959

TOX-F6025, a new chemical

approval of EUP & Temporary Tolerance of 0.05 ppm in

EUP and Temporary tolerance toxicologically supported

180.1001: yes

Existing: new chemical Resulting: 0.92

TMRC: 0.0007 mg/day (1.5 kg)

in setting the ADI:

ADI printout: YES/NO; TOX "one-liner": YES/NO; DER: YES/NO

regulatory actions against registration: 13 week feeding, dog

status:

data: attached reviews **BEST AVAILABLE COPY**

deficiencies in reproduction

consideration application permanent

Second teratology study is

3-29-84

Reviewer: Date:

Section Head: 4-13-84

003786

MOS calculations

Residue (temp tolerance) in rice: 0.05 ppm

Single serving portion: 454 grams

NOEL for teratology: 150 mg/kg/day

$454 \times 0.05 = 22.7$ ppm residue/serving

$\frac{22.7}{1000} = 0.0227$ mg/gram serving (Equivalent to 1500 mg/gram daily intake)

$\frac{0.00227}{60} = 0.00038$ mg/kg/day (for a 60 kg man)

$MOS = \frac{150}{0.0038} = 39600$

Unverified Printout

ACCEPTABLE DAILY INTAKE DATA

003786

NOEL mg/kg	LOEL ppm	S.F.	ADI mg/kg/day	HPI mg/day(60kg)
2.500	100.00	2000	0.013	0.0750

Current Action 332959

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans (Oil)(1+3)	0.050	0.92	0.0069

HPI	TMRC	% ADI
0.0750 mg/day(60kg)	0.0007 mg/day(1.5kg)	0.92

****

DRAFT

BEST AVAILABLE COPY

003786

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute Oral Toxicity in rat

Accession Number: 072016 (7)

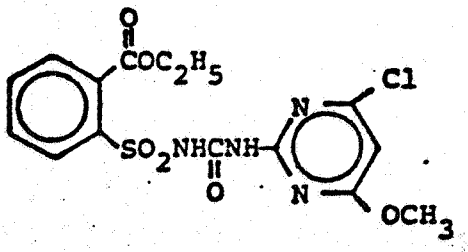
MRID Number:

Sponsor: DuPont

Contracting Lab: Haskell Lab. No. 311-83

Date: 9-1-83

Test Material: 2-(((((4-chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))-benzoic acid, ethyl ester 75%, DPX-F6025 75% Dry Flowable.



Protocol:

"Five male and 5 female 8-week-old rats were fasted approximately 24 hours prior to testing. Single oral doses of the test material, as a solution in corn oil, were administered by intragastric intubation. Surviving rats were weighed and observed during a 14-day recovery period and then sacrificed. All rats were sent to pathology for gross examination."

Doses were as shown below.

Results:

"Dose (mg/kg)	Average Body Weight (g)	Solution (mg/mL)	Average Dose (mL)	Mortality Ratio
5,000	215	300	3.52	1/5
5,000	152	300	2.53	0/5 "

"Lethargy was observed immediately after dosing. Stained and wet perineal area, diarrhea, stained face and lethargy were observed for the next two days after dosing. Weight loss was slight. One male rat died within one day of dosing."

"Gross pathologic examination of the male rat that died showed dark-red and moist lungs, dark-red lymph nodes, pink thymus, and distended G.l. tract. Fourteen days after dosing, one female had a dark-red speen, one male had dark-red and moist lungs, and one female and two males had hydronephrosis."

Conclusions:

"LD 50(male and female rats): more than 5000 mg/kg
Acute oral Toxicity Category: IV

Core Classification:

Guideline

TOXICOLOGY BRANCH
DATA REVEIW

003786

Study Type: Acute dermal toxicity in rabbit

Accession Number: 072016 (8)

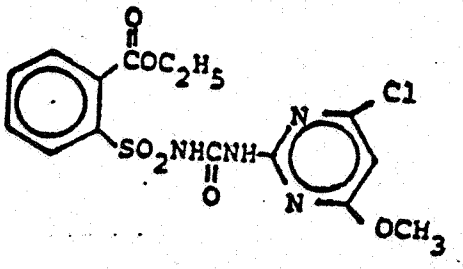
MRID Number:

Sponsor: DuPont, Haskell Lab. No. 283-83

Contracting Lab: Hazleton Lab. No. 201-628

Date: 7-25-83

Test Material: 2-(((((4-chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))-benzoic acid, ethyl eater 75%, DPX-F6025 75% Dry Flowable



Protocol:

"Prior to initiation, the hair was closely clipped from the back of each rabbit, 5 males and 5 females. Just prior to compound application, the skin of all animals was abraded with minor incisions which were sufficiently deep to penetrate the stratum corneum, but not deep enough to disturb the derma or to produce bleeding. The test material was applied to the skin of each rabbit at a dosage level of 2000 mg/kg. Approximately 3.5 ml tap water was mixed with the compond to form a paste. Plexiglas collars were place on each rabbit and remained for a duration of seven days to prevent accidental ingestion of the test material. The test material remained in contact with the skin for twenty-four hours by means of a nonabsorbent binder composed of rubber damming.

"Twenty-four hours following application, the binders were removed, the residual amount of the test material was estimated, and the exposure sites were wiped with gauze to preclude futher exposure sites were wiped with gauze to preclue futher exposure of the animals to the test material."

"All of the rabbits were observed for signs of toxic and pharmacologic effects once daily for fourteen consective days. Dermal responses were graded and scored on Days 1,3,7,10, and 14 according to the system of Draize (1959). Individual body weights were recored at initiation, on Day 7 and termination."
"At termination (Day 14), all suriving rabbits were sacrificed."

Results:

"No deaths occurred among the test animals during the study."

"All rabbits appeared normal and gained weight between initiation and termination of the study."

"Dermal irritation consisted of very slight to well-defeined erythema and very slight to slight edema in most animals on Day 1. All animals were clear of erythema and edema on Day 3. Epidermal scaling was observed on one male at Day 14. The compound adhered to skin of most animals to Day 14."

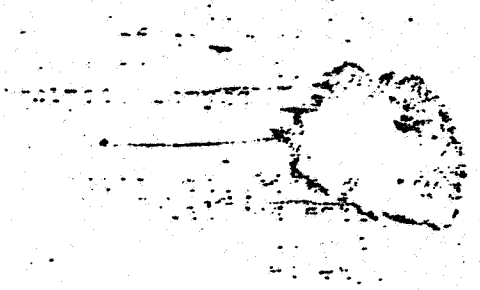
Conclusions:

LD 50 (males and female rabbits): more than 2000 mg/kg. (HDT)

Acute dermal Toxicity Category: III

Core Classification:

Minimum



003786

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Primary eye irritation

Accession Number: 072016 (9)

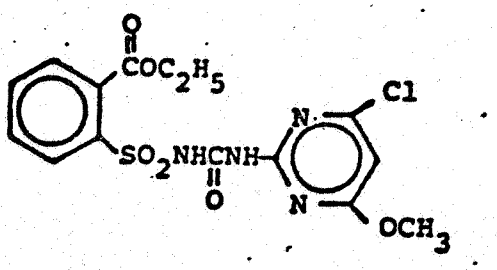
MRID Number:

Sponsor: Dupont, Haskell Lab. No. HLO 272-83

Contracting Lab: Hazleton Lab. No. 201-630

Date: 7-7-83

Test Material: 2-(((((4-Chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))-benzoic acid, ethyl ester 75%, DXP-F6025 75% Dry Flowable



Protocol:

"A 26 mg aliquot of the test material was place into the conjunctival sac of the left eye of each rabbit. The eye was gently held close for approximately one second following instillation. Three treated eyes were rinsed ten seconds after instillation for one minute with lukewarm water. The right eye of each rabbit was not treated, and thus served as a negative control."

"Eye irritation was scored and graded at 24, 48 and 72 hours according to the system of Draize (1959). After the examination at twenty-four hours, the left eye of each rabbit was examined for corneal defects following staining with 2% fluorescein sodium solution."

Results:

"No opacity or iritis was observed in the six unwashed eyes at any observation interval. Mild conjunctival redness (grade one) was obseved in five unwashed eyes at 24 and/or 48 hours. Mild discharge (grade one) was noted in one unwashed eye at 24 and 48 hours. A small amount of compound remained in one unwashed eye at 24 hours."

"In the three washed eyes, opacity and iritis were not observed at any observation interval. Mild conjuntival redness (grade one) was observed at 24 hours in the three washed eyes."

"Fluorescein examination were negative at 24 hours in the unwashed eyes."

Conclusions:

Primary eye irritation category: III

Core Classification:

Guideline

TOXICOLOGY BRANCH
DATA REVIEW

003786

Study Type: Primary dermal irritation in rabbit

Accession Number: 072016 (10)

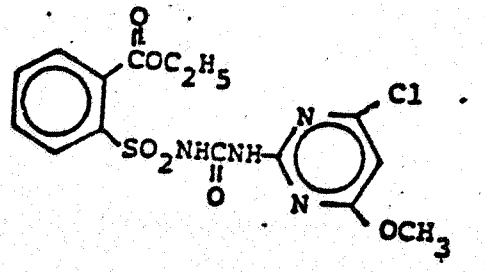
MRID Number:

Sponsor: DuPont, Haskell Lab. No. HLO 282-83

Contracting Lab: Hazleton Lab. No. 201 - 629

Date: 8-5-83

Test Material: 2-(((((4-chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))-benzoic acid, ethyl ester 75%, DPX-F6025 75% Dry Flowable



Protocol:

"Prior to treatment, the dorsal area of each rabbit was clipped free of hair, and four sites were chosen for application. Just prior to compound application, the skin at two sites were abraded with minor incision which were sufficiently deep to penetrate the stratum corneum, but not deep enough to disurb the derma or to produce bleeing. The skin of the other two sites remained intact. The skin was premoistened with water. A 0.5 gm aliquot of the test material was introduced under a one by one inch square gauze patch (two layers thick) which was secured in place with porous tape. The trunk of each rabbit was wrapped with impervious rubber damming. The rabbits were then immobilized in stocks for twenty-four hours without food and water.

"Twenty-four hours following application, the binders and patches were removed, and the exposure sites were wiped with a dry towel to preclude further exposure of the animal to the test material."

Results:

"Mild erythema (grade one) was observed in four animals, both abraded and intact sites, at 24 hours postapplication. Grade one erythema was noted at one intact site in one animal and in one intact and both abraded sites in the remaining animal. Mild edema (grade one) was observed in only one animal, on one abraded site, at 24 hours. No other dermal effects were noted at any observation interval. The primary irritation score ranged from .13 to .63."

