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Re: TSCA Section 8(e) Notification

Dear Sir or Madam:

ChemFirst Inc is submitting this notice in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's Statement of Interpretation and Enforcement Policy, 43 Fed. Reg. 1110 (March 16, 1978).

The basis for this submission is an acute oral toxicity study in rats of N-ethyl-meta-toluidine (NEM), CAS no. 102-27-2. This study was sponsored by ChemFirst Inc and a copy of the report is attached. Rats were given single oral doses of 100, 500, 750, or 1000 mg/kg. Cyanosis was observed in all rats except those in the 100 mg/kg group and one rat in the 500 mg/kg group. The acute oral median lethal dose (LD₅₀) was calculated to be 787 mg/kg.

Exposure is minimized during production and handling of NEM by the use of recommended protective suits, neoprene gloves, and a full-face respirator with organic vapor/acid gas cartridges. Protective clothing and equipment are specified on the Material Safety Data Sheet. NEM is produced by First Chemical Corporation, a ChemFirst Inc. Company, for use as a cure accelerator in polymerizations. The information from this study will be included in the Material Safety Data Sheet for NEM.

Sincerely,

Ellen R. Stephens, Ph.D. DABT
Manager, Toxicology
601-938-2219

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**ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS**

FINAL REPORT

Testing Facility:

Submitted to:

**ChemFirst, Inc.
1001 Industrial Road
Pascagoula, MS 39581-3237**

Attention:

June 1997

ACUTE ORAL TOXICITY STUDY OF N-ETHYL-META-TOLUIDINE IN RATS

Study Initiation Date: March 19, 1997
Treatment Initiation Date: April 2, 1997
Biophase Termination Date: May 21, 1997

SUMMARY

N-Ethyl-meta-Toluidine was administered as a solution in corn oil by oral gavage to four groups of five male and five female Sprague-Dawley rats at doses of 100, 500, 750 and 1000 mg/kg of body weight. The surviving rats were observed for 14 days after test substance administration.

Mortality incidences in the 100, 500, 750 and 1000 mg/kg dose groups were 0/10, 0/10, 3/10 and 9/10, respectively. The acute oral median lethal dose (LD₅₀) of N-Ethyl-meta-Toluidine in male and female rats was calculated to be 787 mg/kg with 95% Confidence Limits of 585 to 1058 mg/kg.

All mortalities except three occurred on days 2 and 3 following dosing. Cyanosis was observed in all rats except those in the 100 mg/kg group and one rat in the 500 mg/kg group. Two rats in the 100 mg/kg group and one in the 500 mg/kg group exhibited no clinical signs for the duration of the study. Other clinical signs observed during the study consisted of salivation, hypoactivity, prostration, ataxia, convulsions, wet/discolored inguinal fur, discoloration around the mouth, clear/red nasal discharge, redness around nose and eyes, discolored paws, alopecia, necrotic tail, cold to touch, pale, dyspnea, irritability, dark/red urine, vocalization, rough hair coat and diarrhea. All rats in the 100 mg/kg dose groups appeared to be normal by day 2. One rat in the 500 mg/kg group and four in the 750 mg/kg group appeared pale through days 6 and 7, respectively, at which time the animals were clear of all clinical signs and remained so for the duration of the study. One surviving rat in the 1000 mg/kg dose group exhibited alopecia on the abdomen/inguinal area that remained present to the end of the study. All surviving rats gained weight during the study. No gross necropsy findings were observed in the 100 and 500 mg/kg groups, while necropsy findings in rats in the 750 and 1000 mg/kg groups consisted of red foci on lungs, red/dark/brown lungs, mottled lungs, dark/brown liver (pale liver in one animal), dark brown heart, dark brown spleen (small spleen in one animal), red surface stomach foci, red fluid in the urinary bladder, foci on thymus, dark brown kidneys, dark brown adrenals and yellow subcutaneous tissues and yellow ovaries in one animal.

STUDY PARTICIPANTS:

This report was prepared by

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ACUTE ORAL TOXICITY STUDY OF N-ETHYL-META-TOLUIDINE IN RATS

I. INTRODUCTION

The purpose of this study was to determine the acute toxicity of N-Ethyl-meta-Toluidine in rats following a single oral dose.

