CASWELL FILE

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

FEB 5 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of Company responses on Harmony

To: V. Walters, PM 25
Registration Division TS-767C

From: Marcia van Gemert, Ph.D. W. van Gemert 1/29/88
Head, Section III
Toxicology Branch, HED

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED

Caswell No: 573S

Project No: 7-1108

Firm: Dupont

In response to some of the reviews of Dupont data on Harmony in Toxicology Branch's memo of 3/17/87 Dupont has submitted additional information.

Purity specifications have been given for all test samples in the studies submitted. These specifications are on attached pages 2 and 3. Some samples were analyzed twice, and thus some measure of compound stability can be determined from these analyses. The types of impurities of three samples of harmony and their structures are on attached pages 4, 5 and 6.

The issues raised concerning specific studies mentioned in the March 17, 1987 memo will be addressed one at a time with each study.

1. Mouse oncogenicity study: report # HLR-685-85 dated 6/26/85. Individual pathology sheets for each animal were missing from the report. These have now been supplied by Dupont and are adequate to insure that the summary tables are accurate. This study can now be upgraded from supplementary to minimum and the NOEL is 25 ppm, with an LEL of 750 ppm based on reduced body weights. There was no evidence of an increase in oncogenicity produced by Harmony from this study.

2. Rat chronic/oncogenicity study: Report # 4980-001, HRL-261-86 dated 6/26/86. EPA noted the following deficiencies that needed to be corrected before the study could be upgraded to core minimum.
   a. Individual pathology sheets needed to be submitted.
   b. An explanation was needed as to why several clinical chemistry parameters such as chloride, phosphorous, total bilirubin and

creatine phosphokinase were not investigated, especially when there were electrolyte effects.

c. An explanation needed to be given as to why ophthalmological examinations were not performed.

d. Clinical chemistry tables needed to be submitted in a clearer form.

e. A decrease in serum sodium levels was seen at all dose levels tested.

Dupont's reply to these specific comments is listed below.

a. Individual pathology sheets have been submitted and the Tox Branch considers them adequate. The microscopic summary table has been checked for nonneoplastic and neoplastic changes against the individual data and no non-neoplastic or neoplastic changes appear to be compound-related.

b. Concerning the missing clinical chemistry parameters, Dupont has explained that chloride closely follows sodium, so the chloride data would not add anything new. They also stated that phosphorous-calcium balance is evaluated primarily in growing animals to detect primary or secondary parathyroid disease. Since no growth problems or parathyroid disease was evident, lack of serum phosphorous levels would not jeopardize this study. They stated that total bilirubin levels are normally very low in rats, and would be grossly evident in serum or skin with icteric serum or jaundice if it occurred. In addition, alkaline phosphatase was performed to assess cholestasis. Creatine kinase was not performed and originates mostly from skeletal muscle. However, they also performed aspartate aminotransferase which would also indicate skeletal muscle lesions. This information was therefore obtained. Tox Branch considers these explanations to be reasonable and will require no further information on these clinical chemistry parameters.

c. Concerning the missing ophthalmological examinations, Dupont stated in its recent submission that ophthalmological exams were in fact performed grossly pretest and weekly on test. These exams were expected to detect:
Inflammation/changes in eyelids or conjunctivae
Corneal or scleral defects
Cataracts
Anterior synechia,
Global degeneration,
hypopyon,
iritis/uveitis,
At necropsy, histopathology was also performed. This appears to be sufficient to determine if ocular damage has occurred from the compound.

d. Clinical chemistry has been submitted in a clearer tabular form. After reviewing these newly submitted tables it doesn't appear that there were any treatment-related effects.

e. Concerning the lack of an NOEL for serum sodium, Dupont has submitted revised sodium tables for males and females. These are on appended pages 7-10. New statistical analyses using both Dunnetts and Mann-Whitney U are also on these tables. From the data on these new tables it appears that the NOEL for serum sodium in male rats is 500 ppm and the NOEL for females is 25 ppm, as originally stated in the study text.

The MTD issue was not raised in the first review because other items had to be cleared up first. It does appear that an MTD has been reached, based on the modest weight loss in the chronic rat study at 2500 ppm and the weight loss seen in both males and females in the 90-day study. This study classification can be upgraded from core supplementary to core minimum with an NOEL of 25 ppm based on decreased serum sodium levels in females. The LOEL is 500 ppm and the compound does not appear to have oncogenic potential.

3. 2-Generation Reproduction Study, # 432-85, Dec. 3, 1985.
EPA had stated that:
a. The study should be run at higher doses,
b. At least 20 pregnant females per group should have been run,
c. Complete postmortem procedures should be done for parental animals,
d. Individual and summarized data should be presented for food consumption, gestation lengths, precoital intervals, and the number of breeding pairs with evidence of copulation.