Conclusions:

Direct dermal irritation category: IV

Core Classification:

Guideline

003786

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Primary skin irritation and dermal sensitization in guinea pig

Accession Number: 072016 (11)

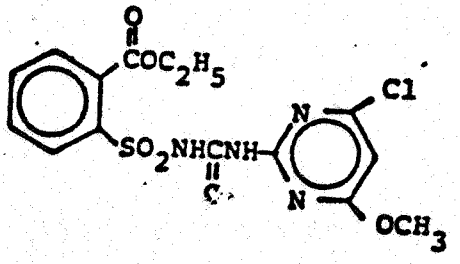
MRID Number:

Sponsor: Dupont, Haskell Lab. No. HLO 350-83

Contracting: Hazleton Lab. No. 201-631

Date: 9-8-83

Test Material: 2-(((((4-chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))benzoic acid, ethyl ester 75%, DPX-F6025 75% Dry Flowable.



Protocol:

Primary Irritation

"Ten test guinea pigs were treated in the primary irritation phase. The control animals were not treated in this phase. The dorsal skin was clipped free of hair and two sites on each animal were chosen for single application. two concentrations (7% and 70% suspension in dimethyl phthalate) of the test material were applied to separate test sites on each animal's back in an area approximately 25 mm in diameter. The resulting irritation scores were compared to the challenge scores as determined at the end of the sensitization period."

Topical Induction of Sensitization

"The same ten animals that were used in the primary irritatic phase and control animals were treated during the induction phase The sacral/hip area of each animals was clipped free of hair and intradermal injection of a 0.1 ml aliquot of the test material suspension (1% in dimethyl phthalate) was attempted after the fin primary irritation reading. Because this was a suspension having the consistency of a paste, this intradermal injection was incomp and unsatisfactory. Control animals received a single 0.1 ml ali intradermal injection of the vehicle. Subsequnet induction sensi zation doses were applied by the topical route. The dorsal skin clipped free of hair and one site on each animal was chosen. Eac site was abraded with minor incisions which were sufficiently dee to penetrate the stratum corneum, but not deep enough to disturb derma or to produce bleeding. A 0.05 ml aliquot of the test mate supension (70%) was applied to the separate test sites on each te animal's back inan area approximately 25 mm in diameter. The veh was applied in the same manner to the control animals. This appl cation was repeated on alternate sides of the back three times a during a three week period for a total of nine topical applicatio

Challenge Phase

"Thirteen days after the final sensitization application was administered, the backs of all animals (both the test and control groups) were shaved. A 0.05 ml aliquot of the challenge test sus sion at 7% and 70% was applied to the assigned two test sites on animal in an area approximately 25 mm in diameter. The ten test animals, as well as the ten control animals, were exposed to the challenge doses."

Observations and Recoreds

"At 24 and 48 hours after each application in the range-findi primary irritation, and challenge phases, the test sites were exmained and scored according to the method of Draize (1959). Af each induction phase treatment, the test sites were observed for necrosis and erythema at 24 hours only.

Throughout the study, all animals were observed for mortalit and moribundity."

Results:

Direct dermal erythema was indicated by slight erythema in some animals.

No dermal sensitization was observed.

Conclusion:

DPX-F6025 was not formed to be dermally sensitizing.

Core Classification:

Minimum

14

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

00378

EPA: 68-01-6561
TASK: 54
February 29, 1984

DL-7

DATA EVALUATION RECORD

DPX-6025 (INF-6025-22)

Mutagenicity/Unscheduled DNA Synthesis

CITATION: Horst AL, McCooey KT, Chromey NC, Sarrif AM. 1983. Unscheduled DNA-synthesis/rat hepatocytes in vitro [INF-6025-22]. Haskell Laboratory Report No. 208-83 prepared by Haskell Laboratory for Toxicology and Industrial Medicine for E.I. du Pont de Nemours and Co., Inc., Wilmington, Delaware.

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Project Scientist
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-29-84

Henry T. Appleton, Ph.D.
Program Manager
Dynamac Corporation

Signature: Henry Appleton
Date: 2/29/84

Cipriano Cueto, Ph.D.
Department Director
Dynamac Corporation

Signature: Cipriano Cueto
Date: 2-29-84

APPROVED BY:

W. Thomas Edwards
EPA Scientist

Signature: W Thomas Edwards
Date: 3-15-84

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity/unscheduled DNA-synthesis.

CITATION: Horst AL, McCooey KT, Chromey NC, Sarrif AM. 1983. Unscheduled DNA-synthesis/rat hepatocytes in vitro [INF-6025-22]. Haskell Laboratory Report No. 208-83 prepared by Haskell Laboratory for Toxicology and Industrial Medicine for E.I. du Pont de Nemours and Co., Inc., Wilmington, Delaware.

ACCESSION NUMBER: 072016. (1-)

MRID NUMBER: Not available.

LABORATORY: Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Co., Inc. Elkton Road, P.O. Box 50, Newark, Delaware 19711.

TEST MATERIAL: The test material was INF-6025-22 (98 percent pure with unknown impurities). The chemical description of this compound is Benzoic acid, 2[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]-amino]sulfonyl-], ethyl ester or 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]-amino]sulfonyl] benzoic acid, ethyl ester.

PROTOCOL:

Hepatocyte Isolation: Livers that had been perfused successfully with Sterile Solution I and Sterile Solution II were obtained from rats weighing 200-300 g. Solution I consisted of Hanks' balanced salt solution ("without calcium, magnesium and phenol red") containing 0.5 mM ethyleneglycol-bis (beta-aminoethyl ether) N,N'-tetraacetic acid (EGTA) and 10.0 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), adjusted to pH 7.35. Solution II, which was used also to produce free hepatocytes, consisted of Williams' Medium E (WME) containing 292 mg/L L-glutamine, 50 µg/ml gentamicin sulfate, 100 units/ml type IV collagenase and 10.0 mM HEPES, adjusted to pH 7.35.

After perfusion, the rat liver was removed in one piece and placed in a sterile Petri dish containing WMEG (WME supplemented with 292 mg/l L-glutamine and 50 µg/ml gentamicin sulfate). After trimming and combining

the liver and placing it into additional Solution II at 37° C, the suspension of hepatocytes that was obtained was pipetted into 50 ml centrifuge tubes, the volumes brought up to 50 ml with WMEG supplemented with 10 percent fetal bovine serum (WMES), centrifuged for 2.5 min. at 50 x g, the supernatant decanted and pellets resuspended in 50 ml of fresh WMES. This hepatocyte suspension was tested for cell viability by preparing a 1:10 or 1:20 dilution and adding 1.0 ml of these diluted cell suspension to 0.2 ml of 4 percent trypan blue and analyzing for dye exclusion using a hemocytometer. Thirty-five mm 6-well tissue culture dishes containing 2.0 ml of WMES were seeded with 5×10^5 cells to 25 mm round plastic coverslips. The dishes were incubated under an atmosphere containing 5 percent CO_2 at 90+ percent humidity and 37° C to permit cell attachment. After 2 hr, the WMES was aspirated and the cells washed with 1.0 ml WMEG. This procedure left only viable cells attached to the coverslips.

Assay for Unscheduled DNA-Synthesis (UDS): After removal of the washing solution described above from the attached cells, 2.0 ml of WMEG containing 5 µCi per ml of [^{3}H-methyl]-thymidine was added. A stock solution of INF-6025-22 in DMSO was further diluted with DMSO to give the desired concentrations, which were administered in 20 µl volumes. In addition, a 20 µl solvent control and a positive control, 1 mM dimethylbenzanthracene (DMBA), were included in each assay. The culture dishes containing the treated cells were then incubated at 37° C for 18 hours. The treatment was then terminated by removing the treatment media, washing the attached cells with WME and placing the coverslips (cell surface up) in clean culture dishes. Two ml of a 1 percent sodium citrate solution were then added in order to swell the cell nuclei. Cells were fixed with ethanol-glacial acetic acid (3:1), the coverslips air dried, and mounted with Permount^R on glass sides (cell surface up).

For UDS quantification, the slides were first dipped into Kodak^R type NTB2 emulsion and then allowed to dry. Then the slides were placed in slide boxes for eight days at 40 °C after which they were developed in Kodak^R D-19 developer, followed by treatment with Kodak^R general purpose hardening fixer and staining with hematoxylin. These steps were performed in the dark.

Evaluation of Data: [^{3}H-methyl]-thymidine incorporation into hepatocyte DNA was detected as silver grains in the developed emulsion layer. Silver grain counts were quantified by measuring light intensity reflected by silver grains with an MPV3 Microscope Photometer (Leitz). Twenty-five randomly chosen nuclei per slide were scored. Background level measurements were taken from the most heavily labelled nuclear-sized cytoplasmic areas that were adjacent to each scored nucleus.

The average of 25 nuclear readings from each slide were analyzed by two way analysis of variance (ANOV) in order to determine dose or trial effects. If significant dose effects were observed, pairwise comparisons were to be made. "Signficance was judged at the 0.05 level; conclusions were based on the results of 2 trials." In order for an assay to be acceptable, at least 3 nontoxic levels (minimum of 25 analyzable cells per slide) had to be produced, and the positive control readings had to be significantly greater than the solvent control.

RESULTS:

The ability of INF-6025-22 to induce UDS in primary rat hepatocyte cultures was assessed in 2 separate assays. In assay I, 8 concentrations of INF-6025-22 ranging from 10^{-5} to 10 mM were tested and in assay II, 5 concentrations of the test material ranging from 10^{-3} to 10 mM were assayed. Solvent controls and treatment with 1 mM DMBA were included.

In the first assay, the average net nuclear count for DMBA-treated cultures was 23.2 and the solvent control was -0.15; in the second assay the average counts for DMBA-treated cultures were 24.5 ± 16.85 and -0.9 ± 6.65 for the solvent controls. For INF-6025-22, the following average net nuclear counts (2 averaged groups) for Trial I were: 1×10^{-5} mM (-1.75); 1×10^{-4} mM (-0.4); 1×10^{-3} mM (-3.3 ± 5.9); 1×10^{-2} mM (-1.0); 1×10^{-1} mM (-0.25); 1.0 mM (-0.35); 5.0 mM (-0.15) and 10 mM (0.95). Table I, adapted from Report No. 208-83 contains this data. The standard deviations for each sample should be noted.

