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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 627. Fosamine Ammonium. Review of Developmental Toxicity Study in Rats

Shaughnessy No. 106701
Tox. Chem. No. 465G
Project No. D179009
Submission No. S418977

TO: Tom Myers, PM Team # 52
Special Review and
Reregistation Division (H7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 9/23/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 12/14/92 12/16/92
Section I, Toxicology Branch I
Health Effects Division (H7509C)

Background and Request:

E. I. du Pont de Nemours and Company has submitted a developmental toxicity study in the rat in support of reregistration of Fosamine Ammonium (MRID 423209-01). The Toxicology Branch (TB-I) has been asked to review and comment on the study.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has reviewed the developmental toxicity study conducted on Fosamine Ammonium in rats. The study is graded Core Guideline and satisfies the regulatory requirement for a developmental toxicity study in rats. The following paragraph summarizes the results of the study.

Fosamine ammonium was tested in a developmental toxicity study in Crl:CD®BR rats at the following dose levels: 0, 50, 350, 1000 and 3000 mg/kg/day. The test material was administered by gavage on days 7-17 of gestation. The maternal NOEL is 1000 mg/kg/day and the maternal LEL is 3000 mg/kg/day based on clinical signs of toxicity (diarrhea) and on decreases in body weight gain and food consumption during the dosing period. The developmental NOEL is 3000 mg/kg/day (HDT).

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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 9/23/92
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner, Head Roger Gardner
Section I, Tox. Branch (H7509C) 12/10/92

DATA EVALUATION REPORT

STUDY TYPE: Teratology - Developmental Toxicity (83-3)

SPECIES: Rat

SHAUGHNESSY NO./CASWELL NO.: 106701 / 465G

ACCESSION NUMBER/MRID NO.: 423209-01

DP BARCODE AND SUBMISSION NO.: D179009

TEST MATERIAL: Fosamine ammonium

SYNONYMS: DPX-R1108-100

MEDICAL RESEARCH NUMBER: 9147-001

REPORT NUMBER: 43-92

SPONSOR: E. I. du Pont de Nemours and Company, Agricultural Products, Newark, Delaware 19714

TESTING FACILITY: E. I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware

TITLE OF REPORT: Developmental Toxicity Study of DPX-R1108-100 in Rats

AUTHOR(S): L. Alvarez

REPORT ISSUED: 5/8/92

CONCLUSION: Fosamine ammonium was tested in a developmental toxicity study in Crl:CD®BR rats at the following dose levels: 0, 50, 350, 1000 and 3000 mg/kg/day. The test material was administered by gavage on days 7-17 of gestation. The maternal NOEL is 1000 mg/kg/day and the maternal LEL is 3000 mg/kg/day based on clinical signs of toxicity (diarrhea) and on decreases in body weight gain and food consumption during the dosing period. The developmental NOEL is 3000 mg/kg/day (HDT).

Classification: Core Guideline

Testing Guideline Satisfied: 83-3

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: ammonium ethyl (aminocarbonyl) phosphonate

Description: Off-white, solid, hygroscopic compound, solubility in H_2O > 10% (w/v), stable at ambient temperature.
Batch #(s), Other #(s): Code Nos. DPX-R1108-100; IN R1108-100 (R1108-100)
Purity: 97.7%
Source: Du Pont Agricultural Products, Wilmington, Delaware 19898
Vehicle (if applicable): Deionized water.
Contaminant: Not provided by Sponsor.

2. Test Animals):

Species and Strain (sexes): Male and nulliparous female Crl:CD®BR rats
Age: ♂: 78 days; ♀: 64 days on receipt.
Weight(s): ♂: 299 - 357 g; ♀: 195 - 251 g on day after receipt. The animals were quarantined for nine days prior to mating.
Source(s): Charles River Breeding Laboratories, Inc., Kingston, New York.