The dupont response to these issues will be taken in the order above.
a. Given the 90-day study's drop in body weight for both males and females, the dose should have been sufficiently high to see effects. EPA agrees with Dupont, and considers the dose selection appropriate.
b. Dupont ran two litters per generation, and this should be sufficient numbers of litters. EPA agrees that with the 2 litters/ generation, sufficient pregnancies were examined to make a sufficiently vigorous exam of potential reproductive effects of the compound.
c. Dupont ran the chronic rat study concurrently with the reproduction study and believed that the histopathology for the chronic study would be sufficient for the reproduction study also. EPA disagrees with Dupont on this matter. All animals used for the chronic study were virgins with no pregnancies and matings. Histopathology done on these chronic study animals would not sufficiently address the concerns for reproductive effects of the compound that might have occurred during mating and pregnancy. Histopathology should be done on control and high dose animals. These tissues should still be available according to GLP protocols, and therefore should be sectioned and evaluated.
d. Individual and summarized individual breeding data were presented, and do not indicate any treatment-related effects. EPA agrees with Dupont on this matter.

4. Concerns for triazine amine were raised by EPA because it is very stable and there is a potential for accumulation in the environment.
Dupont responded to EPA's concerns by submitting studies that were done on triazine amine in connection with premanufacturing notification. These studies included:

Mutagenicity: Ames	Acceptable
Oral LD_{50} : 1680 mg/kg	Minimum
Acute inhalation	Minimum
Acute Dermal LD_{50}	Supplementary
Skin Irritation	Minimum
Eye Irritation	Supplementary
Subacute (10 dose) study	Supplementary

[redacted] given to animals in the toxicology studies. Dupont further stated that residue levels would be extremely low, on the order of 0.0014-0.004 ppm in barley grain, 0.005 ppm in straw and EAB has stated that groundwater levels would be on the order of 1.5 ppb. (Memo from EAB dated 1/24/88.) EPA agrees that these residue and groundwater levels are extremely low, and that with the submitted data, and [redacted] samples fed animals in the chronic studies, sufficient data exist on triazine amine to cover these forseeable exposures. Residue chemistry has confirmed these extremely low residue levels. The DERs for the triazine amine studies are attached.

5. Mutagenicity study in CHO/HPRT assay, # 240-84, Dupont submitted additional information to upgrade the study from unacceptable to acceptable.
EPA response: The data submitted are not sufficient to upgrade the study from unacceptable to acceptable because no justification is given for selecting 7mM as the highest concentration when the test substance could be suspended in DMSO up to 700 mM without "excessive precipitate formation." According to Duponts own criterion, the highest concentration for the assays should give about 10% survival compared to the solvent control, and/or slightly beyond the limit of solubility. Yet the results of the cell survival in the 6 assays at the highest dose of 7mM were:

Trial	Activation	Cell Survival (% of control)
1	no	14.5, 24.7
2	no	58.7, 45.5
1	yes	62.8, 54.5
2	yes	36.4, 45.6
3	yes	89.3, 68.7
4	yes	74.2, 69.8

In only one trial is the criterion ever approached. The study used an insufficient dosage, and therefore will not be upgraded from unacceptable to acceptable.

6. One-year dog study; #201752: Jan 21, 1986.
EPA had noted a number of deficiencies in this study. These deficiencies are listed below.
a. Stability and actual test concentration doses were not submitted with the original report.
b. Several clinical chemistry parameters, such as chloride, phosphorous and alanine aminotransferase were not performed, and an explanation had to be given.
c. Ophthalmological examinations were not performed, and an explanation was needed for why these were not performed.

Dupont responded with the following information:
a. Stability data were given in the original report on pages 130-151. EPA has located this information and is now satisfied.
b. Several clinical chemistry parameters were not done such as chloride, phosphorous and alanine aminotransferase (ALT). Dupont stated that chloride was not done because it usually follows sodium,

and Dupont felt it would be redundent information. Phosphorous is usually used to determine calcium-phosphorous balance in growing animals to evaluate primary and/or secondary parathyroid disease. This would be seen in the calcium results, and no other parameters indicative of disease were apparent. Aspartate aminotransferase was used to substitute for ALT. AST may originate from either muscle or liver, and both ALT and AST may be used to assess hepatic necrosis. EPA is satisfied with these explanations.

C. As stated above in the rat study, Dupont felt that ophthalmological parameters were adequately addressed in weekly examinations, and at the final necropsy. EPA agrees.

Based on the submitted information, this study can be upgraded from supplementary to minimum with an NOEL of 750 ppm, and an LEL of 7500 ppm based on decreased body weights and body weight gains, and increased liver weights in males and females.

7. Mutagenicity: Unscheduled DNA synthesis/ Rat hepatocyte in vitro #337-84: July 27, 1984.

EPA review of April 11, 1985 had stated that this study was unacceptable and the following information should be given by Dupont.

a. Clearly define the cytotoxicity parameters,
b. Report historical data generated by photometric analysis,
c. Report correlation data for the photometric analysis vs. standard methods.

Dupont has stated in their response that:

a. Lactate dehydrogenase activity is released into the medium of cultured hepatocytes when the cells are killed or die. This clearly occurred at or above 2.5 mM Harmony. In addition the test material precipitated at or above 2.5 mM in the treatment medium indicating the limit of solubility had been reached. EPA agrees with Dupont that a high enough dose was used.

b. Historical control data are on file at Dupont and are not needed to interpret the results of this study. EPA agrees.

c. Dupont submitted a lengthy explanation of the use of the photometer for the UDS test. Their explanation is adequate.