In Trial II, the average net nuclear counts for INF-6025-22 (2 averaged groups) were as follows: 1×10^{-3} mM (-2.1); 1×10^{-2} mM (2.7); 1×10^{-1} mM (-4.7) for one group); 1.0 mM (-1.15); 5.0 mM (-2.25) and 10 mM (-1.6). Table II, adapted from Report No. 208-83 contains this data. The standard deviations for each sample should be noted.

DISCUSSION:

The authors concluded that "INF-6025-22 did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes when assayed under the conditions described."

From the analysis of the data presented in this report, our assessment is that the authors' conclusions were correct, however, the standard deviations for the positive controls were very high which indicates that a positive response in the INF-6025-22-treated cultures might have been difficult to detect (i.e., S.D. for the positive control was more than 50 percent of the net grain counts).

CONCLUSIONS:

From the data presented in this report, under the conditions of the two trials, we conclude that INF-6025-22 did not induce unscheduled DNA-synthesis. However, the standard deviations in the positive control, negative control and the samples at various concentrations are quite large, indicative of an experiment which lacks appropriate control on experimental variables.

CLASSIFICATION: Unacceptable.

The assays did not appear to have the required sensitivity and controlled conditions to show a negative response.

TABLE I. Unscheduled DNA Synthesis in Primary Cultures of Rat Hepatocytes: Trial One

Compound	Concentration (mM)	Average Net Nuclear Photometer Reading ± S.D.
DMBA	1mM	19.4 ± 9.4 27.0 ± 19.9
INF-6025-22	0	-0.1 ± 7.7 -0.2 ± 3.9
	1×10^{-5}	-2.1 ± 6.2 -1.4 ± 7.0
	1×10^{-4}	-2.8 ± 5.5 2.0 ± 4.4
	1×10^{-3}	0.0 ± 2.9 -6.6 ± 8.9
	1×10^{-2}	-0.8 ± 9.9 -1.2 ± 3.3
	1×10^{-1}	2.4 ± 3.8 -1.9 ± 3.8
	1.0	-1.1 ± 5.1 0.4 ± 6.8
	5.0	0.1 ± 4.3 -0.4 ± 4.3
	1×10^{1}	2.4 ± 6.7 -0.5 ± 2.4

*Adapted from Haskell Laboratory Report No. 208-83.

TABLE II. Unscheduled DNA Synthesis in Primary Cultures of Rat Hepatocytes: Trial Two

Compound	Concentration (mM)	Average Net Nuclear Photometer Reading ± S.D.
DMBA	1mM	25.4 ± 15.8 23.6 ± 17.9
INF-60-25-22	0	1.0 ± 8.5 -1.9 ± 4.8
	1×10^{-3}	-2.3 ± 5.1 -1.9 ± 6.1
	1×10^{-2}	-0.6 ± 9.2 6.0 ± 7.6
	1×10^{-1}	-4.7 ± 6.3 Missing point
	1.0	-4.7 ± 6.1 2.4 ± 10.8
	5.0	-0.8 ± 5.3 -3.7 ± 13.2
	1×10^{1}	-3.0 ± 4.0 -0.2 ± 4.9

*Adapted from Haskell Laboratory Report No. 208-83.

003786

EPA: 68-01-6561
TASK: 54
March 9, 1984

DATA EVALUATION RECORD

DPX-6025 (INF-6025-30)

Mutagenicity In Vivo

CITATION: Farrow MG, Cortina T, Padillar-Nash H. 1983. In vivo bone marrow chromosome study in rats: HF 14,823 [in INF-6025-30]. Report on Project No. 201-615 prepared by Hazelton Laboratories America, Inc. for E.I. du Pont de Nemours and Company, Wilmington, Delaware.

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Project Scientist
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 3-9-84

Henry T. Appleton, Ph.D.
Program Manager
Dynamac Corporation

Signature: Henry T. Appleton
Date: 3/9/84

Cipriano Cueto, Ph.D.
Department Director
Dynamac Corporation

Signature: Cipriano Cueto
Date: 3-9-84

APPROVED BY:

W. Thomas Edwards
EPA Scientist

Signature: W. Thomas Edwards
Date: 3-15-84

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity in vivo.

CITATION: Farrow MG, Cortina T, Padillar-Nash H. 1983. In vivo bone marrow chromosome study in rats: HF 14,823 [in INF-6025-30]. Report on Project No. 201-615 prepared by Hazelton Laboratories America, Inc. for E.I. du Pont de Nemours and Company, Wilmington, Delaware.

ACCESSION NUMBER: 072016.(15)

LABORATORY: Hazelton Laboratories America, Inc. 2200 Leesburg Turnpike, Vienna, VA 22180, USA.

TEST MATERIAL: The test material was identified as H 14,823 [INF 6025], a white solid whose chemical properties were stated by the testing laboratory as follows: "1. Name: H 14,823; 2. Purity: assumed 100 percent; 3. Method of synthesis: information...on file with the sponsor; 4. Stability: Under the conditions of the assay, the compound was assumed to be stable. Information on stability is on file with the sponsor."

PROTOCOL:

Control Substances: The vehicle control was corn oil (C.F. Sauer Co., Lot Number 80235) which was stated to be 100 percent pure. The positive control chemical was cyclophosphamide (Adams Chemical Co.) whose purity was "Assumed 100 percent."

Test Animals: The animals used in this study were 51 day-old male (95) and female (95) Sprague-Dawley CD rats. They were "received from Charles River Breeding Laboratories, Inc., Kingston, New York, on May 25, 1983."

Maintenance of Animals: For thirteen days prior to study initiation, the animals were acclimated to the laboratory environment, individually housed in wire-mesh cages. Water and food "(Ground Purina Rodent Chow® 5001) were provided ad libitum." The room temperature was kept between 70° and 75° F, the relative humidity kept between 43 and 76 percent, and a cycle of 12 hours light and 12 hours dark were maintained throughout the quarantine and study periods.

Animal Group Assignment: After quarantine, acclimation and health assessment, the 190 albino rats were randomized with the aid of a computer into test groups as indicated in the following table.

Group (No.)	Males	Females	Dosage[a]
Vehicle control (1)	20	20	Corn oil
Positive control (2)[b]	5	5	40 mg/kg
Low dose (3)[c]	20	20	500 mg/kg
Mid dose (4)[c]	20	20	1,500 mg/kg
High dose (5)[c]	20	20	5,000 mg/kg

[a]Volume of 20 ml/kg.
[b]Cyclophosphamide.
[c]INF-6025 (H 14,823).

Preparation and Administration of Test Compound: It was stated that the test material was prepared daily on a weight per volume basis. All treatments with INF-6025, positive control, or vehicle control were by gavage.

Clinical Observations: Twice daily or "prior to sacrifice, observations on appearance, behavior, toxic and pharmacological effects were recorded." Body weights were recorded prior to compound administration for the 6 and 12-hour sacrifices (terminal body weights were deemed inappropriate), and twice (prior to compound administration and prior to colchicine administration) for the 24 and 48-hour sacrifices.

Bone Marrow Cell Sampling: At approximately 4, 10, 22, 46 hours after administration of the test or control materials, appropriate animals received a single ip injection of colchicine (2.0 mg/kg in 5 ml/kg) to arrest cells at metaphase. The animals were sacrificed by CO_2 asphyxiation approximately two hours after colchicine injection. Representative members (5) of each group were sacrificed at 6, 12, 24, and 48 hour intervals and their bone marrow cells were harvested.

Cytogenetic Methodology: The procedures for processing bone marrow cells were modified from Evans[1], and Killian et al.[2] After the rats were sacrificed, the marrow from the femurs were aspirated into 5 ml of Hanks' Balanced Salt Solution (HBSS) which had been maintained at 38° C. The marrow aspirate solution was centrifuged at 1100 rpm, the supernatant removed and then combined with 5 ml of 0.075 M KCl (prewarmed to 37° C). Five drops of freshly made fixative (3:1 methanol: acetic acid) were added to each tube after 25 min., the tubes capped immediately, and gently

[1] Evans HJ. 1976. Cytological methods for detecting chemical mutagens. Vol. 4, Ed. Hollaender, A. Plenum Press, N.Y. pp. 1-30.

[2] Killian DJ et al. 1977. Handbook of mutagen testing. Eds. Kilby BJ et al. Elsevier/North Holland, Amsterdam pp. 243-260.

mixed. The mixture was then centrifuged at 1100 rpm, the supernate decanted, and 5 ml of fixative were added slowly down the sides of each tube and the tubes refrigerated. After resuspension, the last two steps were repeated and the cells were suspended in 0.5 to 2 ml of fresh fixative. From the final cell suspension, several drops were dispersed onto precleaned glass microscope slides and air-dried (2 slides per animal). The slides were stained with Giemsa (Harleco) for 10 min., rinsed twice in glass-distilled water, air-dried and subsequently mounted with coverslips in Coverbond^{R} mounting media.

Cytogenetic Evaluations and Classifications: At least fifty metaphase cells from each rat were examined with an oil objective 100X. In those instances where 50 metaphase spreads could not be located, the cytologists analyzed as many spreads as possible. Cells in mitotic metaphase were the only ones that were analyzed for cytogenetic abnormalities. The following information was recorded for each animal: "numbers and types of chromosomal aberrations, mitotic index, model number for each metaphase and vernier location of each metaphase containing damage." Cytogenetic aberrations were classified as (1) chromatid breaks, (2) chromosomal breaks, (3) chromatid and/or chromosome gaps, (4) exchanges (various types), (5) number of cells with more than 10 aberrations, (6) pulverized cells.

Statistical Analyses: After completion of scoring and slide decoding, data were entered into appropriate groups for statistical analyses. The Kruskal-Wallis analysis of variance and nonparametric pairwise group comparisons (KW-ANOVA) were applied to the mitotic indices, mean model numbers, percent aberrant cells and the mean number of aberrations per cell for each group. Analysis of covariance (ANCOVA) was applied to body weight data. "All analyses were one tailed at the 95 percent confidence level ($p < 0.05$)."

RESULTS:

Gross Clinical Observations and Mortality: Some adverse clinical signs such as depression, ataxia, red nose and/or eyes, and lacrimation were reported in all groups; in group 4 (mid-dose) one animal was dead at 48 hours, and in group 5 (positive control) seven animals were found dead at 48 hours (prior to their scheduled sacrifice). Body weights of male rats in 6, 12, 24, and 48 hr sacrifice groups ranged from 292.1 ± 16.1 g to 317.2 ± 15.1 in the pretreatment males and from 210.2 ± 8.0 to 225.6 ± 7.2 in the pretreatment females.

