UNDATED 137

000134

MEMORANDUM

SUBJECT: PP#352-EUP-105 and 0G2376; Petition for Experimental Use Permit and Temporary Tolerances for DPX-4189 in or on Cereal Grains. CASWELL#194AA

FROM: Charles Frick, Toxicologist
Toxicology Branch, HED (TS-769)

TO: Robert Taylor (25)
Registration Division (TS-767)

THRU: William Burnam, Acting Chief
Toxicology Branch, HED (TS-769)

Action Request:

Proposing EUP and temporary tolerances of 0.05 ppm be established for residues of 2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-aminocarbonyl]benzenesulfon amide in or on cereal grains as follows:

Wheat Grain - 0.05 ppm
Barley Grain - 0.05 ppm

The program requested by E.I. Du Pont De Nemours Co. would use a total of 8,000 pounds of DPX 4189 for use over a three year period. The program would use up to 1,000 pounds beginning in March 1981, 3,000 pounds in 1982, and 4,000 pounds in 1983. It was stated that this will not exceed 8,000, 24,000 and 32,000 acres, respectively, or annual average of about 0.03 percent of the U.S. small grain acreage. Will be used in California, Arizona, West of Cascades, East of Cascades, North Centeral states and Southwest Plain.

Recommendation:

Toxicology Branch cannot envision any hazard associated with the implementation of this action request.

The following studies have been classified as supplementary and will not fulfill regulatory requirements:

1. Primary Skin Irritation and Sensitization Study
2. Eye Irritation (Rabbit)
3. Teratology Study (Rat)

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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Formulation: Du Pont DPX 4189 DF Weed Killer

DPX 4189 (Tech) minimum 91% purity - Herbicide a.i. 82.5%/wt.

[redacted]

Inerts are cleared under 180.1001(c).

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Toxicology Summary

The following studies were conducted with the DPX-4189 formulated product.

Acute Oral LD_{50} (Rat) = 95% Confidence Limits

Females = Lower: 6,806 mg/kg
Upper: 9,198 mg/kg
Slope: 8.0

Males = Lower: 6,996 mg/kg
Upper: 8,891 mg/kg
Slope: 12.7

Category - IV Core-Guidelines

Acute Dermal LD_{50} (Rabbit) = > 2000 mg/kg

Category III Core-Minimum

Eye Irritation (Rabbit) = Category III

Core-Minimum

Primary Skin Irritation (Rabbit) = Not a primary irritant. (0 to 2.63 Draize)

Category IV Core-Minimum

The following studies were conducted with Technical DPX.

Acute Oral LD_{50} (Rat)

Males = Lower: 4,723 mg/kg
Upper: 6,648 mg/kg
Slope: 7.6

Female = Lower: 4,113 mg/kg
Upper: 9,524 mg/kg
Slope: 3.2

Category III Core-Guidelines

Acute Oral LD_{50} (Guinea Pigs) = 1,363 mg/kg

Category III Core-Minimum

Subacute Oral (Rat) = Animals dosed five times a week for two weeks, 2,200 mg/kg/dose, ten doses, mortality 2/10

Core-Minimum

Acute Oral LD_{50} IP (Rat) = 1,450 mg/kg

Core-Minimum

Acute Dermal LD_{50} (Rabbit) = > 3,400 mg/kg

Category III Core-Guidelines

Inhalation LC_{50} (Rats) = > 5.9 mg/L

Category III Core-Minimum

Eye Irritation (Rabbit) = Category not assigned.

Core-Supplementary

Primary Skin Irritation and Sensitization Test (Guinea Pig) - No signs of primary irritation or sensitization.

Core-Supplemental

90-Day Feeding Study (Rat) - NOEL = 100 ppm

Core-Guideline

Two-Year Rat Feeding Study (One-Year Interim Report)- NOEL = 100 ppm (This value may be elevated at study completion.

Core-Guideline

Six-Month Dog Feeding Study - NOEL = 2,500 (Highest Level tested)

Core-Minimum

90-Day Feeding Study (Mouse) Range Finding - NOEL = 2,500 ppm (Highest Level tested = 7,500 ppm)

Two-Year Mouse Oncogenic Feeding Study (One-Year Interim Report) - No conclusion at this point in time.

Three-Generation, Six-Litter Reproduction Study (Rat) Interim Report - No reproductive effects noted through the F_{2b} litters up to and including 500 ppm.

Teratology Study (Rat) - The test compound did not appear to be either embryotoxic or teratogenic at the highest level tested (dietary 2500 ppm)

Core Classification: Supplementary

Mutagenic Test (Salmonella/Microsome Assay - The test material was not mutagenic in this test system.

Core classification not assigned.

Metabolism Study - This study not reviewed by Toxicology Branch but, the summary is included in this review for purposes of information.

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Toxicology Review

The following studies were conducted with DPX-4189 Formulated Product.

Acute Oral LD_{50} (Rats)

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine Elkton Rd., Newark, Delaware, Lab. No. 13,402, Report#300-80.

Protocol:

The test material, as a suspension in corn oil, was administered by intragastric intubation in single doses to 4 groups of fasted young adult male and 5 groups of young fasted adult female ChR-CD rats, 10 animals per group. The surviving animals were weighed and observed during a 14-day recovery period and then sacrificed. All animals were given gross pathological examinations.