This study can be upgraded from unacceptable to acceptable.

Dupont pointed out that the March 17, 1987 Toxicology memo neglected to mention several studies that had been submitted by Dupont and evaluated by EPA as adequate. Two studies were on the end use product 75DF. These are:

1. Primary dermal irritation- #HLO-188-84, Accesson No. 072847(9)

2. Primary eye irritation- #HLO-176-84 Accession No. 072847(8)

An acceptable mutagenicity study on the Technical Harmony was omitted from the reference list. This study is listed below:

1. Mutagenicity- In vivo assay for chromosome aberrations in rat bone marrow cells #HLR-302-84, Accession No. 073010.

CONCLUSIONS:

After review of the submitted information from Dupont, the only remaining outstanding issues with Harmony concern the CHO/HPRT

mutagenicity assay and the 2-generation reproduction rat study. All the other issues concerning toxicity studies have been resolved, including the triazine amine issues connected with residues and groundwater.

Stability: The test material was assumed to be stable throughout the test.

D. Atmosphere Generation

Dust atmospheres of INA-4098-4 were generated with a K-Tron® Bin Feeder equipped with twin feed screws. The feed rate was regulated with a K-Tron® Volumetric Feed Controller. The bin feeder metered the test material into a glass transfer tube. A high pressure air stream blew the test material through a cyclone elutriator and into the exposure chamber. The cyclone removed large particles by inertial impaction, while aerodynamic particles passed through the cyclone and into the exposure chamber. Additional dilution air was added to the dust stream prior to entering the exposure chamber. The atmospheric concentration of INA-4098-4 was controlled by varying the feed rate and the two airflows.

E. Analytical

The atmospheric concentration of INA-4098-4 was determined at approximately 30-minute intervals by drawing calibrated volumes of chamber atmosphere through preweighed, Gelman glass fiber filters. Filters were weighed on a Cahn 26 Automatic Electrobalance®. The atmospheric concentration was calculated from the filter weight differential before and after sampling.

Particle size (mass median aerodynamic diameter and percent respirable) were determined with a Sierra® cascade impactor during each exposure. During most exposures, chamber temperature was measured with a mercury thermometer, relative humidity was measured with a Bendix model 566 psychrometer, and chamber oxygen content was measured with a BioMarine model 225 oxygen analyzer.

F. Records Retention

All raw data and the final report will be stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, or in the DuPont Hall of Records, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

Harmony Reviews

Page _____ is not included in this copy.

Pages 8 through 9 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

X Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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

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SUPPLEMENTAL APPENDIX TABLE 1(continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS: TWO-YEAR FEEDING

TESTS	DOSE (PPM)	SAMPLING TIME 3-MONTH	6-MONTH	9-MONTH	12-MONTH
	0.0	145.(1.)[a]	146.(1.)	143.(1.)	144.(1.)
Na	25.0	145.(2.)	147.(3.)	142.(1.)	145.(2.)
mmol/L	500.0	146.(5.)	146.(2.)	141.(1.)*	143.(2.)
	2500.0	145.(1.)	145.(2.)	140.(1.)*	141.(1.)*
	0.0	5.9(0.3)	5.5(0.2)	5.5(0.2)	5.6(0.3)
K	25.0	5.8(0.5)	5.6(0.3)	5.4(0.1)	5.5(0.3)
mmol/L	500.0	5.5(0.3)*	5.8(0.3)#	5.3(0.4)	5.6(0.4)
	2500.0	5.6(0.3)	5.7(0.7)	5.4(0.4)	5.6(0.2)

[a] Group means and standard deviations(SD)
* Significantly different from controls at 5% level by Dunnett criteria
# Significantly different from controls at 5% level by Mann-Whitney U criteria

H-15172
MR-4980
HC-34

INM-6316
RAT
MALE

SUPPLEMENTAL APPENDIX TABLE 1(continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS: TWO-YEAR FEEDING

TESTS	DOSE (PPM)	SAMPLING TIME 18-MONTH	21-MONTH	24-MONTH
Na mmol/L	0.0	136.(1.)a	142.(2.)	142.(4.)
	25.0	136.(2.)	141.(1.)	138.(2.)*
	500.0	135.(5.)#	140.(2.)	138.(2.)*
	2500.0	133.(2.)#	141.(4.)	135.(2.)*
K mmol/L	0.0	4.9(0.3)	5.2(0.8)	4.8(0.6)
	25.0	5.0(0.2)	4.8(0.5)	5.0(0.7)
	500.0	5.0(0.5)	4.8(0.6)	5.0(0.3)
	2500.0	5.1(0.4)	5.1(0.6)	4.9(0.6)

a Group means and standard deviations(SD)
* Significantly different from controls at 5% level by Dunnett criteria
# Significantly different from controls at 5% level by Mann-Whitney U criteria

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Supplemental Appendix II
appended pg 8
Du Pont HLR-261-86