The minimum body weight change in animals of male INF-6025 treatment groups was 1.6 ± 6.7 g (mid-dose at 24 hr) and the maximum observed change was -37.2 ± 36.0 g (mid-dose at 48 hr). None of the high dose males survived to 48 hr, but the weight change in this group at 24 hr was -4.6 ± 10.5 g). In the female the treatment groups the minimum weight change was -1.2 ± 9.3 g (mid-dose at 24 hr) and the maximum was -16.0 ± 5.7 g (based on 2 survivors in the high dose group at 48 hr). In the mid-dose female treatment group at 48 hr, the maximum change was -8.8 ± 11.8 g (this was the group with 100 percent survival showing the maximum weight change).

Cytogenetics:

In the report, in vivo bone marrow chromosome data was summarized within treatment groups without regard to sex. Negative controls were included at all sacrifice intervals, but the positive control (40 mg/kg cyclophosphamide) was only included at the 24 hr sacrifice. Using this approach, the negative control at all sacrifice intervals had slightly more aberrations per cell than any INF-6025 treatment group. The total number of aberrations per group in the negative control and the various treatment groups was as follows: 6 hr sacrifice - control = 2, low dose = 1, mid-dose = 1, high dose = 0; 24 hr sacrifice - control = 0, positive control = 336, low dose = 0, mid-dose = 0, high dose = 0; 48 hr sacrifice - control = 2, low dose = 0, mid-dose = 0, high dose = 0. The average number of aberrations per cell were: 6 hr sacrifice - control = 0.004, low dose = 0.002, mid-dose = 0, high dose = 0; 12 hr sacrifice - control = 0.010, low dose = 0.002, mid-dose = 0.002, high dose = 0; 24 hr sacrifice - control = 0, positive control = 0.911, low dose = 0, mid-dose = 0, high dose = 0; 48 hr sacrifice - control = 0.005, low dose = 0, mid-dose = 0, high dose = 0. None of the p values in INF-6025 treatment groups for number of aberrant cells per group, percent aberrant cells per group, total number of aberrations per group or average number of aberrations per cell were significantly different from their respective negative control at $p < 0.05$. However, the positive control at 24 hr was reported to be significantly higher for all of these parameters at $p = 0.0001$.

From inspection of the individual animal data there appears to be a positive treatment effect bias forwards the male rats with respect to most parameters. In the group of animals in the positive control analyzed at 24 hr, this bias is seen especially for chromatid breaks in the positive control where 68.8 percent (121/176) of the total occurred in male rats. The numbers in INF-6025 treatment groups were too small to determine if this bias also occurred. However, in the 48 hr sacrifice of group 4, 4/6 chromatid gaps occurred in males and the mortality was more pronounced in male treatment groups; i.e., all 5 males treated with the high dose were dead at 48 hr. whereas 2 females survived.

There were no statistical differences noted between the mean mitotic indices and modal chromosome numbers of the INF-6025 treatment groups and the vehicle controls for the various treatment levels in either sex (i.e., the modal number of chromosomes was always above 41 and below 42 where 42 is the accepted chromosome number in this species), and the mean mitotic indices were not significantly higher than the controls which were 1.1 (6 hr), 1.4 (12 hr), 2.4 (24 hr) and 2.6 (48 hr). The positive control, however, had a mean mitotic index of 0.5 ± 0.6 at 24 hr although the modal chromosome number was 41.21.

DISCUSSION:

The authors noted that there were adverse clinical observations in all treatment groups, including one animal death at 1,500 mg/kg and seven animal deaths at 5,000 mg/kg [all 5 males died and 3/5 females died by

48 hr], and there was body weight loss in the treated animals [more pronounced in males]. They noted no significant differences between treatment groups and the negative controls with respect to modal (chromosome) number, mitotic indices, and frequencies of chromosomal aberrations. They concluded from this data that "H 14,823 [INF-6025-33] is not considered to be clastogenic at any of the levels tested."

After examination of the data, our assessment is that a negative response could only be valid for the 24 hr treatment group because this was the only interval for which a positive control result was reported. Therefore, negative data for all other sacrifice intervals could not be supported because the response sensitivity was not demonstrated.

CONCLUSION:

Under the conditions of the assay, male and female rats treated with INF-6025-33 at 500, 1,500 and 5,000 mg/kg did not display clastogenic chromosomal or chromatid effects when sacrificed 24 hr after treatment. Data at 6, 12, and 48 hr could not be evaluated because a positive control was not reported.

CLASSIFICATION: Unacceptable in its present form, except for the data on animals treated and sacrificed 24 hr after treatment. If data for positive controls are presented, the classification of the study may be reconsidered.

12

00:

EPA: 68-01-6561
TASK: 54
March 9, 1984

DATA EVALUATION RECORD

DPX-6025 (INF-6025-6)

Mutagenicity

CITATION: Donovan SM, Irr JD. 1982. Mutagenicity evaluation in Salmonella typhimurium. Unpublished Report No. 459-82 on compound INF-6025-6 prepared by Haskell Laboratory for Toxicology and Industrial Medicine for E.I. du Pont de Nemours and Co., Inc.

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Project Scientist
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 3-9-84

Henry T. Appleton, Ph.D.
Program Manager
Dynamac Corporation

Signature: Henry T. Appleton
Date: 3/9/84

Cipriano Cueto, Ph.D.
Department Director
Dynamac Corporation

Signature: Cipriano Cueto
Date: 3-9-84

APPROVED BY:

W. Thomas Edwards
EPA Scientist

Signature: W. Thomas Edwards
Date: 3-15-84

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity.

CITATION: Donovan SM, Irr JD. 1982. Mutagenicity evaluation in Salmonella typhimurium. Unpublished Report No. 459-82 on compound INF-6025-6 submitted by E.I. du Pont de Nemours and Co., Inc.

ACCESSION NUMBER: 072016.(12)

MRID NUMBER: Not available.

LABORATORY: E.I. de Pont de Nemours and Co., Inc. Haskell Laboratory for Toxicology and Industrial Medicine Elkon Road, P.O. Box 50, Newark, Delaware 19711.

TEST MATERIAL: The test material used in this study was identified as Benzoic acid, 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)-amino]carbonyl] amino] sulfonyl]-ethyl ester, also called 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl) amino]carbonyl]-amino]sulfonyl]benzoic acid, ethyl ester or INF-6025-6 (Code name). The test material was described as being "about 95 percent INF-6025-6," and is a light-yellow solid which dissolves readily in DMSO at concentration up to 10 mg per 0.1 mL.

PROTOCOL:

Bacterial Strains: The bacteria used in this study were Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 which were described by Ames et al.[1]

Preparation of S-9 Fraction and S-9 Mix: The S-9 was prepared from homogenized liver slices from 8 to 9 weeks old male Charles River CD rats that were administered 500 mg of Aroclor 1254 per kg five days prior to sacrifice. The S-9 mix contained per ml, 0.3 ml of S-9 diluted with PBS (1.6 mg protein), 8 µM $MgCl_2$, 33 µM KCl, 5 µM glucose-6-phosphate, 4 µM NADP and 100 µM sodium phosphate.

---

[1] Ames BN, et al. 1975. Mutations Res. 31:347-364.

Controls: The positive control compounds for the assays without S-9 activation were TA1535 (MNNG)[1], TA1537 (9AAc)[1], TA98 (2NF)[1], and TA100 (MNNG)[1]. The positive control compound for all the assays using S-9 was 2-aminoanthracene (2AA). DMSO (dimethylsulfoxide) served as the negative (solvent) control and was used for dissolving the test material and all positive control chemicals.

Preparation of Test Material: The test material was dissolved in DMSO and delivered to the Petri plates at concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, and 0.5 µg per plate.

Mutagenicity Assays: The plate incorporation assay for solid and nonvolatile liquid materials was performed in the presence and absence of rat liver S-9, essentially as was described by Ames, et al.[2] To 2 ml of top agar (0.6 percent agar, 0.6 percent NaCl, 0.05 mM L-histidine, 0.05 mM biotin were added 0.1 ml of the solvent, or test sample or positive control solution and 0.1 ml of an overnight culture containing ~10^8 bacteria when the assay was performed without activation. For assays with activation 0.5 ml of S-9 mix was added to the bacteria/test -1 sample/top agar mixture. For assays with or without S-9 activation, that top agar mixture was poured onto the surfaces of Petri dishes containing 25 ml of Davis minimal agar. The His^+ revertant colonies were scored after the plates had been incubated for 48 hours at 37° C.

Cytotoxicity Assay: The test sample was assayed for cytotoxicity in strain TA1535 only, by a protocol[2] identical to that for mutagenesis except that 10^3 rather than 10^8 bacteria were plated and excess histidine was present.

Statistical Analysis: The statistical methods used were described by Snee and Irr[3]. The number of revertants per plate were transformed, using the power transformation $Y = X^{0.2}$, after which a t-test was performed to determine significant increases in the mutation frequency at the various dose levels and the significance of the dose-response relationship was tested. "Linear, quadratic, and higher order dose-response effects were tested in an F-test of significance." A test sample was considered nonmutagenic when $p > 0.05$ and there was not a positive correlation between increasing revertants and increasing doses of the test material.

---

[1] MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; 9AAc, 9-aminoacridine; 2NF, 2-introfluorene.

[2] Ames BN et al. 1975. Mutation Res. 31:347-364.

[3] Snee RD, Irr JD. 1981. Mutation Res. 85:77-93.

The test sample was considered mutagenic when $p < 0.01$ that the test samples have more mutants than the control or $p < 0.01$ "that there is not a positive correlation between the number of revertants and increasing concentrations of the test sample."

RESULTS:

INF-6025-6 (designated H-14,552 in the assay) was tested at concentrations from 50 to 10,000 µg per plate, and was reported to be toxic at all doses. The authors conducted partial mutagenesis experiments (TA1535: 10^8 cells per plate and concurrently TA1535: 10^3 cells per plate) because previous members of this class of compounds had been shown to have different cytotoxic effects at different cell densities. The concentrations of INF-6025-6 to be used in all four tester stains "were based on the results of the partial mutagenesis experiments (results not shown)."

In the absence of S-9 activation, the number of mutant colonies per plate (average of two trials using duplicate plates) was as follows for the negative controls: TA1535(37), TA1537(25.8), TA98(32), and TA100 (113.5). For the positive controls without S-9 activation, the average numbers of revertants per plate were TA1535(MNNG = 3589), TA1537(9AAc = 187), TA98(2NF = 1219.8), and TA100(MNNG = 3114.8). For treatment with INF-6025-6 at 0.001 µg/plate the revertant numbers were TA1535(33.5), TA1537(21.8), TA98(33.5), and TA100(109), and at 0.5 µg/plate the revertant numbers were TA1535(12), TA1537(15.3), TA98(25), and TA100 (65.8). At concentrations between 0.001 and 0.5 µg/plate the His^+ revertant values were similar. None of the test compound values for revertants per plate were statistically elevated ($p>0.05$) above the control level of revertants whereas all positive controls were significantly elevated at $p<0.01$.