Results:

Dose(mg/kg)	Sex	Mortality Ratio
9000	M	8/10
7000	M	3/10
6000	M	1/10
5000	M	0/10
9000	F	9/10
8000	F	5/10
7000	F	2/10
6000	F	1/10
5000	F	2/10

Gross pathologic changes observed in the lungs, thymus, spleen, kidneys, stomach, liver, testes, brain and eyes. Most frequently observed clinical signs included: stained and wet perineal, stained face, weakness, and moderate weight loss.

All deaths occurred within 4 days after dosing. One male rat at the highest dose level showed corneal opacity.

LD_{50} (95% Confidence Limits) = Females - Lower: 6,806 mg/kg
Upper: 9,198 mg/kg
Slope: 8.0

Males - Lower: 6,996 mg/kg
Upper: 8,891 mg/kg
Slope: 12.7

Category - IV

Core-Classification: Core-Guidelines

Acute Dermal LD_{50} (Rabbit)

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine Elkton Rd., Newark, Delaware, Lab. No. 13402, Report No. 211-80.

Protocol:

Five adult male and 5 adult female albino rabbits were clipped free of hair over the back and trunk area and fitted with plastic collars. Doses of 2,000 mg/kg of formulation were applied to abraded skin on the back of each animal under two pieces of gauze. After a 24-hour exposure period, the wrappings were removed. Rabbits were observed and weighed over a 14-day recovery period and then sacrificed. Two rabbits of each sex were sent to pathology for gross examination at 15 days post exposure.

Clinical Signs: 1-8 days; slight edema and sporadic weight loss for 14-days.

Pathology:

Treatment area appeared stained brown, scaley and swollen. No other compound related abnormalities were noted. One male animal died within 3 days of treatment, no cause given.

Dermal LD_{50} = > 2,000 mg/kg

Category - III

Core-Classification - Core-Minimum Data

Eye Irritation

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Haskell No. 13402; Study No. 130-80.

Protocol:

The right eyes of nine male albino rabbits were treated with 0.1 ml (approx. 30 mg) test material, and left eye served as control. After 20 seconds, 3 treated eyes were washed with water for one minute - the other eyes were not washed. The eyes were examined with a bright light and a hand-slit lamp at 1, 2 and 3 days and scored according to Draize.

Results:

Test material caused transient local to moderate area of slight corneal clouding and mild to no conjunctival irritation in the unwashed eyes of 6 rabbits. It also cause the same grade of irritation in the 3 rabbits with washed eyes. No iritic involvement occurred in washed or unwashed eyes.

All eyes were normal within 2-3 days after treatment.

Category - III

Core-Classification - Core-Minimum Data

Primary Skin Irritation (Rabbit)

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Haskell No. 13,402; Report No. 264-80.

Protocol:

Six male albino rabbits were clipped free of hair on the trunk and lateral areas and placed in confining stocks. Doses of 0.5 gm solid test material, slightly moistened with saline, were applied to 2 intact and 2 abraded skin areas under gauze squares. After 24 hours, the rabbits were removed from the stocks, patches removed and test sites wiped to remove any test material.

Observations were made upon removal of patches and at 72 hours and at 4, 6 and 7 days after treatment and scored according to Draize.

Results:

This formulation was not found to be a primary irritant when tested on the intact and abraded skin of six rabbits.

The range of the Primary Irritation Scores was 0.0 to 2.63 (Draize).

Category - IV

Core-Classification - Core-Minimum Data

The following studies were conducted with technical DPX.

Oral LD_{50} - Rat

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Haskell No. 12,361; Report No. 399-79.

Protocol:

Test material (Technical), as a suspension in corn oil, was administered by intragastric intubation in single doses to 4 groups of male and 6 groups of female young adult ChR-CD rats, 10 animals per group (all animals were fasted). Survivors were weighed and observed over a 14-day recovery period and then sacrificed. Gross pathology was done on all animals.

Results:

Dose (mg/kg)	Sex	Mortality
7,000	M	7/10
6,000	M	7/10
5,000	M	4/10
4,000	M	1/10
10,000	F	8/10
7,000	F	8/10
7,000	F	3/10
6,000	F	3/10
5,000	F	5/10
4,000	F	3/10

Rats at all dose levels showed stained faces and wet or stained perineal areas; humped posture was seen at all levels except 10,000 mg/kg females. Although not seen in all test groups; eyes half closed, lethargy, chromodacryorrhea, salivation and diarrhea were generally present. Other signs observed frequently included prostration, hematuria, weakness, piloerection, lacrimation, stained underside, body and feet. All deaths occurred within 1 to 4 days after dosing with moderate weight losses for 1-4 days and sporadic weight losses through the 14th in the survivors.

Gross pathological abnormalities were noted in the following organs of male and female rats dosed at 10,000 - 4,000 mg/kg: thymus, liver, lungs, brain, heart, spleen, kidney, eye, pancreas, testes, gastrointestinal tract, skin, uterus and stomach. Animals were fasted prior to dosing.

LD_{50} = Females - Lower: 4,113 mg/kg
Upper: 9,542 mg/kg
Slope: 3.2

Males - Lower: 4,723 mg/kg
Upper: 6,648 mg/kg
Slope: 7.6

Category - IV

Core-Classification - Core-Guideline

Oral LD_{50} - Guinea Pigs

Study conduted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Haskell No. 12,700-02, Report No. 308-50.

Protocol:

Test material, as suspension in corn oil, was administered by single dose, intragastric intubation to 3 groups of 10 young adult DUH male guinea pigs. (A range finding study produced death at 2,250 mg/kg and above after dosing from 670 to 7,500 mg/kg, 1 animal per dose level.) Surviving animals were weighed and observed during a 14-day recovery period and then sacrificed. Two surviving animals from each of the test groups were sent to pathology for gross examination.