H-15172
MR-4980
HC-34

INM-6316
RAT
FEMALE

SUPPLEMENTAL APPENDIX TABLE 2(continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS: TWO-YEAR FEEDING

TESTS	DOSE (PPM)	SAMPLING TIME 3-MONTH	6-MONTH	9-MONTH	12-MONTH
Na mmol/L	0.0	143.(2.)[a]	144.(2.)	146.(4.)	143.(3.)
	25.0	144.(1.)	144.(1.)	145.(2.)	140.(2.)*
	500.0	143.(2.)	143.(1.)	143.(1.)*	139.(1.)*
	2500.0	144.(1.)	144.(1.)	138.(2.)*	140.(2.)*
K mmol/L	0.0	5.3(0.4)	5.0(0.4)	4.8(0.6)	4.9(0.4)
	25.0	5.2(0.4)	5.0(0.3)	4.8(0.2)	4.7(0.5)
	500.0	5.1(0.2)	4.9(0.4)	5.0(0.3)	5.0(0.5)
	2500.0	5.2(0.4)	4.8(0.3)	4.6(0.5)	4.3(0.4)*

[a] Group means and standard deviations(SD)
* Significantly different from controls at 5% level by Dunnett criteria

Supplemental Appendix II
Appendix pg 9
Du Pont HLR-261-86

H-15172
MR-4980
HC-34

INM-6316
RAT
FEMALE

SUPPLEMENTAL APPPENDIX TABLE 2(continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS: TWO-YEAR FEEDING

TESTS	DOSE (PPM)	SAMPLING TIME 18-MONTH	21-MONTH	24-MONTH
Na mmol/L	0.0	141.(3.)[a]	143.(2.)	138.(1.)
	25.0	137.(2.)*	141.(2.)	137.(2.)
	500.0	134.(2.)*	141.(2.)	136.(2.)
	2500.0	136.(5.)*	139.(2.)*	133.(2.)*
K mmol/L	0.0	4.7(0.7)	4.8(0.6)	4.3(0.4)
	25.0	4.4(0.4)	4.8(0.6)	4.1(0.6)
	500.0	4.6(0.3)	5.0(0.4)	4.4(0.4)
	2500.0	4.8(0.9)	4.8(0.6)	4.3(0.3)

[a] Group means and standard deviations(SD)
* Significantly different from controls at 5% level by Dunnett criteria

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TOXICOLOGY BRANCH: DATA REVIEW

Reviewed by: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

TB Project:

Date: 12/29/87

Thru: Judith W. Hauswirth, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division
12/31/87

CHEMICAL: 1,3,5,-Triazin-2-amine, 4-methoxyl-6-methyl-(INA-4098; Haskell No. 12, 598)

Caswell:
EPA Chem:

STUDY TYPE: Mutagenicity -- Reverse mutation in bacteria (Samonella-Ames Test)

CITATION: Mutagenic Activity in the Salmonella/Microsome Assay

ACCESSION NO.:

MRID:

SPONSOR: E.I. du Pont de Nemours & Co

TESTING LAB.: Haskell Lab. for Toxicology and Industrial Medicine

STUDY NO.: HLR 716-78 (MR NO. 0581-781)

STUDY DATE: November 22, 1978

TB CONCLUSIONS/EVALUATION: ACCEPTABLE. Negative in repeat experiments at concentrations up to 10,000 ug/plate (non-toxic, but limit dose) with and without metabolic activation.

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DETAILED REVIEW

TEST MATERIAL: 1,3,5,-triazin-2-amine, 4-methoxy-6-methyl-,100% (aka Haskell No. 12, 598).

Procedure: In an initial dose-selection cytotoxicity experiment, cultures of Salmonella typhimurium strain TA 1535 were exposed to test material in the absence and presence of the 9000x supernatant of liver homogenate from Aroclor 1254-treated rats (S9), plus generating co-factors (S9 mix).

The mutagenesis assay was performed twice with S. typhimurium strains TA 1535, TA 1537, TA 98, and TA 100 exposed to test article at concentrations of 500, 1000, 2500, 5000 and 10,000 ug/plate, both with and without S 9 mix. Revertent colonies were counted after 48 hour incubation.

Both positive (known mutagens) and negative (solvent) controls were included in each assay.

Data were expressed both as revertents per average of duplicate plate (presented in the report), and revertents/n-mole or ug of test sample (not presented).

Results: In the cytotoxicity test with TA 1535, the test material was stated not to be toxic up to 10,000 ug/plate. [Data, however, for this test were not presented.]

In the two mutagenesis experiments, the highest dose was also 10,000 ug/plate. At no test dose with or without activation in either test were the reversion frequencies significantly increased (twice or more) over the solvent frequency. In contrast, all positive controls responded with highly significant increases in revertents (Report Tables I to IV, attached to this review.

TB Evaluation: Acceptable. Negative in repeat experiment at concentrations up to the limit dose (10,000 ug/plate).