In the presence of S-9, the number of mutant colonies per plate (average of two trials using duplicate plates) was as follows for the negative controls: TA1535(21.3), TA1537(20.3), TA98(34.3), and TA100(118.8). For the positive control, 2AA, the values were TA1535(95.5), TA1537(823.5), TA98(2981.5), and TA100(1716). For INF-6025-6 treatment with S-9 at 0.001 µg/plate the revertant numbers were TA1535(24.8), TA1537(18.3), TA98 (35.5) and TA100(110.3), and at 0.5 µg/plate the revertant numbers were TA1535(10.3), TA1537(16.0), TA98(22.0), and TA100(61.0). At concentrations between 0.001 and 0.5 µg per plate the His^+ revertant values were similar. At none of the test compound levels were the revertants per plate statistically elevated above the revertant values of the negative control ($p > 0.05$) whereas all positive controls were significantly elevated at $p < 0.01$.

DISCUSSION:

The authors concluded that "INF-6025-6 was not mutagenic when tested in Salmonella typhimurium according to the described protocol." Their data showed that the test material was not mutagenic for S. typhimurium strains

TA1535, TA1537, TA98 or TA100 at six INF-6025-6 concentrations ranging from 0.001 to 0.5 µg/plate in the absence or presence of rat-liver S-9 activation.

We consider that the authors' conclusions were correct based on the data reported. It should be noted that strain TA1538, usually us as part of the 5 tester strain-battery was not included in this assay nor required by the protocol. However, TA 98 has the same histidine mutation as TA1538 and therefore, the latter strain is not required. Essential cytotoxicity data used to set the maximum dose for the mutagenicity assays was not presented; "concentrations for the mutagenesis experiments in all 4 strains were based on the results [not reported] of the partial mutagenesis experiments." Data used as a basis for choosing the dose-range for each of the test strains are required before it can be determined that the mutagenesis assays were conducted using appropriate level.

CONCLUSIONS:

Under the conditions of the study, the test material, INF-6025-6, was not mutagenic for the four S. typhimurium strains assayed at 0.001 through 0.5 µg/ml in the presence or absence of rat liver S-9 activation. However, the absence of cytotoxicity data used as a basis for selecting the dose-range for each of the test strains limits the acceptability of these findings.

CLASSIFICATION: The report of this study is unacceptable in its present form; however, this classification will be reconsidered if the missing cytotoxicity information is made available.

003786

EPA: 68-01-6561
TASK: 54
March 9, 1984

DATA EVALUATION RECORD

DPX-6025 (INF-6025-22)

Mutagenicity (CHO/HGPRT)

CITATION: Summers JC, Waterer JC, Chromey NC, Sarrif AM, and Krahn DF. 1983. CHO/HGPRT assay [of INF-6025-22] for Gene Mutation Haskell Laboratory Report No. 270-83 prepared by Haskell Laboratory for Toxicology and Industrial Medicine for E.I. du Pont de Nemours and Co., Inc.

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Project Scientist
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 3-9-84

Henry T. Appleton, Ph.D.
Program Manager
Dynamac Corporation

Signature: Henry T. Appleton
Date: 3/9/84

Cipriano Cueto, Ph.D.
Department Director
Dynamac Corporation

Signature: Cipriano Cueto
Date: 3/9/84

APPROVED BY:

W. Thomas Edwards
EPA Scientist

Signature: W. Thomas Edwards
Date: 3-15-84

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity.

CITATION: Summers JC, Waterer JC, Chromey NC, Sarrif AM, and Krahn DF. 1983. CHO/HGPRT assay [of INF-6025-22] for Gene Mutation Haskell Laboratory Report No. 270-83 prepared by Haskell Laboratory for Toxicology and Industrial Medicine for E.I. du Pont de Nemours and Co., Inc.

ACCESSION NUMBER: 072016( )

MRID NUMBER: Not available.

LABORATORY: E.I. du Pont de Nemours and Co., Inc. Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Delaware 19711.

TEST MATERIAL: The test material used in this study was INF-6025-22 (98 percent INF-6025-22) which is chemically defined as Benzoic acid, 2[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]-amino]sulfonyl]-ethyl ester or 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino-]carbonyl]-amino] sulfonyl]benzoic acid, ethyl ester.

PROTOCOL:

Cell Line: The Chinese hamster ovary BH4 clone of the cell line CHO-K1 was used in this study. These cells were cultured as monolayers using Ham's F12 medium without antibiotics or hypoxanthene but containing 5 percent dialized heat-inactivated fetal bovine serum (BH1FBS). For assays, 50 U/ml of penicillin and 50 ug/ml of streptomycin were added to the culture medium. Incubation was at 37° C in 5 percent CO_2 and 90+ percent relative humidity. The cultures were treated with 0.05 percent trypsin to remove them from dishes for subculturing. Cells were required to be free of mycoplasma.

Liver S-9 Preparation: Liver slices from 8 to 9 week old male Charles River CD® rats, injected ip with Aroclor® 1254 five days before sacrifice, were used to prepare liver homogenates. The S-9 fractions obtained from these homogenates were quick frozen and stored in liquid nitrogen vapor or at -70°C. The protein concentration and cytochrome P-450 level in the microsomal fractions of each S-9 were determined. The S-9 fractions were also assayed at 0, 0.2, 0.5, and 1.0 mg level of S-9

per ml of treatment-medium and at two concentrations of the test chemical to establish a survival range between 30 and 70 percent relative to the solvent control.

Solvents and Positive Controls: The solvent to be used in the study was selected on the basis of INF-6025-22 solubility. From a solvent preference order of phosphate buffered saline (PBS), ethanol, dimethyl sulfoxide (DMSO) and acetone, DMSO was chosen because it had the highest solubility level for the test material. The positive control for testing without S-9 was 0.35 mM ethylmethanesulfonate (EMS, in PBS) and for testing with S-9, 0.01 mM, 1,2-dimethylbenz[a]anthracene (DMBA, in DMSO) was used.

Test Material Preparation: The test material, INF-6025-22 was solubilized in DMSO and administered in volumes of 20 to 40 µl so that final concentrations in treatment media were 0.1, 1.0, 2.5, 5.0, 7.5, and 8.5 mM. The code number H-14,743 was assigned to all INF-6025-22 treatment concentrations.

Treatment Medium: For assays without S-9 activation, 3 ml of the medium contained 5 percent DHIFBS, 50 U/ml penicillin, 50 µg/ml streptomycin and 20-40 µl of the test material in F12 (w/o hypoxanthene) with 2.5×10^{-2}M HEPES (pH 7.2) as a buffer. For assays with S-9, 3 ml of the medium contained "S-9 (3 mg protein, unless otherwise specified)," 50 U/ml penicillin, 50 µg/ml streptomycin, 5.6×10^{-3} M magnesium chlor- ide, 5×10^{3}M glucose-6-phosphate, 1.5×10^{-3} M NADP, and 20 to 40 µl of test chemical in F 12 (w/o hypoxanthene) with 2.5×10^{-2} M HEPES (pH 7.2) as a buffer.

Mutagenicity Assay: In 5 ml of culture medium were plated 5×10^{-5} cells per 25 cm^2 flask. After incubation, culture medium was removed the next day, treatment medium was added, and the cells incubated for 18-19 hr without activation for 5 hours with S-9. Next, the treatment medium was removed and the flasks washed with culture medium; cultures were incubated an additional 21-25 hours before they were subcultured for assessment of cylotoxicity and mutagenicity (resistance to 6-thioguanine, 6-TG).

For cytotoxicity assessment, 200 cells each were plated in 6 dishes (60 mm), incubated for 7 days, and the colonies stained and counted. Survival was expressed as a percentage of cells plated and as a percent of cells in the solvent control.

For mutagenicity assessment, cells from each treatment flask were plated at concentration of 1×10^{6} into one 100 mm dish. By subculturing twice, these cells were maintained in the exponential growth phase for 7 days. On day 7, the cells were plate in 5 plates at 2×10^{-5} cells per 100 mm dish in culture medium fortified with 1×10^{-5} M 6-TG. Survival, as previously described, was determined concurrently. After 7 days of incubation, the colonies were stained and counted, and the mutant

frequency (colonies/10^6 surviving cells at the time of selection) calculated.

Data Evaluation and Analysis: The data was transformed using the formula, "Y = (mutation frequency +1)$^{0.15}$" provided it satisfied the assumptions required to perform parametric statistical analyses. A two variable (dose and experiment) Analysis of Variance (ANOVA) Model was used because this procedure allowed for unequal numbers of doses and unequal numbers of test results for each trial. Using one analysis, a t-test of significance was noted to compare a chemical dose to the solvent control for a significant increase in the mutation frequency. ANOVA was applied to evaluate dose-response relationships. When there were linear, quadratic or higher order effects, they were tested using an F-test. A test sample was classified as nonmutagen when $p>0.05$ that any sample did not have a geater number of mutants than the solvent control and there was not a positive correlation between increased mutagenesis and the dose. A test sample was classified as mutagen when $p<0.01$ that the number of mutants at one or more concentration was greater than the solvent control and when $p < 0.01$ that a dose response for mutagenesis occurred. The details of this analysis have been previously published[1].

RESULTS:

The absolute plating efficiency was determined using variable S-9 concentrations, 0.0 to 1.0 mg protein/ml and variable concentrations of the test material. At 0 mg of S-9 and 0 mM INF-6025-22, the average plating efficiency (PE) was 75.7, while at the same S-9 concentration but at 7.5 mM INF-6025-22 the average PE was 72.0. At 1.0 mg of S-9 and 0 mM INF-6025-22, the average PE was 77.6, but at the same S-9 concentration with 7.5 mM INF-6025-22 the average PE was 53.1. At intermediate S-9 concentrations and/or INF-6025-22 levels, similar or intermediate results were obtained with a tendency toward reducing the PE as the concentrations of INF-6025-22 of S-9 were increased. From two trials, it could be seen that cytotoxicity was concentration related and that INF-6025-22 became slightly more toxic after S-9 activation.