II. MATERIALS AND METHODS

- A. Test Substance: N-Ethyl-meta-Toluidine (NEM), CAS No. 102-27-2, was received March 5, 1997. The test substance was a clear liquid and was stored in the original container at room temperature. The Material Safety Data Sheet (MSDS) indicated that the test substance is stable. Complete characterization and attendant documentation of the stability and purity of the test substance were the responsibility of the Sponsor. The corn oil vehicle (Sigma Chemical Co., St. Louis, MO; Lot No. 125H0598) was a purchased product and, as such, was considered characterized by its labelling.
- B. Dosage Formulation: Solutions of the test substance were prepared in corn oil at 1, 5, 7.5 and 10% (w/v) concentrations for the 100, 500, 750 and 1000 mg/kg dose level groups, respectively. The doses were administered at a constant dosing volume of 10 ml/kg of body weight. Test substance/corn oil formulations were prepared on the day of dosing for the respective dose groups.
- C. Animals: Male and female Sprague-Dawley rats (CrI:CD^RBR), approximately six weeks of age, were purchased for use in this study. The animals used in the 1000 mg/kg dose group were received on March 19, 1997 and animals used in the 100 and 500 mg/kg dose groups were received March 26, 1997. The body weights of a random sample (approximately 36%) from the March 26 shipment ranged from 134 to 150 g the day following receipt. The animals used in the 750 mg/kg dose group were received on April 23, 1997 and their body weights ranged from 137 to 154 g the next day. The rats were held in quarantine for approximately 2 weeks during which time they were observed daily for survival and at the end of the quarantine period were examined carefully to ensure their health and suitability as test subjects. Rats selected for the study were identified by a uniquely numbered metal tag inserted through the pinna of the right ear and by a cage card bearing the corresponding identification number.

- D. Food and Water: Certified Purina Rodent Chow 5002 (PMI Feeds, Inc., St. Louis, MO) was provided *ad libitum*, except for approximately 18 - 19.5 hours immediately prior to dosing and approximately 4 hours after dosing. municipal water was available *ad libitum* by means of an automatic watering system.
- E. Environment: The rats were housed individually in stainless steel wire mesh cages suspended over absorbent animal cage boards. The animal room temperature and relative humidity ranged from 21.0 to 25.0°C and 35 to 70%, respectively, during the treatment phase of the study. Fluorescent lighting was provided for 12 hours followed by 12 hours of darkness.
- F. Methods:
1. Assignment to Groups: Rats selected for testing were assigned at random to groups of five males and five females each. There was no control group.
 2. Fasting: Approximately 18 - 19.5 hours prior to test substance administration, all food was removed from the cages and the test animals fasted until approximately 4 hours after dosing.
 3. Dosing: The test substance was administered in solution in corn oil at doses of 100, 500, 750 and 1000 mg/kg of body weight via oral gavage using a syringe equipped with a stainless steel, ball-tipped intubation needle. Test substance was administered to the 1000 mg/kg dose group on April 2, 1997, the 100 and 500 mg/kg dose groups on April 9, 1997, and the 750 mg/kg group on May 7, 1997.
 4. Clinical Observations: Study animals were observed at frequent intervals on the day of dosing and at least once per day for the balance of the 14-day observation period.
 5. Body Weights: All study animals were weighed prior to fasting (randomization weights), immediately prior to dosing (fasted weights) and at death (carcass weight). Dosage calculations were based upon the fasted body weights. Rats were also weighed 7 and 14 days following test substance administration.
 6. Necropsies: All surviving test rats were euthanized by carbon dioxide asphyxiation at the end of the observation period [*i.e.*, April 16, 1997 (1000 mg/kg dose group), April 23, 1997 (100 and 500 mg/kg dose groups), and May 21, 1997 (750 mg/kg

dose group)]. A gross necropsy was performed on all animals which died during the study and on all animals which were sacrificed at the end of the observation period.

7. LD₅₀ Calculation: The acute oral LD₅₀ and associated 95% Confidence Limits for the combined sexes were calculated using an in-house developed computerized program incorporating the probit method of Miller and Tainter, *Proceedings of the Society for Experimental Biology and Medicine*, 57: 261-264, 1944.