TABLE I

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 98, AND TA 100 WITH METABOLIC ACTIVATION

Trial

Compound Added			Histidine+ Revertants Per Plate*			
			TA 1535	TA 1537	TA 98	TA 100
DMSO			31	6	44	169
12,598		µg/plate				
"	500	"	25	9	40	167
"	1000	"	21	9	37	172
"	2500	"	31	7	35	179
"	5000	"	37	7	42	153
"	10000	"	23	11	33	156
2AA	5	"				2058
"	10	"	1015	867	2218	

DMSO = Dimethylsulfoxide (solvent control)
12,598 = 1,3,5-Triazin-2-amine, 4-methoxy-6-methyl-
2AA = 2-Aminoanthracene (positive control)
" = Average of 2 plates

TABLE II

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 98, AND TA 100 WITHOUT METABOLIC ACTIVATION

Trial I

Compound Added			Histidine+ Revertants Per Plate*			
			TA 1535	TA 1537	TA 98	TA 100
DMSO			27	6	21	158
12,598		µg/plate				
"	500	"	28	9	27	151
"	1000	"	23	5	20	125
"	2500	"	28	5	19	134
"	5000	"	28	10	18	139
"	10000	"	33	5	18	147
MNNG	2	"	2002			2225
9AAc	50	"		1071		
2NF	25	"			2257	

DMSO = Dimethylsulfoxide (solvent control)
12,598 = 1,3,5-Triazin-2-amine, 4-methoxy-6-methyl-
MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine (positive control)
9AAc = 9-Aminoacridine (positive control)
2NF = 2-Nitrofluorene (positive control)
* = Average of 2 plates

TABLE III

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 98, AND TA 100 WITH METABOLIC ACTIVATION

Trial 2

Compound Added			Histidine+ Revertants Per Plate*			
			TA 1535	TA 1537	TA 98	TA 100
DMSO			27	6	31	149
12,598		µg/plate				
"	500	"	30	8	26	169
"	1000	"	22	6	34	175
"	2500	"	29	7	33	174
"	5000	"	29	10	28	158
"	10000	"	24	10	27	154
2AA	5	"				1918
"	10	"	1032	631	2147	

DMSO = Dimethylsulfoxide (solvent control)
12,598 = 1,3,5-Triazin-2-amine, 4-methoxy-6-methyl-
2AA = 2-Aminoanthracene (positive control)
" = Average of 2 plates

TAB E IV

MUTAGENIC ACTIVITY IN *SALMONELLA TYPHIMURIUM* STRAINS TA 1535, TA 1537, TA 98, AND TA 100 WITHOUT METABOLIC ACTIVATION

Trial 2

Compound Added			Histidine+ Revertants Per Plate*			
			TA 1535	TA 1537	TA 98	TA 100
DMSO			23	9	18	126
12,598		µg/plate				
"	500	"	23	8	18	131
"	1000	"	31	12	16	132
"	2500	"	26	8	18	140
"	5000	"	22	8	25	134
"	10000	"	28	8	18	123
MNNG	2	"	2682			2732
9AAc	50	"		1535		
2NF	25	"			2236	

DMSO = Dimethylsulfoxide (solvent control)
12,598 = 1,3,5-Triazin-2-amine, 4-methoxy-6-methyl-
MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine (positive control)
9AAc = 9-Aminoacridine (positive control)
2NF = 2-Nitrofluorene (positive control)
* = Average of 2 plates

Reviewed by: Marcia van Gemert, Ph.D. M. van Gemert 1/29/88
Head, Section III, Tox. Branch (TS-769C)
Secondary Reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch,(TS-769C) 2/2/88

DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: 1,3,5 Triazin -2-amine,4-methoxy-6-methyl

SYNONYMS: triazine amine

STUDY NUMBER: 6316/Tox 2

SPONSOR: Dupont

TESTING FACILITY: Haskell Laboratories

TITLE OF REPORT: HLR-27-80 acute oral LD_{50} toxicty test in rats

AUTHORS: G. Kennedy

REPORT ISSUED: Jan. 25, 1980

CONCLUSIONS: LD_{50} was calculated to be 1680 mg/kg. Prominant symptoms included stained and wet face and perineal area, salivation, diarrhea, prostration, and severe weight loss.

CLASSIFICATION: Core minimum although only male animals were used.

A. MATERIALS:

1. Test Compound: Triazine Amine
   Description: not given
   Batch #: 12,598
   Purity: 98%

2. Test Animals:
   Species: rats, male
   Strain: Chr-CD
   Age: young adults
   Weight: not given
   Source: not given.

Study Design: 10 males/dose were given single doses by intragastric intubation. Surviving animals were weighed and observed during a 14-day recovery period and sacrificed. Two surviving animals/ group were given gross necropsy.

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Results: According to the study text the following symptoms were evident:

Dose	Mortality	Clinical signs
3000	10/10	stained face and perineal area, eyes half-closed, diarrhea, congestion, weakness, lethargy, prostration, moribundity and severe weight loss. All mortalities occurred within 5 days after dosing.
2500	10/10	Stained and wet perineal area, stained face, eyes half-closed, salivation, lethargy, prostration, and severe weight loss. All mortalities occurred within 5 days after dosing.
2000	6/10	Stained and wet perineal area, eyes half-closed, lacrimation, salivation, ataxia, weakness, prostration, and severe weight loss. All mortalities occurred within 4 days post-dosing
1400	3/10	Stained perineal area, diarrhea, weakness, lethargy, prostration and severe weight loss. All mortalities occurred on the 3rd day post-dosing.