In the non-activated assay, the average mutation frequency in the solvent control was 6.9×10^{-6}, resulting in a transformed average of 1.3644. Treatment with 0.35 mM EMS gave a mutation frequencies of 103.3 and 61.4×10^{-6} and transformed averages of 2.0079 and 1.8592 in trials 1 and 2, respectively; these gave T-test values of 2.56 and 3.42 and p values of 0.0203 and 0.0033 for EMS in trails 1 and 2, respectively.

---

1 Snee RD and Irr JD. 1981. Mutation Res. 85:77-93.

The average frequencies mutation x 10^{-6} at variable INF-6025-22 doses in the absence of S-9 were 0.100mM(9.3), 1.0 mM(11.4), 2.5 mM(11.1), 5.0 mM(12.9), 7.5 mM(4.4) and 8.5 mM(1.6). T-values were 0.100 mM(0.40), 1.0 mM(0.71), 2.5 mM(0.67), 5.0 mM(0.90), 7.5 mM(-0.53) and 8.5 mM (-1.27). The p values were 0.100 mM(0.69), 1.0 mM(0.49), 2.5 mM(0.51), 5.0 mM(0.38), 7.5 mM(0.60), 8.5 mM(0.22). By analysis of variance, there was no significant dose response at the 95 percent confidence limit ($p = 0.62$).

In the S-9 activated assay, the average mutation frequency in the solvent control was 18.6 x 10^{-6}, resulting in a transformed average of 1.5627. Treatment with 0.01 mM DMBA gave mutation frequencies of 106.5 and 157.5 x 10^{-6}, and transformed averages of 2.0171 and 2.1380 in trials 1 and 2, respectively; these gave t-test values of 1.82 and 3.30 and p-values of 0.0858 and 0.0042 for DMBA in trials 1 and 2, respectively.

The average mutation frequencies x 10^{-6} at variable INF-6025-22 doses in the presence of S-9 were 0.100 mM(8.8), 1.0 mM(13.9), 2.5 mM(34.9), 5.0 mM (14.0), 7.5 mM(39.9) and 8.5 mM(3.6). T-values were 0.1 mM(-0.98), 1.0 mM (-0.40), 2.5mM(-0.39), 7.5mM(1.14), and 8.5mM(-1.20). The p-values were 0.1 mM(0.34), 1.0 mM(0.6938), 2.5 mM(0.36), 5.0 mM(0.70), 7.5 mM(0.27), and 8.5 mM(0.25). By analysis of variance, there was no significant dose response at the 95 percent confidence limit ($p = 0.49$).

DISCUSSION:

From these data and their analyses, the authors concluded that INF-6025-22 was not mutagenic in the CHO/HGPRT assay at concentrations ranging from 0.1 mM to 8.5 mM in either activated or nonactivated mutagenicity trials. Thus mutagenic activity was not detected under the conditions of their assays.

In our assessment of the data, the assays without S-9 activation were unquestionably negative; overall, the assays with S-9 activation were negative for dose-response and at most dose points. However, at 2.5 mM and 7.5 mM INF-6025-22, the average mutation frequencies were virtually double that of the solvent control. Although these increases were not statis- tically significant, they should not be totally dismissed. By the criteria set to evaluate this assay, the test material would be considered a nonmutagen in the presence of S-9.

CONCLUSIONS:

The test material, INF-6025-22 (98 percent pure) did not appear to be mutagenic to the CHO cell line CHO-K1, clone BH4 in the presence or absence of S-9 activation under the conditions of the study.

CLASSIFICATION: Acceptable.

003786

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 90-Day feeding and one generation reproduction study in rats

Accession Number: 072017 (16)

MRID Number:

Sponsor: DuPont

Contracting Lab: Haskell Lab. No.

Date: 9-1-83

Test Material: 2-(((((4-chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))-benzoic acid, ethyl ester, DPX-F6025, INF-6025.

"The test materials (INF-6025-17, INF-6025-18 and INF-6025-25) were supplied by the Agricultural Chemicals Sections as off-white crystalline solids (Biochemicals Department Notebook References 8868-155, 8868-159, and 8868-184-1, respectively), and assigned Haskell Laboratory identification numbers H-14,697-01, H-14,697-02, and H-14,697-04, respectively. Approximately 1.14 kg of H-14,697-01 (90.6% purity) and 2.39 kg of H-14,697-02 (93.2% purity) were mixed to make a single homogeneous sample numbered H-14,697-03 with a purity of 92.5%. H-14,697-03 was used in the preparation of test diets through 12/29/82; H-14,697-04 (99.6% purity) was used for the remainder of the study." No reason was given for changing from Technical grade to analytical grade material."

During the first 70 days technical grade DPX-F6025 was used then analytical grade DPX-F6025 was used for the remainder of the studies.

Protolcol:

"Groups of 16 male and 16 female Crl:CD® rats from Charles River Breeding Laboratory were fed for approximately 90 days that contained 0, 100, 2,500, or 7 500 ppm. Clinical indices were measured in urine and blood at approximately one, two, and three months after study initiation. At the end of the 90-day period, ten rats per sex from each diet group were sacrificed, necropsied, and select tissues examined histologically. The remaining six rats per sex diet group were mated for the purpose of a one-generation, one-litter reproduction substudy."

Group and dosages were as follows:

"Treatment Group (No. Rats)		Dietary Concentration of INF-6025* (ppm)
Male	Female	
I (16)	II (16)	0 (Control)
III (16)	IV (16)	100
V (16)	VI (16)	2,500
VII (16)	VIII (16)	7,500

*Weight/weight concentration active ingredient INF-6025 (adjusted for % purity)"

I. 90-Day feeding

"During the 90 feeding phase of the study all rats were weighed once weekly and were observed at least twice daily for abnormal behavior or appearance and moribundity. The amount of food consumed by each group was determined weekly during the 90-day feeding phase of the study. Food consumption and body weight data were used to calculate, mean daily food consumption, food efficiency (utilization of food for body growth), and mean daily intake of INF-6025).

"At approximately one, two, and three months after the study's intitiation hematological, clinical chemical, and urine analytical evaluations were conducted on the rats in each treatment group designated for evaluation of subchronic toxicity. Rats were individually housed in metabolism cages to facilitate the collection of urine. Following a 24-hour acclimation period, the rats were deprived of food overnight (approximately 16 hr.) during which time urine was collected. Blood samples were subsequently collected via tail cut from the fasted rats.

"The hematological parameters examined at each interval consisted of erythrocyte, leukocyte, differential leukocytes, and platelet counts, hemoglobin contents, and mean corpuscular volumes (MCV). Hematocrits, mean corpuscular hemoglobin contents (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated. Blood smears for reticulocytes were prepared and counted. Clinical chemical evaluation of serum consisted of measures of alkaline phosphatase (AP), alanine-amino transferase (ALT), and aspartate-amino transferase (AST) activities, blood urea nitrogen (BUN), total serum protein, albumin, globulin (calculated), creatinine, cholesterol, glucose, calcium, sodium and potassium. Urine analysis consisted of quantitative measures of urine volume, pH, and osmolality and semi-quantitative measures of glucose, protein, bilirubin, urobilinogen, ketones, and occult blood. Urine color and appearance were recorded and sediment from the urine samples was examined microscopically.

"On days 95 and 96 of the study, the surviving male and female rats, respectively, from each treatment group designated to be evaluated for subchronic toxicity were sacrificed and necropsied. The order of sacrifice was random among all rats within a sex. All rats that died or were also necropsied. Brain, heart, liver, spleen, kidneys, lungs, thymus, adrenals, pituitary and testes of all necropsied rats expcept those found dead were weighted and, for rats in the terminal sacrifice, organ weight/final body weight ratios were calculated. The following tissues taken from rats designated to be evaluated for subchronic toxicity (tissue integrity permitting) or were sacrificed prior to the end of the 90-day feeding study, and from rats in the high-dose and conrtrol groups were examined microscopically: thymus, spleen, femoral bone marrow, lymph nodes, heart, aort-thoracic, trachea, lungs, salivary glands, esophagus, stomach, small intestine (duodenum, jejunum and ileum), large intestine (colon and cecum), liver, pancreas, kidneys, bladder, pituitary, thyriod- parathyroid, adrenals, testes, epididymides, prostates, ovaries, corpus and cervix uteri, Vagina, brain, eye, muscle (thigh), bone (femur), and all gross lesions. All gross lesions, heart, liver, kidney, and target organs from rats in the low-and intermediate-dose groups were also examined histopathologically. The other tissues listed above from rats in the low- and intermediated-dose groups were collected, processed to the block stage of preparation, but were not evaluated histologically.

"Tissue samples (brain, liver, kidney, spleen, muscle, testis, and fat), blood, urine, and feces were collected from rats subjected to the terminal sacrifice and pooled by test group. These sample were frozen and sent to the Biochemicals Department for residue analyses. The result of these analyses will appear in a separate Biochemicals Department for residue analyses. The results of these analyses appear in a separate Biochemicals Department report."

II. <u>One-generation Reproduction</u>

"The six designated rats in each test group were used to initiate a one-generation, one-litter reproduction study at the end of the 90-day feeding study. Throughout the reproduction study, all rats received their respective treatment group's diet. Each female (F_0) rat was housed with a randomly selected male rat from the same test group for a period of fifteen days. Females were examined for copulation plugs daily and the date of first observation of the plug was recorded. After the mating period, the female rats were separated from the male rats, and housed individually in polypropylene pans with Ad-sorb-dri® cage bedding. Six days later, all female rats were examined a least three times daily for the birth of pups (F_{1A}).

Total pup weights for each litter were determined approximately 24 hours and 4-days after birth. Litters that contained more than 10 pups were reduced to this number, four days after birth by random selection of equal number of male and female pup (when possible). Extra pups were sacrificed and discarded without pathological evaluation. Pups that died prior to weaning on day 21 postpartum were discarded without pathological evaluation. The male and female parent rats were sacrificed after all pups were weaned (test-day 155) and discarded without pathological evaluation.

"The following formulas were used for the generation of reproduction and lactation indices:

Fertility Index (%)	=	$\frac{\text{Total number of litters delivered}}{\text{total number of females mated}}$	x 100
Gestation Index (%)	=	$\frac{\text{Total number of litters with at least one live pup}}{\text{Total number of litters delivered}}$	x 100
Percent Pups Born Alive (per litter)*	=	$\frac{\text{Number of pups born alive}}{\text{Number of pups born}}$	x 100
Viability Index (%) (per litter)**	=	$\frac{\text{Number of pups alive at four days postpartum}}{\text{Number of pups born alive}}$	x 100
Lactation Index (%) (per litter)**	=	$\frac{\text{Number of pups alive at weaning}}{\text{Number of pups alive after reduction (reduction occurs at four days postpartum)}}$	
Litter Survival (%)	=	$\frac{\text{Total number of litters at weaning}}{\text{Total number of litters delivered}}$	x 100

* Indices are calculated on a per litter basis for female rats bearing litters. The percentage is calculated for each litter and the average percentage for each group is reported.