- G. Archives: Raw data generated during the study and a copy of the final report will be retained in the archives for one year, at which time the Sponsor will be consulted to determine final disposition.

III. RESULTS

- A. Mortality: Mortality incidences are summarized in Table 1. None of the rats in the 100 and 500 mg/kg dose groups died. Three of ten rats in the 750 mg/kg dose group and nine of ten rats in the 1000 mg/kg dose group died. None of the deaths occurred on the day of dosing. Three of the twelve deaths occurred on day 2, while six occurred on day 3. The remaining three deaths occurred on days 4, 5 and 7.
- B. Clinical Observations: Clinical signs observed during the study are summarized in Table 2 and individual clinical observations are given in Table 3. Cyanosis was observed in all rats, except those in the 100 mg/kg group and one rat in the 500 mg/kg group; this clinical sign was observed on the day of dosing and continued through day 5 in two rats from the 1000 mg/kg group. Other clinical signs included salivation, hypoactivity, prostration, ataxia, convulsions, wet/discolored inguinal fur, discoloration around the mouth, clear/red nasal discharge, redness around nose and eyes, discolored paws, rough hair coat, cold to touch, pale, dyspnea, irritable, dark/red urine, vocalization, necrotic tail, alopecia and diarrhea. All rats in the 100 mg/kg dose group were clear of all clinical signs by day 2. One female rat in the 500 mg/kg group and four in the 750 mg/kg group appeared pale through days 6 and 7, respectively, at which time the animals were clear of all clinical signs and remained so for the duration of the study. One surviving rat in the 1000 mg/kg group exhibited alopecia on the abdomen/inguinal area that remained present to the end of the study.
- C. Body Weights: Body weight data are summarized in Table 4. All surviving rats in the 100, 500, 750 and 1000 mg/kg groups gained weight during the study.

D. Gross Pathology: A summary of gross necropsy observations is provided in Table 5. No gross lesions were observed in any of the rats in the 100 and 500 mg/kg dose groups. Necropsy observations for rats in the 750 and 1000 mg/kg groups included red foci on lungs, red/dark/brown lungs, mottled lungs, dark/brown liver (pale liver in one animal), dark/brown heart, dark/brown spleen (small spleen for one animal), external surface stomach foci, red fluid in urinary bladder, foci on thymus, dark brown kidneys, dark brown adrenals and yellow subcutaneous tissues and yellow ovaries in one animal.

IV. EVALUATION

Based on the results of this study, the acute oral median lethal dose (LD_{50}) of N-Ethyl-meta-Toluidine in male and female rats was calculated to be 787 mg/kg with 95% Confidence Limits of 585 to 1058 mg/kg (Table 6).

V.

VI. TABLES

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 1

Summary of Mortality Data
(5 rats/sex/group)

Dose Group	Mortality Incidence		
	Males	Females	Combined
100 mg/kg	0	0	0
500 mg/kg	0	0	0
750 mg/kg	1	2	3
1000 mg/kg	5	4	9

**ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS**

TABLE 2

Summary of Clinical Observations
(5 rats/sex/group)

<u>Observation</u>	<u>Incidence</u>							
	<u>100 mg/kg</u>		<u>500 mg/kg</u>		<u>750 mg/kg</u>		<u>1000 mg/kg</u>	
	<u>M^a</u>	<u>F^b</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
No signs observed	5	5	5	5	5	5	5	5
Found dead	-- ^c	--	--	--	1	2	5	4
Cyanosis	--	--	5	4	5	5	5	5
Prostrate	--	--	--	--	--	--	--	1
Hypoactive	--	--	1	1	3	5	5	5
Ataxia	--	--	--	--	--	--	1	--
Wet inguinal fur	--	4	1	2	1	1	1	4
Discoloration around mouth	--	--	1	--	1	1	3	2
Clear nasal discharge	--	--	--	--	1	--	--	--
Redness around nose	--	--	3	2	2	5	2	3
Discolored paws	--	--	--	--	1	4	2	3
Discolored inguinal fur	--	1	1	1	--	3	2	3
Rough hair coat	--	--	--	2	1	1	1	2
Diarrhea	4	2	1	--	--	--	--	1
Vocalization	--	--	--	--	--	4	--	--
Convulsions	--	--	--	--	1	--	--	--
Salivation	--	--	--	--	1	--	--	--
Cold to touch	--	--	1	--	1	--	2	1
Redness around eyes	--	--	1	--	--	--	--	--
Pale	--	--	--	1	2	4	--	1
Dark/red urine	--	--	--	1	1	3	--	--
Irritable	--	--	--	--	--	--	1	--
Dyspnea	--	--	--	--	1	--	2	1
Necrotic tail	--	--	--	--	1	--	--	1
Red nasal discharge	--	--	--	--	1	--	--	--
Alopecia	--	--	--	--	--	--	--	1