Gross Pathology: one rat at 1400 and 1 rat at 2000 mg/kg had slightly heavy livers.

Conclusions: LD_{50} is calculated to be 1680 mg/kg.

Reviewed by: Marcia van Gemert, Ph.D. M. van Gemert 1/29/88
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T. Farber 2/2/88

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation study in rats TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: INA-4098

SYNONYMS: Triazine Amine

STUDY NUMBER: HLR-288-85

SPONSOR: Dupont

TESTING FACILITY: Haskell Laboratories, Newark, Delaware

TITLE OF REPORT: Inhalation median lethal concentration (LC_{50}) of INA-4098-4

AUTHORS: C. Hutt

REPORT ISSUED: June 7, 1985

CONCLUSIONS: LC_{50} > 5.0 mg/l
LC_{50} could not be calculated due to the low order of toxicity by inhalation.

CLASSIFICATION: core minimum, although only male rats were used.

A. MATERIALS:

1. Test Compound: INA-4098-4, Triazine amine
   Description: white solid
   Batch #: 4098-4
   Purity: 95%

2. Test Animals:
   Species: rats, male
   Strain: Crl-CD (SD) Br
   Age: 8 weeks old
   Weight: 229-273 gms
   Source: Charles River Breeding Laboratories, Kingston N.Y.

Study design:
10 rats/group were restrained in perforated stainless steel cylinders with conical nose pieces. Exposure was for a single 4-hour period nose only. Rats were weighed prior to exposure and observed during exposure. Surviving rats were weighed and observed daily for 14 days post exposure, weekends and holidays excluded. Atmosphere generation and analytical methodology are on appended page 1.

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Results:

Concns (mg/l)	Mortality (deaths/# exposed)
2.6	0/10
2.7	3/10
3.2	6/10
4.6	7/10
5.0	4/10

Chamber temperatures ranged from 23-25°C, relative humidity ranged from 21-61%, oxygen content was 21%. Because of the low number of deaths with 5.0 mg/l the LC_{50} could not be calculated.

Clinical signs-

During the exposure to 2.6 mg/l rats had a lessened startle response with faces stained with the test material. At higher doses the rats could not be seen during exposure because test material coated the chamber. After exposure some rats in all groups were lethargic and had dry red nasal or ocular discharges and partially closed eyes. Most deaths occurred 1-2 days post exposure although 2 rats exposed to 3.2 mg/l died 4 days post exposure. Significant weight loss was noted in all surviving rats, losing 10-16% of body weight one day post exposure. Some rats continued to lose weight up to day 4 post exposure. Labored breathing and lung noises were noted with wet or stained perineum, diarrhea and hunched posture. At doses greater than 2.5 mg/l there was lethargy, limpness and no righting reflex seen, with partially closed eyes and discolored fur. These signs were evident days 1-3 post exposure.

Conclusions:
LC_{50} could not be calculated due to the low order ot toxicity seen by inhalation.

$LC_{50} > 5.0$ mg/l

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DATA EVALUATION REPORT

STUDY TYPE: Rangefinding Acute LD_{50} in rats TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: 1,3,5,-triazin-2-amine, 4-methoxy-6-methyl

SYNONYMS: triazine amine

STUDY NUMBER: 6316/tox-2

SPONSOR: Dupont

TESTING FACILITY: Haskell Laboratories

TITLE OF REPORT: HLR-674-78 Acute oral toxicity test in rats

AUTHORS: M. Kaplan

REPORT ISSUED: Nov. 10, 1978

CONCLUSIONS: This was a quick dose-range-finding study to set the doses for an LD_{50}. The LD_{50} from this study was calculated to be approximately 1500 mg/kg.

CLASSIFICATION: Supplementary

A. MATERIALS:

1. Test Compound: Triazine Amine
   Description: not given
   Batch #: 12,598
   Purity: 98%

2. Test Animals:
   Species: rats
   Strain: Chr-CD
   Age: adult male
   Weight: not given
   Source: not given

Study design:
The test material was suspended in acetone/corn oil at 15% and given by gavage in a single dose per animal/dose level. Survivors were held for 14 days and sacrificed.

Doses tested were 5000, 3400, 2250, 1500, 1000, 670 mg/kg with 1 animal/dose administered the test material.

Results: One animal died at 5000, 3400, 2250 and 1500 mg/kg. The animals tested at the 1000 and 670 mg/kg dose levels survived to day 14.

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Clinical signs:

Lethal doses: the study reported "eyes half-closed, salivation, belly-to-cage posture, rapid and labored breathing, stained perineal area and face, nose shoveling, lethargy and moribundity."

Nonlethal doses: weight loss for 3-4 days after dosing at 1000 and 670 mg/kg.