Results:

Dose (mg/kg)	Mortality Ratio
2,000	7/10
1,500	6/10
1,000	3/10

Gross pathology - lungs were pale red at all levels tested in 1-2 animals; guinea pigs dosed at 2,000 and 1,000 mg/kg showed lungs that were hyperinflated with red gray mottling in 1-2 animals. The most commonly observed clinical signs were: stained perineal area, stained face, eyes half-closed and weight loss. All deaths occurred within 9 days after dosing.

LD_{50} = 1,363 mg/kg

Category - III

Core-Classification - Core-Minimum Data

Subacute Oral - Rat

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, Haskell No. 10,539-02, Report No. 97-77.

Protocol:

Test material, as a 30% suspension in corn oil, was administered by intragastric intubation to a group of ten young adult male ChR-CD rats, five time a week for two weeks; an additional group of ten rats served as controls and were intubated with corn oil. Five control and four test rats were sacrificed 14 days after the last dose.

Results:

Dose (mg/kg)	No. of Doses	Mortality
2,200	10	2/10

Pathology:

It was stated that no compound-related gross or histologic changes were detected four hours or 14 days after the last dose; however, two of the ten animals died during the testing period.

It was noted the following tissues were examined histologically.

lung	adrenal	testes
liver	pancreas	epididymis
kidney	stomach	heart
trachea	small intestine	brain
thyroid	cecum	spleen
thymus	lymph node	bone marrow
sternal bone	eye	

Core-Classification - Core-Minimum Data

LD_{50} acute IP - Rat

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine; Newark, Delaware; Haskell No. 12,361; Report No. 403-79.

Protocol:

Test material, as a suspension in corn oil, was administered by intraperitoneal injection to 4 groups of 10 young adult ChR-CD male rats in single doses. The surviving animals were weighed and observed during a 14 day recovery period and then sacrificed. One or two surviving animals from each group were examined for gross pathology.

Result:

Dose (mg/kg)	Mortality Ratio
2,500	10/10
1,600	9/10
1,400	2/10
1,200	1/10

Gross pathological observations revealed the following: compound was found throughout organ surfaces, adhesions were seen in the liver and diaphragm, thymal lymph nodes were slightly enlarged and lungs were hyperinflated.

Clinical signs included: Eyes half-closed, stained and wet perineal area, stained face, lacrimation, chromodacryorrhea, diarrhea, weakness, lethary and weight loss.

LD_{50} = 1,450

95% Confidence Limits: Lower: 1,352 mg/kg
Upper: 1,575 mg/kg
Slope: 21.0

Core-Classification - Core-Minimum

Acute Dermal LD_{50} - Rabbits

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware; Haskell No. 12,361, Report No. 415-79.

Protocol:

Fifteen adult male and ten adult female albino rabbits were clipped free of hair over the back and trunk area and fitted with plastic collars. The test material was applied to abraded skin on the back of each animal under gauze pads. The animals trunk was then wrapped. After a 24-hour exposure period, the wrapping were removed and the treated site was washed with water and dried. Surviving animals were sacrificed at 14 days. Four male and two female rabbits were subjected to autopsy for gross examination.

Results:

Rabbits Treated	Dose (mg/kg)	Mortality	Clinical Signs
5 males	2,000	1/5	The four surviving animals had initial weight loss. No clinical signs for duration of test. The animal found dead on day 5 of the test showed initial weight loss but, other than slight skin irritation no other clinical signs were noted.

Gross Pathology:

No compound-related abnormalities were noted.

Rabbits	Dose (mg/kg)	Mortality	Clinical Signs
10 males	3,400	0/10	One animal had diarrhea the day after treatment. Other than slight skin irritation no other clinical signs were noted.
10 females	3,400	0/10	No clinical signs in any animal for duration of test.

Gross Pathology:

No compound-related abnormalities were noted.

Dermal LD_{50} = > 3,400 mg/kg

Category - III

Core-Classification - Core-Guideline

LC_{50} - Rats

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Haskell No. 12,700; Report No. 129-80.

Protocol:

Ten male and ten female young, adult ChR-CD rats were exposed to a dust atmosphere of test compound for 4 hours in a 30 liter battery jar.

Samples of chamber atmosphere were collected approx. every half hour on a Gelman glass fiber filter. Atmosphere concentration was calculated from weight gain of the filter; results are expressed as a Time-Weighed Average.

Upon completions of the exposure the animals were removed from the restraints, wiped free of excess dust, examined for clinical signs and returned to cages for a 14-day observation period.

At 14 days all animals were sacrificed; three animals per sex were subjected to necropsy. The following tissues were examined microscopically; trachea, lungs, liver, kidneys, testes, epididymides, ovaries and uterine horns.

Results:

Chamber concentrations ranged from 2.9 mg/L to 12.0 mg/L with a Time-Weighted Average of 5.9 mg/L. Mass median particle diameter was 6.0 micron (Range 5.8-6.1 micron). No unusual clinical signs were observed at any time during the study.

Pathological findings were not compound-related.

LC_{50} = > 5.9 mg/L

Category - III

Core-Classification - Core-Minimum

Eye Irritation - Rabbit

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware; Haskell No. 10,539; Report No. 744-76.