3. Study Design:

This study was designed to assess the developmental toxicity potential of fosamine ammonium when administered by gavage to rats on gestation days 7 through 16, inclusive.

a. Mating:

Natural or artificial insemination? Natural.
Describe technique used: Females were cohabitated with males (1:1) until copulation was confirmed by the presence of a copulatory plug in the vagina or on the cageboard. The day copulation was confirmed was designated as day 1 of gestation.

b. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	25
Low Dose	50	25
Mid Dose 1	350	25
Mid Dose 2	1000	25
High Dose	3000	25

c. Dosing:

All doses were in a volume of 10 ml/kg of body weight/day. Dosing was based on the most recent gestation day body weight.

1) Basis For Selection of Dose Levels: Dose levels were selected on the basis of a pilot study in which groups of 7 mated rats were dosed with 0, 500, 1000, 3000 or 5000 mg/kg/day fosamine ammonium on days 7 - 16 of gestation (day 1 was considered to be the day in which copulation was confirmed). Two deaths were observed at the 5000 mg/kg dose level and significant decreases in food consumption and increases in clinical signs of toxicity were observed at the 3000 and 5000 mg/kg dose levels. No other effects were observed, including reproductive or fetal parameters.

2) Preparation: The dosing formulations were prepared each morning. Weighed amounts of the test material were dissolved in deionized water to produce the formulations with nominal concentrations of 0, 5, 35, 100 and 300 mg/ml for the control, 50, 350, 1000 and 3000 mg/kg dose groups, respectively.

3) Frequency of Preparation: Daily.

4) Storage Conditions: The test material was stored at room temperature in a capped glass or plastic container.

5) Stability Analyses: One the third day of dosing, 2 of 3 samples from each dose level were analyzed immediately for concentration of the test material. The third sample was retained for approximately 5 hours at ambient

temperature and then analyzed to establish the stability of the test substance in the vehicle.

6) Homogeneity Analyses: Not conducted.

7) Concentration Analyses: Samples from each dose level were taken on the third, tenth and seventeenth days of dosing. Duplicate samples were analyzed to verify concentration.

d. Maternal Examinations:

1) Clinical Observations and Mortality: The animals were examined for clinical signs of toxicity on the day after arrival, weekly before mating, each morning on days 1-22 and each afternoon on days 7-16 (the dosing period). Observations for morbidity and mortality were made daily.

2) Body Weight Determinations: Body weights were recorded on the day after arrival, weekly before mating, and on days 1, 7-17 and 22 of gestation.

3) Food Consumption: Feed was weighed on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 22 of the gestation period.

4) Gross Necropsy:

Animals which died or were sacrificed in moribund condition prior to end of exposure period and were subjected to complete gross pathological examinations: This was not mentioned in the report, probably because none of the dams either died or were sacrificed in moribund condition prior to the terminal sacrifice.

Animals sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All females, including apparently "non-pregnant" ones were examined.

5) Uterine Examinations: The following parameters were measured:

Number of corpora lutea
Number of live fetuses
Number of dead fetuses
Early and late resorptions
Total implantations
Individual fetal weights
Gravid uterine weights
Empty uterine weights
Very early resorptions in uteri of "non-gravid" rats

e. Fetal Examinations:

The fetuses were examined in the following manner: live fetuses were weighed, sexed and examined for external alterations. The maximum stunted weight (MSW) was calculated for each litter (total weight minus lightest weight divided by remaining number of fetuses and multiplying by 0.666). The report stated that a fetus weighing the same or less than the MSW was considered to be stunted and its weight was omitted when the mean weight for the litter was calculated. Starting with the first live fetus, every other fetus in each litter was decapitated and examined for visceral alterations and for verification of sex. In addition, all stunted and externally malformed fetuses were examined for visceral alterations. Retarded renal development was classified using the schema of Woo and Hoar (1972). Individually selected heads were fixed in Bouin's fluid and examined. The remaining fetuses were sacrificed, fixed in 70% ethanol, eviscerated, macerated in 1% aqueous KOH, stained with alizarin red S and examined for skeletal alterations.

f. Historical Control Data:

Historical control data were not provided to allow comparison with concurrent controls.

g. Statistical analysis:

The following statistical analysis methods were employed: For litter parameters, the proportion of affected fetuses/litter or the litter mean was used as the experimental unit for statistical

evaluation. The following summary of the statistical tests used is quoted from the report.