** Indices are calculated on a per litter basis for female rats bearing litters with at least one live pup. The percentage is calculated for each litter and the average percentage for each group is reported."

Statistical Analyses

"Body weights organ weights were subjected to analyses of variance. The least significant difference [LSD] from control values and Dunnett's statistics were calculated whenever the ratio of variances (F-ratio) indicated significant differences existed among the test groups. Significance was judged at the $p \leq 0.05$ probability level. Fisher Exact, Kroskal-Wallis, and/or Mann-Wallis, and/or Mann-Whitney U tests were used to evaluate measures of reproduction and lactation performance."

Results:

A. 90 Day feeding

One rat" (female 7,500 ppm group), was found dead on Day 68 of the study. The probable cause of death was not determined. Two rats, a female (2,500 ppm group) and a female (7,500 ppm group) were interchanged and fed the incorrect diets following the one-month clinical examination. These rats were sacrificed on Day 29 of the study. Tissues from these were held in block stage and excluded from histologic evaluation."

"The mean body weight and overall weight gains of male rats in the 7,500 ppm group were significantly lower than the control group from weeks 3-13 of the study. Significantly lower body weights and/or overall weight gains were also observed in the female 2,500 and 7,500 ppm groups from weeks 5-13 and 2-13, respectively. These parameters were not affected in the male 100 and 2,500 ppm groups and the female 100 ppm group."

"No meaningful differences in diet consumption were observed among male or female treated groups when compared to their respective control groups. Food efficiency was significantly lower in the male 7,500 ppm group and the female 2,500 and 7,500 ppm groups compared to their respective control group."

"Rats in this study did not demonstrate any adverse clinical signs with the exception of alopecia which was observed in two males and two females in the 7,500 ppm groups."

"Numerous, statistically-significant changes in hematological and clinical chemical parameters were reported for both male and female rats at each of the evaluation periods during the study. Decreased erythrocyte counts and hematocrits and decreased or increased or increased MCH and MCHC were belived to have resulted from non-compound-released *in vitro* hemolysis." The biological significance of other hematological and of clinical chemistry parameters is unclear, although there may be some dose-effect relationships at the 2500 and 7500 ppm dosage levels.

"Numerous, statistically-significant differences in absolute and relative organ weights were observed between rats in the control and treatment groups. However, none of the organs affected demonstrated any compound-related histopathological effects; "except livers of males which moderately (and statistically significantly) increased in weight in the highest dosage group. "The liver was the only organ affected histologically. Margination of hepatocyte cytoplasmic contents in the centrilobular areas occurred in the male 2,500 and 7,500 ppm groups and the females 7,500 ppm group."

B. One generation reproduction

"Indices that were affected included a statistically-significant reduction in the litter weights on day four postpartum in the 2,500 and 7,500 ppm groups. Mean male and female pup weights at weaning were also significantly lower in the 7,500 ppm group relative to the groups. All other reproduction and lactation parameters measured were comparable among treated and control groups. No adverse clinial signs were observed among the dams but body weights of dams in the 7,500 ppm group were significantly lower than those in the control group."

Conclusions:

A. 90-Day feeding, rat

NOEL: 100 ppm
LEL: 2500 ppm (body weight changes and histopathology lesions)

B. One-generation reproduction, rat

Using the analytical grade test material no harmful effects were found at the 100 ppm a.i. treatment level. The lowest fetal effect level was 2500 ppm (reduced liver weights). Body weights of dams were reduced at 7500 ppm. EPA guidelines require that the technical material be tested.

Core Classification:

A. 90- Day feeding, rat

minimum

B. One - generation reproduction, rat

"minimum"

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 13-weeks feeding, dog

Accession Number: 072094

MRID Number:

Sponsor: DuPont

Contracting Lab: Hazleton, No. HLO 463-83

Date: 11-1-83

Test Material: 2-[[[[(4-Chloro-6-methoxy-2-pyrimidinyl)-amino]carbonyl]amino]sulfonyl]-, ethyl ester (96%), DPX-F6025, Technical.

Protocol:

"The sixteen males and sixteen females selected for use in this study were stratified by weight and assigned to the following groups using a table of random permutations of nine.

Group No.	No. of Animals		Treatment Level
	Males	Females	ppm
1	4	4	0
2	4	4	100
3	4	4	1500
4	4	4	7500

All of the dogs were observed twice daily for mortality and moribundity and once daily for appearance, behavior, fecal elimination and signs of toxic and pharmacologic effects. These observations were recorded daily. Individual food consumptions were recorded daily and individual body weights were recorded weekly beginning one week prior to the initiation of treatment."

"The following clinical laboratory studies were performed on all dogs twice prior to treatment (Weeks -4 and -1) and during study Weeks 4, 8, and 13.

Hematology:

Hemoglobin (HGB)
Hematocrit (HCT)
Erythrocyte count (RBC)
Platelet count (PLATELET)
Total leukocyte count (WBC)
Differential leukocyte count
Reticulocyte count (RETIC)[a]
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Erythrocyte and leukocyte morphology

Serum Chemistry:

Total cholesterol (T CHOL)
Blood urea nitrogen (BUN)
Alanine aminotransferase (ALT)
Asparate aminotransferase (AST)
Lactate dehydrogenase (LDH)
Alkaline phosphatase (ALK PHOS)
Total protein (T PROT)
Albumin (ALBUMIN)
Albumin/globulin ratio (ALB/GLOB RATIO)
Globulin (GLOBULIN)
Glucose (GLUCOSE)
Sodium (SODIUM)
Potassium (POTAS)
Calcium (CALCIUM)
Total bilirubin (T BILI)
Creatinine (CREAT)
Uric acid (URIC AC)

[a] Slides for reticulocyte counts were conducted at weeks 4, 8, and 13 as a result of depressed red cell parameters at these intervals."

"Urinalysis

Volume (U VOL)
Color and appearance (APPEAR)
Specific gravity (SP GR)
Protein (PROTEIN)
ph (PH)
Glucose (GLUCOSE)
Bilirbin (BiLI)
Ketones (KETONES)
Occult blood (OC BLD-U)
Urobilinogen (UROBIL)
Osmolality (U OSMOL)
Microscopic examination of the sediment

The clinical pathology samples were collected by jugular puncture (hematology and clinical chemistry) or cage pan run-off (urinalysis). The dogs were food and water fasted overnight prior to collection of blood and urine."

"Following thirteen weeks of study, all dogs were. sacrificed. A complete gross examination was performed on each dog and necropsies were performed on each."

Organ/body weight ratios were caleulated for the following.

Brain (including brainstem)	Test with epididymides
Heart	Ovaries
Liver	Adrenals
Thyoid with parathyroids	Pituitary
Kidneys	

The following tissues from each dog were preserved in 10% neutral buffered formalin:

brain (fore-,mid-,hind-)	large intestine (cecum, colon, rectum
pituitary	urinary bladder
thoracic spinal cord	prostate
lumbar spinal cord	ovaries
salivary glands (mandibular)	uterus- one section from each horn
thyriod (parathyroids)	femur
trachea	bone marrow (femur)
thymus	bone marrow smear (rib)
tongue	lungs (two sections, one from each of left and right lobes with mainstem bronchi)
esophagus	liver (two lobes)
heart with aorts	kidneys
cervix	
vagina	
spleen	

adrenals	stomach - one section from each of
pancreas	the cardiac, fundic and pyloric
gallbladder	portions
nerve	skin
mammary gland	muscle
tonsil	mediastinal, mesenteric, and
small intestine (duodenum,	mandibular, lymph node
jejunum and ileum)	rib with osteochonral junction
	all unusual lesions

"The eyes and testes with epididymides were preserved in in Bouin's fixative. Bone marrow smears (preapared from rib marrow) were prepared from each dog and stained for possible future evaluation."

Results:

"There were no deaths or moribund sacrifices during the thirteen weeks of study."

"Treatment-related clinical signs were noted in the high-dose dogs which included pale or yellow gums; yellow discoloration in sclera of the eye; thin, and languid appearance."

"Statistical evaluation of the mean total food consumption revealed significantly lower values for the high-dose males and females than for the controls. Mean total food consumption of the low- and mid-dose dogs were comparable to control."

"Body weight losses were evident in six of the eight high-dose animals. The mean body weight for the high-dose females at week 13 and mean body weight changes for the high-dose males and females were significantly decreased when compared to the controls. Although no statistical significance was noted, the high-dose males' mean body weight at week 13 was notably lower when compared to the controls. Mean body weights and mean body weight changes of the low- and mid-dose groups were comparable to the controls."

"Urinalysis values were largely unremarkable with the exception of decreased urine osmolality and specific gravity in the high-dose groups which were considered to be most likely related to increased urine volume at the sampling interval."

"Clinical chemistry changes consisted of decreases in albumin, A/G Ratio, calcium and uric acid, and increasas in globulin, alkaline phosphatase, cholesterol, aspartate aminotransferase and alanine aminotransferase. These changes were reasonably consistently observed in the high-dose groups, and were occasionally noted in albumin, A/G ratio, and alkaline phosphatase at the mid-dose level. Although the nutritional status of these animals may have been somewhat compromised as a result of their decreased food consumption, these findings are generally indicative of a primary compound effect on the liver."

"Evalution of the hematology data revealed decreased mean hemato- crit, hemoglobin, and erythrocyte counts in the mid- and high-dose dogs at all post-initiation sampling intervals. Statistical significance in the mid-dose group was confined to hematocrit at the Week 13 interval. These findings combined with the finding of Prussian Blue (iron) postive pigment in sinusoidal cells of the liver, suggested increased red blood cell turnover, probably due to hemolysis."

"Extramedullary hematopoeisis was observed in the spleens from all high-dose males and from three high-dose females. The observation of entrapped megakaryocytes within alveolar capillaries of the lung in high-dose males was related to the extramedullary hematopoeisis. Hyperplasia was observed in femoral bone marrow from three high-dose males and all high-dose females and in the rib marrow of three males and one female from the high-dose."

"The moderate anemia detected in the high-dose group was accompanied by an increase in reticulocytes and polychromatophilic cells, possibly indicating a compensatory attempt by the bone marrow. Other associated hematologic changes included increases in mean corpuscular volume and mean corpuscular hemoglobin, nucleated erythrocytes, echinocytes, Howell-jolly bodies, and anisocytes."