Protocol:

Ten milligrams of the undiluted solid chemical was placed into the right conjunctival sac of each of two albino rabbits. After 20 seconds, one treated eye was washed with tap water for one minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris and conjunctiva were made at one and four hours, and at one, two, and three days.

Results:

Test compound produced very mild temporary conjunctival irritation with no corneal or iritic involvement in both the unwashed and washed rabbits eyes. Both treated eyes were normal within 4 hours.

Category - Not assigned.

Core-Classification - Supplementary Data

Primary Skin Irritation and Sensitization Tests on Guinea Pigs.

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware; Haskell No. 10,539; Report No. 794-76.

Protocol:

The test for primary irritation was conducted by applying, and lightly rubbing in 1 drop (0.05 ml) each of a 30% and 3% suspension (wt./vol.) of the test material in propylene glycol on the shaved intact shoulder skin of 10 male albino guinea pigs. To test for the sensitization potential, a series of four sacral intradermal/injections was given, one each week over a three-week period, which consisted of 0.1 ml of a 1% solution (wt./vol.) of test material in dimethyl phthalate. Following a two-week rest period, the test animals were challenged for sensitization by applying, and lightly rubbing in, one drop (0.05 ml) each of a 30% and 3% suspension (wt./vol.) of test material in propylene glycol on the shaved intact shoulder skin. A group of 10 previously unexposed guinea pigs received similar applications at the time of challenge to provide a direct comparison of the challenge reactions on skin of similar age.

Results:

No signs of irritation or sensitization were noted.

Comments:

It is not clear how many injections were given in the sensitization study or why the challenge was made with a topical dose of 0.05 ml. No positive controls were used.

In the irritation study is not clear why the dose was only 1 drop of material (Diluted) rubbed into the unabraded skin of the animal.

Studies are classified as supplemental.

Ninety-Day Feeding Study - Rat

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Haskell No. 80-80; Project No. 3067.

This study makes mention of a previously run 90-day rat feeding study - No data was presented but the following summary was provided:

Male and female weanling rats were fed for approx. 90 days with diets that contained 0, 100, 500 or 5,000 ppm. Hematological, urine analysis clinical chemistry and histopathological examinations were conducted on the control and high dose group (5,000 ppm). The following significant changes were observed in the high dose group: increased plasma creatinine and increased relative number of lymphocytes in both male and female animals; decreased rate of body weight gain by female rats; decreased relative number of eosinophils and monocytes in female rats; and decreased total serum proteins in male rats. Male rats also exhibited increased relative heart and thymus weights and increased absolute and relative testes weights. Other organ weight anomolies were noted. In conclusion it was noted that in the absence of dose-response relationships and any gross or microscopic findings, the observed organ weights changes were not considered to be compound related. It was also stated that in order to further investigate and to establish a no observed effect level for the clinical pathological abnormalities noted in the above study, a subsequent 90-day rat feeding study was conducted - the protocol and results of this subsequent study are as follows:

Protocol:

Four hundred-seventeen male and 421 female rats were received from Charles River Breeding Laboratories Inc. After a pretest period of 11 days, 40 rats of each sex were selected on the basis of weight gain and freedom from any clinical signs of disease or injury. Each group of rats was designated to receive a ground Purina Laboratory Chow diet to which was added 0, 100, 500 or 2,500 ppm as follows:

Male	Female	Dietary Concentration
Group*	Group	
I	II	0 ppm
III	IV	100 ppm
V	VI	500 ppm
VII	VIII	2,500 ppm

*Ten animals each group.

Blood was taken from the tails of 10 male and 10 female rats in the groups fed 0, 100, 500, and 2500 ppm approx. 1, 2, and 3 months after the test compound was added to their diet. Hematology measurements on the blood including the following: erythrocyte count, hemoglobin, hematocrit, leukocyte counts, and relative number of neutrophils, lymphocytes, eosinophils, monocytes and basophils. Mean cell volume, mean cell hemoglobin, and mean corpuscular hemoglobin concentrations were calculated.

Clinical chemistry measurements included alkaline phosphatase, glutamic-oxalacctic transaminase, glutamic-pyruvic transaminase, BUN, Creatinine, glucose, total protein and uric acid.

A 24 hour sample of urine was collected from each rat subjected to the hematology examination. This was examined for volume, pH, sugar, blood urobilinogen, bilirubin and protein. Sediment from pooled samples were examined microscopically.

Organs that were weighted are marked with an asterisk in the following list.

Tissues examined microscopically included brain*, spinal cord, sciatic nerve, heart*, aorta, mesenteric vessels, spleen*, femoral bone marrow, sternal bone with marrow, thymus*, lymph nodes, eye, skin, skeletal muscle, salivary and exorbital lacrimal galnds, esophagus, stomach*, duodenum, jejenum, ileum, cecum, colon, pancreas, pituitary*, adrenal*, thyroid and parathyroid glands, trachea, lungs*, liver*, kidney*, urinary bladder, prostate, epididymis, testes*, mammary gland, ovary, uterus, vagina and all gross lesions.

All animals were examined at least once a week and the amount of diet consumed by each group was determined at each weighing period. Mortality during test period was reported.

Results:

Body Weights and Weight Gain

A marked decrease in mean body weights was noted in the growth curves of all female rats on the eighty-fourth day of the study. It was stated that this was a result from a malfunction in the caging system's automatic watering device which prevented the female rats from receiving water from a 36-48 hour period. Afterwards weight gains were comparable to control group.