"When Bartlett's test for homogeneity of variances was significant ($p \leq 0.05$), analyses of maternal body weight and feed consumption data were done using Jonckheere's test. Where the data were tied and the standard large sample versions of Jonckheere's and Cochran-Armitage tests were not applicable, exact p values were calculated using permutation methodology.

Parameter	Trend Test
Maternal weight Maternal weight changes Maternal feed consumption	Linear contrast of means from ANOVA
Live fetuses Dead fetuses Resorptions Nidations Corpora lutea Incidence of fetal alterations	Jonckheere's test
Incidence of pregnancy Clinical observations Maternal mortality Females with total resorptions Abortions/Early deliveries	Cochran-Armitage test
Fetal weight	Linear contrast of least square means from ANCOVA

h. Compliance:

A signed Statement of No Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

Signed documentation of quality assurance was provided.

B. RESULTS:

1. Dosage Preparation: The results of the stability analysis are as follows:

On the first day of sampling, the mean measured concentrations at time 0 ranged from 98 to 113 percent of the nominal concentrations over all the dose levels tested. At five hours post-sampling time, the mean measured concentrations ranged from 101 to 106 percent of the nominal concentrations. The stability of the test solutions appeared to be stable over 5 hours.

The results of the concentration analyses are as follows:

Again, on the first day of sampling, the mean measured concentrations ranged from 98 to 113 percent of the nominal concentrations over all the dose levels tested. At the next sampling time, the mean measured concentrations ranged from 98 to 119 percent of the nominal concentrations over all the dose levels tested. At the final sampling time, the mean measured concentrations ranged from 98 to 103 percent of the nominal concentrations over all the dose levels tested. In general, it appears that the actual concentrations were within the nominal concentrations.

2. Maternal Toxicity:

a. Clinical Observations and Mortality: There were no mortalities during the study. During days 7-16 there was a statistically significant increase in the incidence of diarrhea in the 1000 and 3000 mg/kg dose groups and in vaginal discharge in the 3000 mg/kg dose group (Cochran-Armitage test). In addition, on days 17-22, there was a statistically significant increase in diarrhea in the high dose group (Cochran-Armitage test). The actual incidences indicate that only the incidence of diarrhea in the 3000 mg/kg group may be biologically significant. The following table summarizes these particular signs.

Summary of Clinical Observations

Day of Gestation	Observation	Group / Dose / # Exam.	I 0 25	II 50 25	III 350 25	IV 1000 25	V 3000 25
7-16	Diarrhea		0	0	0	2*	25*
7-16	Vaginal discharge		0	0	0	0	2*
17-22	Diarrhea		0	0	0	0	20*

* Significant difference from control value (Cochran-Armitage test), $p \leq 0.05$.

b. Body Weight Determinations: Statistically significant decreases in body weight gain were observed in the high dose group on days 7-9, 11-13 and 15-17. Statistically significant increases in body weight gain were observed in the high dose group on days 9-11 and 13-15. The overall mean body weight gains during the dosing period (days 7-17) and during the post-dosing period (days 17-22) were similar to controls at all dose levels. The authors stated that although not statistically significant, there were some decreases in body weight gain in the mid-dose group as well. This effect appears to be marginal at best. The NOEL for bodyweight gains is therefore 1000 mg/kg/day and the LEL is 3000 mg/kg/day.

The investigators supplied the following data:

Table I: Mean Body Weight Gains (grams)[a]

Daily Dose (mg/kg)	Days of Gestation									
	1-7	7-9	9-11	11-13	13-15	15-17	7-17	17-22	1-22	Corrected[1]
0	37.5	4.9	10.3	13.7	4.6	24.8	58.3	92.3	188.0	79.8
50	34.8	7.1	10.5	14.0	7.7	21.5	60.8	92.7	188.3	76.8
350	35.3	5.8	10.1	13.4	9.7	20.7	59.7	89.9	185.0	79.7
1000	33.2	2.8	10.5	12.0	8.8	20.8	54.9	95.2	183.3	76.6
3000	32.8	1.6*	14.9*	11.2*	11.9*	18.5*	58.1	93.3	184.2	75.9