Discussion:
The study states that the LD_{50} is approximately 1500 mg/kg. However, inadequate numbers of animals were used. This was a rangefinding LD_{50} and was used to set the doses for the LD_{50} study.

The LD_{50} was calculated to be around 1500 mg/kg.

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DATA EVALUATION REPORT

STUDY TYPE: Acute dermal LD_{50} TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: 1,3,5-triazine-2-amine,4-methoxy-6-methyl

SYNONYMS: triazine amine

STUDY NUMBER: 62-81, 6316-tox 2

SPONSOR: Dupont

TESTING FACILITY: Haskell Laboratories

TITLE OF REPORT: HLR-62-81. Rabbit skin absorption- Approximate Lethal dose (ALD)
AUTHORS: L. Silber

REPORT ISSUED: Feb. 20, 1981

CONCLUSIONS: Dermal LD_{50} > 5000 mg/kg
is considered nontoxic by the dermal route
Toxicology category IV

CLASSIFICATION: supplementary, only male animals were used, and only 6 animals/group were used.

A. MATERIALS:

1. Test Compound: triazine amine
   Description: not given
   Batch #: 12,598
   Purity: 98%

2. Test Animals:
   Species: rabbits, males
   Strain: New Zealand
   Age: not given
   Weight: 2.1-3.3 kg.
   Source: not given

Study design:
6 males/group were shaved over the back and trunk areas and fitted with plastic collars. Test doses were applied to the back under a 3" by 3" 12-ply gauze pad opened to full length. The rabbits trunks were then wrapped with plastic wrap, stretch gauze bandage and elastic adhesive tape. After 24 hours exposure the wraps were removed and test site washed with water and dried. Observation was for 14 or 15 days prior to sacrifice.

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Results:

# rabbits tested	Dose mg/kg	Gms	Weight (gms) initial	final	Mortality
6	5000	13.54	2707	3028	0
6	3400	9.89	2908	3125	0
6	2250	5.91	2628	2919	0

Symptoms:

5000 mg/kg: slight erythema at 1-2 days, slight edema at 1 day, scaling 5-9 days.
3400 mg/kg: slight erythema at 1-3 days, slight edema at 1 day scaling at 8 days
2250 mg/kg: slight erythema 1-4 days, slight edema at 1 day.

Conclusions:
Dermal LD_{50} > 5000 mg/kg
Considered nontoxic by the dermal route
Tox category IV
Core classification- Supplementary, only male animals were used and only 6/group were tested.

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DATA EVALUATION REPORT

STUDY TYPE: Skin Irritation and Sensitization in Guinea Pigs TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: 1,3,5-triazin-2-amine-4-methoxy-6-methyl

SYNONYMS: triazine amine

STUDY NUMBER: 694-78

SPONSOR: Dupont

TESTING FACILITY: Haskell Laboratory, Newark Deleware

TITLE OF REPORT: HLR- 694-78 primary skin irritation and sensitization test in guinea pigs

AUTHORS: R.L. Ferens

REPORT ISSUED: Dec. 1, 1978

CONCLUSIONS: No skin sensitization was seen on intact guinea pigs skin with either a 3 or 30% suspension in dimethyl phthalate. However, the material did cause some skin irritation.

CLASSIFICATION: Minimum, although positive control data on the test material would be appropriate.

A. MATERIALS:

1. Test Compound: Triazine amine
   Description: not given
   Batch #: 12,598
   Purity: 98%

2. Test Animals:
   Species: Guinea pigs
   Strain: albino, not given
   Age: not given
   Weight: not given
   Source: not given

Study Design:

A range-finding study for dermal irritation was conducted with 3 male albino guinea pigs. The highest dose of 30% suspension in dimethyl phthalate didn't cause skin irritation. In the main study, 10 guinea pigs (sex unspecified) were exposed to 3% or 30% suspension in dimethyl phthalate which was lightly rubbed onto the shaved intact skin of the shoulder.

The induction phase for sensitization consisted of a series of 4 sacral intradermal injections of 0.1 ml of a 1.0% solution in

dimethyl phthalate once per week beginning day 2 post primary irritation application. After a 13 day rest, test animals were challenged for sensitization by applying a lightly rubbing in 0.05ml. of a 30% and a 3% suspension on shaved intact shoulder skin. 10 unexposed guinea pigs served as controls, receiving the identical topical treatments.

Results:

Control animals # animals	Initial weights	Final weights
10	517 gms	714 gms
Test Animals		
10	514 gms	719 gms

Skin reaction

		Control		Experimental	
		30%	3%	30%	3%
Primary Irrit. test	24h			10 neg.	10 neg
	48h			10 neg	10 neg
Challenge test	24h	10 neg	10 neg	1+, 9 neg	10 neg
	48h	10 neg	10 neg	1+, 9 neg	10 neg

No sensitization occurred with a 3 or 30% suspension in DMP. However, the material can cause some skin irritation.