Clinical Observations and Mortality

All animals survived the study period. No outstanding clinical observations were noted.

Clinical Chemistry

Male rats dosed at 500 and 2,500 ppm test compound exhibited slight decreases in plasma creatinine (original 90 day study showed creatinine increase) decreased urinary pH and slightly increased hematocrits. Female rats at 2,500 ppm dose exhibited slightly decreased erythrocyte counts.

Some glucose variations were noted between test groups however, they were not dose related or consistent and probably not significant.

Pathology

Gross pathologic and histopathologic examination of rats on the test material for 98 days revealed several commonly encountered abnormalities and lesions. However, no significant differences were observed between control and the treated groups in either the incidence or severity of these findings which were believed to be incidental or the result of intercurrent disease and unrelated to exposure of the test material.

Conclusion

The conclusion reached by the originators of this study is as follows:

> In the absence of compound-related histopathological changes in the kidney, the decreased urine pH observed in male rats fed 500 or 2,500 ppm of test compound is of unclear biological significance; however, when incorporated into a nutritionally adequate diet at a concentration of 100 ppm and fed to weanling ChR-CD rats for 98 days, the test compound did not produce any detectable adverse changes in the nutritional, biochemical, hematological, clinical, gross pathological or histopathological parameters examined.

This reviewer concurs with the above conclusion.

NOEL = 100 ppm

Core-Classification - Core Guideline

One-Year Interim Report of a Two-Year Rat Feeding Study

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Haskell No. 3067; Report No. 283-80.

Protocol:

Three hundred sixty-eight male and 371 females rats were used (Charles River Breeding Labs. Mass.) at the end of a 12 day pretest period, 320 rats of each sex were selected on the basis of weight gain and freedom from signs of disease or injury were divided by randomization into four groups of 80 males and 80 females and housed in pairs.

Design:

Test Groups		Dietary Concentrations
Male	Female	
I	II	0 ppm
III	IV	100 ppm
V	VI	500 ppm
VII	VIII	2500 ppm

All rats were examined at least once daily for abnormal behavior and clinical signs of toxicity. Rats were weighed once a week during the first six months and once every two weeks during the last six months of this study interval. Mortality of the animals during the test period was recorded.

Three and six months after the studys start and just prior to the study's one-year interim sacrifice, 10 rats selected from each of the groups were subjected to clinical chemistry and urinalysis. Tail blood was evaluated for alkaline phosphatase activity, SGOT, SGPT, BUN, creatinine and total protein. Urine, from the same animals was collected in the same time interval and examined for volume, pH, sugar, protein, bilirubin, urobilinogen and occult blood. The blood from this same group of animals was examined for the following parameters: erythrocyte, leukocyte and differential leukocyte counts, hematocrits and hemoglobin concentrations. Mean corpuscular hemoglobin, mean volume were calculated.

Fifty-two weeks into the study, 10 rats not subjected to the clinical tests were selected from each test group, sacrificed and necropsied. The brain, heart, spleen, thymus, stomach, pituitary, adrenals, lungs, liver, kidneys, and testes were weighed and mean final body weights, organ weights, and organ to body weight ratios were calculated. The tissues noted above and the following tissues were examined microscopically for histopathologic changes: spinal cord, sciatic nerve, aorta, mesenteric vessels, sternebrae and humoral bone marrow, lymph nodes, eye, skin with underlying mammary tissue, skeletal muscle, salivary and exorbital lacrimal glands, esophagus, duodenum, jejunum, ileum, cecum, colon, pancreas, thyroid and parathyroid glands, trachea, urinary bladder, prostate, epididymis, mammary gland, ovary, uterine horns, vagina and all masses.

Results

Mean Body Weights and Weight Gain

Mean body weights of male rats fed 2500 ppm were consistently lower than the control group from weeks 5-52. In the 500 ppm male rats, it was noted that this group had significantly decreased mean body weights on the fifty-second week of the study. The male rats at 100 ppm and the female rats at all dose levels were comparable to their respective control groups throughout the 52 weeks study period.

Clinical Observations and Mortality

It was noted that, with the exception of a greater incidence of stained or discolored fur in the female rats' test groups, the incidence and severity of the clinical abnormalities observed did not differ appreciably from those observed among the control groups. Several animals exhibited palpable masses during this study interval however, not enough animals were involved to make any conclusions at this point in time.

Mortality during this period was as follows:

Groups	No. Dead/No. Animals Started On Test
I	1/80
II	0/80
III	1/80
IV	1/80
V	3/80
VI	3/80
VII	1/80
VIII	1/80

Nothing extraordinary noted in mortality rate at this point in time.

Clinical Laboratory Measurements

Male rats fed 500 and 2500 ppm of test compound for 52 weeks exhibited decreased erythrocyte counts and increased hematocrits which resulted in an increase in the calculated values for mean cell volume and mean cell hemoglobin and a decrease in the calculated mean corpuscular hemoglobin concentrations for these groups. It was suggested this might be a compound related reticulocytosis however, in the absence of a reticulocyte count this could not be substantiated (reticulocyte counts will be made during the remainder of the study).

No gross or histopathological changes that could be attributed to the presence of test compound were observed.

NOEL = 100 ppm (This value may be elevated at study completion)

Core-Classification: = Core-Guideline

Six-Month Dog Feeding Study

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Haskell No. 108-80; Project No. 3246.