"Enlargement of the spleen and liver was consistently noted in the high-dose animals. Additional findings in the high-dose group included small testes and/or prostate, small uteri and thymuses."

"Evaluation of the organ weight data revealed significantly higher and dose-related mean absolute liver weights for the mid- and high-dose males and high-dose females. In addition, dose-related and statistically significant increases of mean relative liver weights were noted for the mid- and high-dose animals when compared to the controls."

"Treatment-related hepatic changes included significantly enlarged livers, increased alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and significantly decreased total cholesterol. Histomorphologic alterations in high-dose dogs included hepatocytic swelling, periportal/perivascular mixed inflammatory cell infiltration, bile retention, prussian Blue (iron) positive material in sinusoidal cells and hepatocytes, increased amount of PAS (Periodic-Acid Schiff) positive-diastase resistant material in sinusoidal cells and hepatocytes, and extramedullary hematopoiesis. Mid-dose males demonstrated hepatocytic swelling and Prussian Blue (iron) positive pigment in sinusoidal cells while only one mid-dose female demonstrated hepatocytic swelling. Choleliths were observed in the gallbladder of two high-dose males and one high-dose female."

"It was postulated that the presence of iron in the liver is secondary to the anemia and not a direct result of the hepatic toxicity. The cholelithiasis in three high-dose animals is likely to be secondary to the hepatic changes."

"Also seen the high-dose animals as possible secondary effects were thymic atrophy, epididymal hypospermia, testicular hypospermato- genesis, and hypoplasia/atrophy of the prostate, uterus, cervix and vagina. Additional histopathology findings of high-dose and some mid-dose animals included medial mineralization of the section of aorta attached to the heart and a nonsuppurative skeletal myositis/fasciitis, sometimes accompanied by mineralization. One mid-dose male and minimal thymic atrophy."

"Decreased number of sperm were observed in the epididymides of three high-dose males with minimal to slight numbers of abnormal sperm forms present. Bilateral hypospermatogenesis was observed in the testes of two males with minimal numbers of abnormal sperm froms present. Minmal multifocal bilateral atrophy of the testes was also obseverd in one of the animals with hypospermatogenesis."

"Moderate or moderately severe hypoplasia/atrophy was observed in the prostate from all high-dose males. This alteration was also observed as minimal and slight in two mid-dose males, slight and moderate in two low-dose males and minimal in one control."

"The uterus cervix and vagina of three high-dose females demonstrated hypoplasia/atrophy. These organs were small compared to controls, but were otherwise morphologically normal."

"Medial mineralization in conjunction with intimal proliferation, fibrosis or aortitis/periaortitis was observed in the section of aorta attached to the heart in one high-dose male and female and two mid-dose males. The medial mineralization observed in one mid- and low-dose female occurred independently of the other changes. Although Medial mineralization, intimal proliferation, fibrosis and aortitis/periaortitis were not observed in control animals in this study, medial mineralization has been observed in a control male in a study of similar duration. However, the location of these lesions and the severity observed in high-dose dogs of both sexes and mid-dose males in conjunction with the occurrence of an atrial thrombus near the atrioventricular valve in a mid-dose female, suggested that these changes may have been related to hemodynamic or hypoxic alteration."

"Nonsuppurative myositis/fasciitis was observed in the routine sections of skeletal muscle from two high-dose males and one mid- and high-dose female. In the male dogs there was concurrent mineraliztion."

"Although the majority of clinical findings were confined to the high-dose group, the toxic effects included mid-dose animals, as the mid-dose group exhibited many pathological changes generally consistent with, but in a lesser degree than those findings in the high-dose animals."

Conclusions:

13-weeks feeding, dog NOEL: 100 ppm
LEL: 1500 ppm (hematologic changes organ weight changes, and atrophy of thymus and prostate)

Core Classification:

Guideline

003786

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Teratogenicity in rats

Accession Number: 072018 (17)

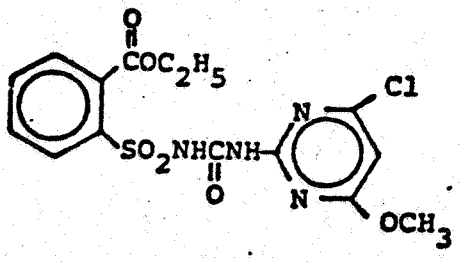
MRID Number:

Sponsor: DuPont

Contracting Lab: Haskell Lab. No. 336-83

Date: 9-1-83

Test Material: 2-(((((4-chloro-6-methoxypyrimidine-2-yl)-aminocarbonyl))-aminosulfonyl))-benzoic acid, ethyl ester 96% a.i., DPX-F6025, INF-6025.



Protocol:

"Groups of 25 pregnant rats were given by gavage nominal dosages of 0,30, 150 or 600 mg INF-6025/kg body weight in a 0.5% methyl cellulose vehicle from Day 7 through 16 of qestation. The actual dosages administered, calculated as the mean of three test suspension samples at each dose level, were 0, 30, 152 and 682 mg INF-6025/kg body weighgt."

"Female rats about 60 days old (nulliparous) were received from Charles River Breeding Laboratories, Inc. Kingston, New York. Their body weights ranged from 163.3g to 191.5g. Male rats used in this study were of the same strain and from the same supplier. They were 85 days old upon arrival and their body weights ranged from 304.3g to 386.2g."

"The dams were weighed upon arrival, just before breeding, days 6 thourgh 17 of gestation, and day 21 of gestation. Individual clinical signs were observed arrival, before breeding, each morning from Day 1 through 21 of gestation and each afternoon from Day 7 through 16 of gestation (dosing period). Feed consumption was measured throughout gestation. The dams were coded from just before sacrifice on Day 21 of gestation until all raw data were collected and all structural alterations classified, so that personnel involved did not know the group to which any dam or fetus belonged."

"After sacrifice by cervical dislocation, the dams were exmained for gross pathologic changes, the liver was removed and weighed, and the number of corpora lutea were counted at a magnification of 2.5X (Ednalite)®). The number and position of all live, dead and resorbed fetuses were recored. The gravid ana empty uterus was weighed. A uterus that appeared to be empty was stained with an ammonium sulfide solution to determine the presence of very early resorptions. "Apparently" non-pregnant females that were found to be pregnant after staining were used only to determine the incidence of pregnancy.

All live and dead fetuses were weighed, and the live fetuses were examined for extrernal alterations and sexed at a magnification of 2.5X (Ednalite®). Fetal sex also was determined during the internal examination. About one-half of the live fetuses were examined for visceral alteration (1). The heads of these fetuses were fixed in Bouin's solution and examined (2). In addition, the eyelids were peeled and the intact eyes measured for seven control and seven high level litters, and for all fetuses classified as malformed, stunted, or having "no" or "small" eye bulge (s).

All fetueses, except for the heads of those fixed in Bouin's solution, were fixed in 70% ethanol, eviscerated (if not done previously), macerated in 1% aqueous KOH solution, and stained with alizarin red S to examine the skeletons for alterations. When indicated at sacrifice, tissues were fixed in bouin's solution for storage or histopathologic examination."

"The litter (i.e. proportion of affected fetuses/litter or litter mean) was used as the experimental unit for the purpose of statistical evaluation (3). The level of significance selected was $p \leq 0.05$. The statistical tests used to analyze the parameters studied are listed below:

Parameter	Test
incidence of pregnancy	Cochran-Armitage (5)
incidence of litters with total resorptions	Fisher's exact test (6)
clinical signs	
maternal death	
feed consumption	Test for linear trend using orthogonal polynomial of dose ranks (7).
maternal body weight	
maternal body weight gain	Dunnett's test, when one-way AVOVA was significant (8)
absolute and relative liver weight	

mean number of corpora lutea/dam	Jonckheere's test (9) Mann-Whitney U test (4,10)"
mean number of implants/ dam	
mean number of resorptions/ dam	
mean percent of resorptions/dam	
mean number of live fetuses/dam	
mean number of male and female fetuses/dam	
mean fetal body weight/ litter	
incidence of structural alterations	

Result:

"Maternal toxicity at the 600 mg/kg level was demonstrated by a significant ($p<0.05$) increase in alopecia and significant ($p\leq0.05$) decreases in feed consumption and body weight gain during the treatment period (Days 7-16 of gestation). Dams in the 150 mg/kg group also showed a significant ($p\leq0.05$) decrease in body weight gair during the treatment period. The adverse effects observed in the 150 and 150 and 600 mg/kg groups were dose-related. No adverse effects were detected among dams given 30 mg INF-6025/kg body weight when compared with control dams."

"No adverse effect that was dose-related was noted for any reproductive parameter. A significant decrease in fetal body weight that was dose-related was demonstrated between the control and 600 mg/kg group."

"A significant increase, that was dose-related, in the frequency of "developmental variations" was noted among fetuses from the 600 mg/kg group when compared with the control value. Among the "developmental variations" the incidence of bipartite and dumbelled centra were significantly greater among fetuses in the 600 mg/kg group when compared with the control value. A significant increase, that was dose-related, in the frequency of "variations due to retarded development" was note for fetuses in both the 150 and 600 mg/kg group when compared with the control group. Among the "variations due to retarded development," a significant ($p\leq0.05$) increases that was doses-related was observed in the frequency of partially ossified or unossified centra among fetuses in the 600 mg/kg group.

In addition, the incidence of partially ossified or unossified sternebra was significantly higer and dose-related for fetueses in both the 150 and 600 mg/kg groups when compared with the control fetuses. When "developmental variations" and "variations due to retarded development" were combined and statistically analyzed, a significant increase in the total number of variations was noted among fetuses in the 600 mg/kg group when compared with the control value."

Conclusions:

As stated above, "maternal and embryo-fetal toxicity was noted at 150 and 600 mg INF-6025/kg body weight. A weak teratogenic response was detected at 600 mg INF-6025/kg body weight, a level that was overtly toxic to the dam. The apparent no-effect level for both the dam and conceptus was 30 mg INF-6025/kg body weight). In this study, INF-6025 did not represent a unique hazard to the conceptus."

Teratogenicity NOEL: 150 mg/kg
LEL: 600 mg/kg (skeletal anomalies indicating teratogenicity)

Fetotoxicity NOEL: 30 mg/kg
LEL: 150 mg/kg (variations due to retarded development)

Maternal Toxicity NOEL: 30 mg/kg
LEL: 150 mg/kg (reduced weight gain)

Core Classification:

Mininum