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DATA EVALUATION REPORT

STUDY TYPE: Eye irritation in rabbits TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: 1,3,5-Triazin-2-amine,4-methoxy-6-methyl

SYNONYMS: triazine amine

STUDY NUMBER: 6316/tox-2, HLR-675-78

SPONSOR: Dupont

TESTING FACILITY: Haskall Laboratories

TITLE OF REPORT: HLR -675-78 Eye irritation test in rabbits

AUTHORS: R.L. Ferenz

REPORT ISSUED: Nov. 10, 1978

CONCLUSIONS: Test compound was mildly irritating to both washed and unwashed eyes causing slight corneal opacity and mile conjunctival irritation. The iris was not involved and eyes returned to normal within 7 days.

CLASSIFICATION: Core supplementary, at least 6 animals should be used, only one animal with unwasyed test compound was used.

A. MATERIALS:

1. Test Compound: Triazine amine
   Description: not given
   Batch #: 12,598
   Purity: 98%

2. Test Animals:
   Species: Rabbits
   Strain: albino, no strain given
   Age: not given
   Weight: not given
   Source: not given

Study design:

The right conjunctival sac of the eyes of two rabbits were treated with 10 mg of solid triazine amine. 20 seconds post treatment one treated eye was washed with tap water for 1 minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris and conjunctiva were made with a hand-slit lamp at one and four hours, and at one, two, three, seven and 14 days; Fluor-i-strip stain and a biomicroscope were used at examinations after the day of treatment.

Results:

Treatment	Ocular effects		
	Cornea	Iris	conjunctiva
unwashed	small area of slight opacity 1-2 days		Redness: mild 1-4 hrs Swelling: slight at 4 hrs Discharge: mild 4 hrs-1 day
Washed	questionable opacity 1-4 hrs; moderate area of slight opacity at 1 day receding to a small area 2-3 days		Redness: mild 1 hr-1 day Swelling: none Discharge: none

Conclusions: the test compound was mildly irritating to both washed and unwashed eyes causing slight corneal opacity and mild conjunctival irritation. The iris was not involved and eyes returned to normal within 7 days.

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DATA EVALUATION REPORT

STUDY TYPE: Subacute oral test in rats TOX CHEM NO: 573S

ACCESSION NUMBER: MRID NO: 403403-20

TEST MATERIAL: 1,3,5-triazin-2-amine,4-methoxy-6-methyl

SYNONYMS: triazine amine

STUDY NUMBER: HLR-129-79

SPONSOR: Dupont

TESTING FACILITY: Haskell Laboratories

TITLE OF REPORT: HLR-129-79 Ten-dose oral subacute test in rats

AUTHORS: R.L. Trivits

REPORT ISSUED: April 20, 1979

CONCLUSIONS: Only one dose group (300 mg/kg) was used with 6 male rats/group used. Animals were dosed for 14 days with 10 doses one/day excluding weekends. No mortality occurred, however, liver and heart appear to be target organs. NOEL < 300 mg/kg

CLASSIFICATION: Supplementary

A. MATERIALS:

1. Test Compound: Triazine amine
   Description: not given
   Batch #: 12598
   Purity: 98%

2. Test Animals:
   Species: rats, male
   Strain: Chr-CD
   Age: Young adult, age unspecified
   Weight: not given
   Source: not given

Study design:

Six/group were given 300 mg/kg/day in a 2% suspension of triazine amine in acetone/corn oil 15:85 intragastrically 5 times/week for 2 weeks. 6 rats served as controls and were intubated with vehicle (acetone/corn oil, 15?85) only. Animals were weighed daily (weekends excluded) and observed for clinical signs. Four hours post 10th dose three control and 3 test rats were sacrificed. The remaining animals were examined grossly, selected tissues were weighed and some tissues and organs were evaluated histologically.

Those that were examined histologically included:

Liver*
Kidney*
heart
Spleen*
brain
adrenal glands
stomach,
small and large intestines,
pancreas
lungs
mediastinal tissue,
trachea
thyroids
thymus*
sternum with bone marrow
eyes
abdominal skin
testes*
epididymus

* = weighed also

Results:

Dose mg/kg	# of doses	mortality	clinical signs
300	10	0/6	First week: stained face, wet stained perineal area, stained underside, unkempt fur, stained head, weakness and moderate weight loss (around 10% from initial body weight) Second week: Unkempt fur, weakness, humped posture and moderate weight loss (around 15% from initial body weight) Recovery period: Piloerection, humped posture and stained nose (body weight gain greater than in the controls)

Pathology:

Heart and liver appeared to be the target organs- 3/3 rats exhibited myocardial congestion with focal edematous interstitium, myocardial degeneration and myocarditis. 1/3 rats had periportal hypertrophic hepatocytes of the liver. Hematopoetic alterations (small constricted red pulps, devoid of extramedullary hemopoiesis in the spleen, hyperplastic bone marrow with fatty replacement, atrophic thymus with lymphocytic depletion) were observed and are conjectured to be due to stress or secondary to the cardiovascular effects.

During the recovery period, all effects disappeared by 14 days in the recovery group.

Conclusion: Only one dose was used, which was considered toxic to both heart and liver. the NOEL is < 300 mg/kg Core supplementary

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