Protocol:

Sixteen male and 16 female pure-bred beagle dogs approx. 9 to 11 months old were divided into four groups of four males each and four groups of four females each. The groups received one of four test diets as follows:

Group		Diet
Male	Female	
I	II	Dog meal (control)
III	IV	Dog meal + 100 ppm A.I.
V	VI	Dog meal + 500 ppm A.I.
VII	VIII	Dog meal + 2500 ppm A.I.

Diets were prepared fresh each week.

All dogs were weighed once a week during the study.

Individual diet consumption was measured weekly throughout the study. From the diet consumption and body weight data, food efficiency and dose of test compound were calculated.

Dogs were examined daily for any abnormal behavior or clincial signs of toxicity.

Hematological examinations were conducted on each dog two times before study initiation and after approx. one, two, three and six months of feeding. The analysis included, erythrocyte count, hemoglobin, hematocrit, total leukocytes count and relative number of neutrophils, lymphocytes, eosinophils, moncytes and basophils. From this data was calculated, mean cellular volume, mean cellular hemoglobin concentration indices. At the same time blood was examined for, glucose, BUN, creatinine, cholesterol, uric acid, total protein, albumin, alkaline phosphatase, SGOT, LDH and albumin/globulin ratio.

Urine (24 hour) was checked for volume, osmolality and pH; blood, sugar, protein, bilirubin, urobilinogen, ketones, color, appearance and a microscopic examination of sediments.

After approx. six-months of continuous feeding, all dogs were sacrificed by electrocution for gross and microscopic pathological appraisal. The following organs were weighted at necropsy:

thymus	kidneys	brain
spleen	testes	
heart	prostate	
lung	thyroid	
stomach	parathyroid	
liver	adrenals	
gall bladder	pituitary	

The following organs and tissues were collected for histological examination: back and abdominal skin with underlying mammary tissue, skeletal muscle (posterior thigh muscle) bone (sternebrae), bone marrow (sternebrae and femur), thymus, spleen, lymph nodes (salivary, mediastinal and mesenteric) heart, aorta, trachea, lung, salivary gland, tonsil, esophagus, stomach, small intestines (duodenum, jejunum and ileum) large intestines (cecum, colon and rectum), liver with gall bladder, pancreas, kidneys, urinary bladder, testes with the epididymides, prostate, ovaries, fallopian tubes, uterine horns and body, vagina, thyroids with parathyroids, adrenals, pituitary gland, brain, spinal cord (cervical), peripheral (sciatic) nerve, eyes and lacrimal gland.

Results

Mean Body Weight and Weight Gain - Nothing extraordinary noted.

Food Consumption, Efficiency and Intake of Test Material - No significant differences among treatment groups with respect to dose were apparent.

Clinical Observations - Two female animals fed diets which contained 2,500 ppm of test material developed alopecia on the back during the second week of testing. Both animals recovered within one month and did not experience repeated alopecia - No other significant clinical observations were noted.

Mortality - No mortalities occurred during the study.

Clinical Pathology

The relative numbers of leukocytes and monocytes in male dogs assigned to receive diets that contained 500 ppm test material were significantly elevated during the pretest period with respect to control values. During the test period, the relative numbers of lymphocytes and eosinophils in male dogs fed the 500 and 2,500 dosage, were significantly higher than control values. It was pointed out that these changes were not considered to be compound related - this reviewer concurs. All other hemotological parameters evaluated were within the normal ranges as established by the control group.

Urine Analysis - Nothing extraordinary noted.

Clinical Chemistry

Serum glutamic-oxaloacetic transaminase activities in male dogs in the 100 and 500 ppm groups were lower and glucose values for female dogs assigned to receive diets containing 2,500 ppm were higher than average during the pretest period. Serum glutamic-pyruvic transaminase activities were higher than historical averages in all dogs during the pretest period and in all but the control females during the test period. An apparent increase in serum glutamic-pyruvic transaminase activities in female dogs fed diets which contained 2,500 ppm test material resulted from lower than average enzyme activities in control females during the test period and was not considered to be attributable to the feeding of the test compound. All groups of male dogs fed diets which contained the test compound had lower albumin concentrations throughout the test period. The depressed albumin level did not appear to be dose-related and were not considered compound related. All other clincial chemistry determinations fell within the normal ranges as established by the control group.

Pathology - Gross and microscopic findings were not extraordinary.

Conclusion - No significant effects related to the test compound were noted up to an including 2,500 ppm* via dietary administration. *Highest level tested.

Core-Classification: Core-Minimum

Ninety-Day Range-finding Feeding Study in the Mouse

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Haskell Lab. No. 3154, Report No. 69-80.

This study was designed to evaluate the subchronic toxicity of test compound when it was incorporated into the diets of male and female mice and to establish dietary levels for a two-year study.

Protocol

After a pretest period of 11 days, 50 mice of each sex were selected on the basis of weight gain and freedom from clinical signs of disease into 5 groups of 10 males and 5 groups of 10 females as follows:

Groups		
Male	Female	Dose
I	II	control
III	IV	2,500 ppm
V	VI	5,000 ppm
VII	VIII	7,500 ppm
IX	X	

All animals were weighed once a week during the study.

Group diet consumption was measured weekly and from this the body weight, food efficiency and dose were calculated.

Animals were examined daily for any abnormal behavior or clinical signs of toxicity.

Hematological exams were run on each mouse after 87 days on the study. This included, hemoglobin concentration, hematocrit, erythrocyte count, leukocyte count and differential leukocyte count. The indices, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated.

Alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase activities, total protein, BUN, and plasma creatinine were run on all animals after approx. 90 days. Of this series only the total protein was determined because of equipment breakdown.

After 91 and 92 days on test surviving animals were subjected to grosss pathological examinations.

The brain, heart, liver, kidneys and testes were weighed.

Microscopic examination of tissues from the control and high dose group included the following: stomach, duodenum, jejunum, ileum, cecum, colon, pancreas, liver, gall bladder, kidney, heart, brain, esophagus, spleen, bone marrow, thymus, testes, epididymis, urinary bladder, seminal vesicle, prostate, lung, eye, lymph node, thyroid, salivary gland, trachea, adrenal, ovary, uterus, skin and medinstinal tissue.

Results

Weight Gain - The final body weights of male mice fed 2,500 or 7,500 ppm were significantly lower (6%) then those of the control males. This effect was not considered biologically significant by the experimenters.

Clinical Toxicity - No clinical signs of toxicity were noted.

Mortality - One male mouse (500 ppm) died on day 87 - death was not considered compound related.

Hematology - Male mice fed 5,000 or 7,500 ppm had lower erythrocyte counts than control males; mean corpuscular volumes and mean corpuscular hemoglobins were higher in these mice.*

Female mice fed 5,000 or 7,500 ppm had fewer neutrophilic granulocytes and more lymphocytes than control females.

The above hematologic changes were statistically significant when compared to the controls.

No effect were apparent at the 500 or 2,500 ppm dose levels.

*The same macrocytic changes were noted in the one-year rat feeding study.

The absolute liver weights of male mice fed 7,500 ppm were increased. The relative liver weights of females fed 5,000 ppm were decreased.

No pathological effects were noted in these organs so the weight changes were not considered to be compound related.

One-Year Interim Report on an ongoing Two-Year Mouse Feeding Study (Oncogenic)

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware. Haskell Lab. No. 203-80, Report No. 3307.

Protocol

Each group consisted of 80 animals.

Male	Female	Dietary Concentration
I	II	0 ppm
III	IV	100 ppm
V	VI	500 ppm
VII	VII	5000 ppm

Animals used were CD-1 Charles River mice.

Animals were examined at least once daily for abnormal behavior, clinical signs of toxicity and palpable masses. Mice were weighed once a week during the first 26 weeks of the study and once every two weeks during weeks 26-52 on test. Food efficiency and intake of test compound were calculated. Mortality was recorded.

Approximately one, three, six and twelve months after the start of the study ten mice from each test group were subjected to hematological examinations. Parameters examined included erythrocyte and total leukocyte counts; relative number of neutrophils, lymphocytes, eosinophils, monocytes and basophils; hematocrits; hemoglobin and total protein. Mean corpuscular volume, mean corpuscular hemoglobin were calculated.

Results

Body Weight

Mice in the 5,000 ppm groups had consistently lower mean body weights than the control animals. Compared to control groups, mean total body weight gains by mice in the 5000 ppm groups were decreased approx. 9-16% after both 26 and 52 weeks of the study.

Female mice in the 100 and 500 ppm groups also exhibited slight but statistically significant decreases in mean body weights and mean total body weights and mean total body weight gains during the test period. Mean daily food consumption but not food efficiency was decreased in all female test groups when compared to the female control group.

Tumors

At approx. 44 and 32 weeks into the study, one male and one female mouse in the 500 ppm dose groups exhibited palpable masses which were located in the perineal area.

Mortality

Treatment Group	No. Dead/No. on Test
I	4/80
II	3/80
III	8/80
IV	6/80
V	11/80
VI	9/80
VII	12/80
VIII	11/80

The above data is suggestive of a compound related effect.

Male mice fed for 52 weeks with diets that contained 500 or 5000 ppm of test material exhibited increased monocyte counts. Females at the 500 or 5000 ppm levels showed increased mean corpuscular volume. It was stated that because of the absence of any other dose-related abnormalities in the hematological profile the increased mean corpuscular volume and elevated monocyte counts were considered not to have clinical significance.

Apparent statistically significant ($p < .05$ level of probability) increases in erythrocyte counts and hematocrits observed during the second six months of the study in male mice fed diets of 5000 ppm test compound were found to be related to low control values and were not considered to be compound related.

Summary of Progress on a Three-Generation Six-Litter Reproduction Study (Rats).

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine. Study or project number not given.

Protocol

After 98 days of continuous feeding with diets that contained 0, 100, 500 or 2,500 ppm of test compound, 20 rats were selected from each of eight study groups contained in a long-term feeding study in male and female ChR-CD rats with the test compound. These four groups of 20 each were selected for the three-generation six-litter reproduction study.

Male and female F_0 rats within each dietary group were mated to produce F_{1a} litters. During the mating and reproduction phases, F_0 rats continued to receive their respective test group's diets. Approximately one week after weaning the F_{1a} litters, the F_0 rats were mated a second time to produce F_{1b} litters. At weaning, the F_0 rats were returned to their respective groups in the long-term feeding study.

Four groups of twenty male and twenty female weanling rats selected from the F_{1b} rats were then mated twice to produce F_{2a} and F_{2b} litters. Weaning of the F_{2b} litters was completed on 1/18/80, and four groups of twenty male and twenty female rats selected from F_{2b} litters of each study group have been started on their 90-day feeding phase.