^a M = Male

^b F = Female

^c -- = Zero incidence

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3

Individual Clinical Observations - 100 mg/kg group

MALES

ANIMAL NUMBER

Day	<u>481</u>	<u>482</u>	<u>483</u>	<u>484</u>	<u>485</u>
1	Diarrhea	Diarrhea	No signs observed	Diarrhea	Diarrhea
2-14	No signs observed				

FEMALES

ANIMAL NUMBER

Day	<u>486</u>	<u>487</u>	<u>488</u>	<u>489</u>	<u>490</u>
1	Diarrhea Wet inguinal fur	Wet inguinal fur	Wet inguinal fur	Diarrhea Wet inguinal fur Discolored inguinal fur	No signs observed
2-14	No signs observed	No signs observed	No signs observed	No signs observed	No signs observed

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 500 mg/kg group

MALES

ANIMAL NUMBER

<u>Day</u>	<u>491</u>	<u>492</u>	<u>493</u>	<u>494</u>	<u>495</u>
1	Cyanosis Cold to touch Hypoactive Redness around eyes Redness around nose	Cyanosis Diarrhea Wet inguinal fur Discolored inguinal fur Redness around nose Discoloration around mouth	Cyanosis Redness around nose	Cyanosis	Cyanosis
2	Redness around eyes	Wet inguinal fur Discolored inguinal fur	No signs observed	No signs observed	No signs observed
3-14	No signs observed	No signs observed	No signs observed	No signs observed	No signs observed

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 500 mg/kg group

FEMALES

ANIMAL NUMBER

Day	<u>496</u>	<u>497</u>	<u>498</u>	<u>499</u>	<u>500</u>
1	Cyanosis Wet inguinal fur Rough hair coat Redness around nose	Cyanosis Hypoactive Rough hair coat Redness around nose Wet inguinal fur	Cyanosis	No signs observed	Cyanosis
2	Cyanosis Wet inguinal fur Discolored inguinal fur	No signs observed	No signs observed	No signs observed	No signs observed
3	Rough hair coat Redness around nose Pale Red urine	No signs observed	No signs observed	No signs observed	No signs observed
4	Pale	No signs observed	No signs observed	No signs observed	No signs observed
5	No signs observed	No signs observed	No signs observed	No signs observed	No signs observed
6	Pale	No signs observed	No signs observed	No signs observed	No signs observed
7-14	No signs observed	No signs observed	No signs observed	No signs observed	No signs observed

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 750 mg/kg group

MALES

ANIMAL NUMBER

Day	<u>661</u>	<u>662</u>	<u>663</u>	<u>664</u>	<u>665</u>
1	Cyanosis Salivation Clear nasal discharge	Cyanosis Hypoactive	Cyanosis	Cyanosis Discoloration around mouth Discolored paws	Cyanosis Red nasal discharge
2	Cyanosis	Cyanosis	Cyanosis	Cyanosis Convulsions Discoloration around mouth Hypoactive Discolored paws Cold to touch Redness around nose Wet inguinal fur Dyspnea	Cyanosis
3	Cyanosis Hypoactive Redness around nose	No signs observed	No signs observed	Death	Cyanosis Dark urine
4	Hypoactive Redness around nose Pale	No signs observed	No signs observed	-- ^a	Dark urine Pale
5	Pale Rough hair coat	No signs observed	No signs observed	--	Dark urine
6-7	Pale Tail (tip) necrotic	No signs observed	No signs observed	--	No signs observed
8-14	No signs observed	No signs observed	No signs observed	--	No signs observed