[1] = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.
a = Data extracted from (report number 43-92 and table 1, appendices C, D & E)

c. Food Consumption: Statistically significant decreases in food consumption were observed for days 7-9 for the 1000 and 3000 mg/kg/day groups. In addition, statistically significant decreases in food consumption were observed for the high dose group for days 7-17 and significant increases were observed for the high dose group for days 17-22. The decrease in food consumption at the 1000 mg/kg level is within 90% of the control value and is probably not biologically signficant. The NOEL for decrease in food consumption is therefore 1000 mg/kg/day and the LEL is 3000 mg/kg/day.

The investigators supplied the following data:

Table II: Food Consumption Data (unit)[a]

Daily Dose (mg/kg)	Days of Gestation							
	1-7	7-9	9-11	11-13	13-15	15-17	7-17	17-22
0	25.6	25.6	26.8	26.3	24.3	28.7	26.3	30.2
50	25.1	25.1	26.6	26.8	25.3	28.0	26.4	30.5
350	25.6	25.3	26.9	26.9	26.9	29.2	27.0	31.0
1000	25.7	23.2*	26.2	26.2	25.8	27.4	25.7	31.8
3000	25.6	19.8*	24.5	24.5	26.1	27.9	24.4*	34.2*

[a] = Data extracted from (report number HLR 43-92 and table 2 and appendix F)
* Statistically significant (linear contrast of means from ANOVA); $p \leq 0.05$.

d. Gross Pathology: No treatment-related observations in gross pathology were observed. The only observations that were observed were lack of pregnancy in some animals and a dark red mottling, visible on the cut surface of the kidneys of one high dose animal.

e. Cesarean section Observations: No significant treatment-related differences were observed. The following table summarizes the data.

Table III: Cesarean Section observations[a]

	Control	LDT	MDT1	MDT2	HDT
Dose (mg/kg)	0	50	350	1000	3000
#Animals Assigned	25	25	25	25	25
#Animals Mated	25	25	25	25	25
Pregnancy Rate	24	23	23	24	23
(percent)	(96)	(92)	(92)	(96)	(96)
Maternal Wastage					
#Died	0	0	0	0	0
#Died/pregnant	0	0	0	0	0
#Non pregnant	1	2	2	1	2
#Premature Delivery	0	0	0	0	1
Total Corpora Lutea	458	446	451	463	433
Corpora Lutea/dam	19.1	19.4	19.6	19.3	19.7
Total Implantation	418	400	380	396	372
Implantations/Dam	17.4	17.4	16.5	16.5	16.9
Total Live Fetuses	386	373	356	377	350
Live Fetuses/Dam	16.1	16.2	15.5	15.7	15.9
# With Total Resorptions	0	0	0	0	0
Total Resorptions	32	28	24	20	22
Mean Early	1.3	1.2	1.0	0.8	1.0
Mean Late	0.0	0.0	0.1	0.0	0.0
Resorptions/Dam	1.3	1.2	1.0	0.8	1.0
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam	0.0	0.0	0.0	0.0	0.0
Mean Fetal Weight (gm)	5.46	5.45	5.36	5.36	5.34
Sex Ratio (% Male)	50	52	52	50	47
No. of stunted fetuses	1	1	1	1	1

[a] = Data extracted from (report number HLR 43-92 and table 4 or appendix I)

3. Developmental Toxicity: No statistically significant differences in any external, visceral or skeletal malformations or variations were observed at any dose level when compared to controls. The following tables summarize selected results with fetal (litter) incidences.