Observations

Mean total body weight gains of rats in these treatment groups were comparable to those of the control group. Mean body weight and total body weight gain of male F_0 rats in the 2,500 ppm group were 9% and 12% lower, respectively, than those of the control animals at the end of the 48-day feeding period. With the exception of a lower initial mean body weight in female rats in the 500 ppm group, no alterations in body weight or weight gain of F_{1b} rats were observed during the feeding phase of the reproduction study.

No significant clincial observations were noted.

It was seen that slight alternations in reproductive and lactation performances were observed in F_{1b} rats in the 2,500 ppm group during the production of F_{2a} litter. It was stated that, at this point in time a compound related effect cannot be concluded.

Teratology Study - Rat

Study conducted by Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Rd, Newark, Delaware, Lab. No. 583-78, Project No. 2738.

Protocol

Test animals were Charles River-CD rats.

Group	No. of Rats	Dosage
I	27	control
II	27	100 ppm
III	27	500 ppm
IV	27	2500 ppm

Animals were weighed on day of arrival and days 6, 10, 16 and 21 of gestation.

All animals were sacrificed by $ChCl_3$ inhalation on the twenty-first day of gestation. At the time of sacrifice, the abdominal wall of the female was opened and both ovaries and uterus were removed and inspected. The uterus was then opened and the fetuses removed and examined. The following observations and measurements were recorded:

Number of corpora lutea in each ovary
Number of implantation sites in each horn
Number and location of all live and dead fetuses
Weight of each live fetus
Crown-rump length of each live fetus
Any gross anomaly which could be observed under a long focal length of 2 1/2x.

About one-half of the fetuses from each litter were preserved in 95% alcohol for subsequent maceration in 1% aqueous KOH clearing and staining with Alizariin Red and examination to detect skeletal abnormalities. The remaining fetuses were fixed in Bouin's fluid for free-hand razorblade sectioning by the Wilson method and examined for visceral and neural anomalies. The uterus and ovaries of rats in all groups were examined for gross changes.

Results

Body Weights - Not extraordinary (Dams)

Food Consumption - Not extraordinary

Clinical Signs and Mortality - No signs of clinical toxicity were noted and all animals survived the test period.

Pregnancy and Fetal Development - Compound did not seem to alter the mean litter size, the incidence of resorptions per litter or the average fetal body measurements.

Fetal Anomalies and Malformations - Small subcutaneous hematomas and petechial hemorrhages on various parts of the body were found in fetuses from litters of all groups including the control group. The 500 ppm group had a signficantly greater incidence of petechial hemorrhages than did the other groups.

Visceral anomalies such as apparent hydronephrosis, undescended testes and liver peliosis were found in all groups in small numbers. Two anomalies, subcutoneous edema (100 ppm) and an eye with dark material (blood) between the cornea and lens (control) were sigular occurrences. Four major abnormalities were present in two of the test groups, each involving only one fetus. One fetus had hydrocephalus and the other showed multiple defects consisting of heart and lung abnormalities and exencephaly. Both of these were from mothers fed 100 ppm test compound. The other two were from mothers fed 500 ppm test compound. One with an umbilical hernia and the other with a great vessel defect (absent left pulmonary ortery).

No malformations or major abnormalities of the skeletal system were noted. The incidence of minor anomalies and common variants noted were similar in all groups.

Conclusion - Under the conditions of this test, the test compound did not appear to be either embryotoxic or teratogenic in ChR-CD rats. At the highest dose tested (2500 ppm). This feeding study did not demonstrate any maternal toxicity. When this study is repeated it is recommended that higher dosage levels be employed and the route of administration be intubation.

Core-Classification - Supplementary

Mutagenic Testing in the form of the Salmonella/Microsome Assay.

Study conducted by Haskell Laboraotry for Toxicology and Industrial Medicine, Elkton Rd, Newark, Delaware. Lab. No. 121-77, Report No. 058-629.

Protocol

Five histidine-requiring strains of Salmonella typhimurium were used in the assay. Strains TA-1535, TA-100, TA-1537, TA-1538 and TA-98 were employed in this assay.

The tests were performed in the presence and absence of a rat-liver homogenate activation system (S.9). In the absence of metabolic activation, 0.1 ml of a solution of the test material and approx. 10^8 bacteria were added to 2 ml of top agar (0.6% agar, 0.6% Nacl, 0.05 mM L-histidine, 0.05 mM biotin). The metabolic activation system involved the addition of 0.5 ml of S.9 mixture of the chemical-top agar solution.

It was stated that prior to testing, the compound was tested for toxicity to the tester strains. This data was not provided and as only 30 micrograms was the highest concentration tested, this data would be of value.

Solvent Control = DMSO
Positive Control = 2-Aminoanthracene

Results

On the basis of this test, the test material was not mutagenic in the microbial assays either in the presence or absence of a live microsomal system.

Core-Classification - Not assigned

Metabolism Study - Rat

The study was not reviewed by Toxicology Branch but the summary is given below for the purposes of information.

Radiolabeled chemical (^{14}C-phenyl tag) - At the end of 72 hours, 95% of the ^{14}C-dose was elimated in urine and 4% in feces; <1% was retained in tissues/carcass. Major components in urine were intact DPX-4189 (86%), 2-chlorobenzenesulfonamide (5%), and minor metabolites (4%). Biological half-life was <6 hours.

Acceptable Daily Intake Data

NOEL = 100 ppm (Rat 5.0 mg/kg)
Safety Factor 2000x
PADI = 0.0025 mg/kg/day
MPI = 0.1500 mg/day (60 kg)
TMRC = 0.0078 mg/day (1.5 kg)
% ADI = 5.20