Table IV: External Examinations

Dose (mg/kg)	0	50	350	1000	3000
Incidence of Fetal Malformations					
#pups(litters) examined	386(24)	373(23)	356(23)	377(24)	350(22)
#pups(litters) affected	0(0)	0(0)	0(0)	1(1)	0(0)
Imperforate anus	0	0	0	1(1)	0
Tail absent	0	0	0	1(1)	0
Incidence of Fetal Variations					
#pups(litters) examined	386(24)	373(23)	356(23)	377(24)	350(22)
#pups(litters) affected	0(0)	2(2)	0(0)	0(0)	0(0)
Subcutis edema	0	1(1)	0	0	0
Subcutis hemorrhage	0	1(1)	0	0	0

Table V: Visceral Examinations

Dose (mg/kg)	0	50	350	1000	3000
Incidence of Fetal Malformations					
#pups(litters) examined	200(24)	195(23)	183(23)	197(24)	183(22)
#pups(litters) affected	1(1)	0(0)	0(0)	0(0)	1(1)
Dextrocardia	0	0	0	0	1(1)
Great vessel malformation	1(1)	0	0	0	0
Incidence of Fetal Variations					
#pups(litters) examined	200(24)	195(23)	183(23)	197(24)	183(22)

Table V: Visceral Examinations

Dose (mg/kg)	0	50	350	1000	3000
#pups(litters) affected	3(3)	2(2)	7(6)	4(4)	3(3)
Variable origins of greater vessels	0	0	1(1)	0	0
Common pulmonary trunk: greater vessels	3(3)	2(2)	6(5)	4(4)	3(3)
Variations Due to Retarded Development					
#pups(litters) affected	14(11)	7(6)	17(11)	6(5)	10(7)
Kidney - small papilla:size 1	2(2)	2(2)	2(2)	0	1(1)
Kidney - small papilla:size 2	12(10)	5(5)	15(10)	6(5)	9(7)

Table VI: Skeletal Examinations

Dose (mg/kg)	0	50	350	1000	3000
Incidence of Fetal Malformations					
#pups(litters) examined	386(24)	373(23)	356(23)	377(24)	350(22)
#pups(litters) affected	0(0)	1(1)	0(0)	2(2)	2(2)
Rib fusion	0	1(1)	0	0	1(1)
Vertebral fusion	0	0	0	1(1)	1(1)
Vertebra absent	0	0	0	1(1)	0
Hemivertebra	0	0	0	0	1(1)
Incidence of Fetal Variations					
#pups(litters) examined	386(24)	373(23)	356(23)	377(24)	350(22)
#pups(litters) affected	0(0)	6(3)	0(0)	1(1)	3(2)
Rib - rudimentary cervical	0	5(3)	0	1(1)	2(1)

Table VI: Skeletal Examinations

Dose (mg/kg)	0	50	350	1000	3000
Sternebra - misaligned	0	1(1)	0	0	1(1)
Variations Due to Retarded Development					
#pups(litters) affected	26(10)	23(15)	12(9)	21(12)	27(14)
Rib - wavy	1(1)	2(2)	1(1)	1(1)	2(2)
Skull - partially ossified	11(6)	3(3)	2(2)	6(5)	8(5)
Skull - unossified	10(5)	6(6)	2(1)	3(3)	7(4)
Sternebra - partially ossified	3(2)	10(8)	4(3)	6(3)	7(3)
Vertebra - partially ossified	3(3)	3(3)	3(3)	7(7)	5(4)
Vertebra - unossified	1(1)	0	0	0	0

C. DISCUSSION:

1. Maternal Toxicity: The NOEL for maternal toxicity is 1000 mg/kg/day and the LEL is 3000 mg/kg/day based on clinical signs (diarrhea), decrease in body weight gain during the dosing period decrease in food consumption during the dosing period.

2. Developmental Toxicity:

   a. Deaths/Resorptions: No treatment-related effects were observed for deaths and/or resorptions.

   b. Altered Growth: No treatment-related effects were observed for altered growth.

   c. Developmental Anomalies: No treatment-related effects were observed for developmental anomalies.

   d. Malformations: No treatment-related effects were observed for malformations.

D. Study Deficiencies: There were no study deficiencies.

E. Core Classification: Core Guideline Data.

Maternal NOEL = 1000 mg/kg/day
Maternal LOEL = 3000 mg/kg/day
Developmental Toxicity NOEL = 3000 mg/kg/day
Developmental Toxicity LOEL = N/A