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 750 mg/kg group

Day	FEMALES				
	666	667	668	669	670
1	Cyanosis	Cyanosis	Cyanosis Redness around nose	Cyanosis Discolored paws Hypoactive Redness around nose	Cyanosis Discolored paws Redness around nose
2	Cyanosis Redness around nose	Cyanosis Redness around nose Discolored paws	Cyanosis Discolored paws	Cyanosis Discolored paws Redness around nose Wet inguinal fur Discoloration around mouth	Cyanosis Discolored paws Redness around nose Hypoactive Discolored inguinal fur
3	Cyanosis Hypoactive Redness around nose Rough hair coat Dark urine Vocalization	Cyanosis Hypoactive Vocalization	Cyanosis Redness around nose Hypoactive Vocalization Discolored paws	Cyanosis Redness around nose Discolored paws Discolored inguinal fur Vocalization	Death
4	Hypoactive Redness around nose Dark urine Vocalization Pale	Pale Redness around nose Dark urine Vocalization	Pale Redness around nose Discolored paws Discolored inguinal fur Vocalization	Pale Redness around nose Discolored paws Discolored inguinal fur Vocalization	.. ^a
5	Redness around nose Vocalization Pale	Vocalization Pale	Death	Dark urine Pale	--
6-7	Vocalization Pale	Pale	--	Pale	--
8-14	No signs observed	No signs observed	--	No signs observed	--

^a .. = animal died; no data

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 1000 mg/kg group

MALES

ANIMAL NUMBER

Day	<u>581</u>	<u>582</u>	<u>583</u>	<u>584</u>	<u>585</u>
1	Cyanosis Hypoactive Cold to touch Discoloration around mouth	Cyanosis Hypoactive Irritable	Cyanosis Hypoactive Cold to touch Discoloration around mouth	Cyanosis Hypoactive Wet inguinal fur Discolored inguinal fur Discolored paws Discoloration around mouth	Cyanosis Hypoactive Redness around nose Discolored paws
2	Cyanosis	Death	Cyanosis Redness around nose Discoloration around mouth Ataxia Discolored inguinal fur Dyspnea Death	Cyanosis Hypoactive Discolored paws	Cyanosis Hypoactive Redness around nose Discolored paws
3	Cyanosis Rough hair coat Hypoactive Dyspnea Death	-- ^a	--	Death	Death
4-14	--	--	--	--	--

^a -- = animal died; no data

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 1000 mg/kg group

FEMALES

ANIMAL NUMBER

Day	586	587	588	589	590
1	Cyanosis Hypoactive	Cyanosis Hypoactive Wet inguinal fur Discolored inguinal fur Discolored paws Redness around nose Discoloration around mouth	Cyanosis Hypoactive Wet inguinal fur Discolored inguinal fur Discolored paws	Cyanosis Hypoactive Wet inguinal fur Discolored inguinal fur	Cyanosis Hypoactive Discoloration around mouth Discolored paws
2	Cyanosis Hypoactive Wet inguinal fur Redness around nose	Cyanosis Hypoactive Wet inguinal fur Discolored paws	Death	Cyanosis	Cyanosis Redness around nose Discoloration around mouth Discolored paws
3	Death	Cyanosis Hypoactive Discolored inguinal fur Rough hair coat	-- ^a	Cyanosis	Cyanosis Redness around nose Discoloration around mouth Discolored paws Rough hair coat
4	--	Cyanosis Hypoactive Discolored inguinal fur Rough hair coat Diarrhea Dyspnea	--	Cyanosis	Death
5	--	Cyanosis Hypoactive Discolored inguinal fur Rough hair coat Diarrhea Dyspnea	--	Cyanosis	--

^a -- = animal died; no data

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 3 (cont.)

Individual Clinical Observations - 1000 mg/kg group

Day	FEMALES (cont.)		ANIMAL NUMBER
	<u>586</u>	<u>587</u>	
6	-- ^a		<u>588</u> --
		Cold to touch Dyspnea Diarrhea Hypoactive Discolored inguinal fur Pale Rough hair coat Necrotic tail	<u>589</u> No signs observed
7	--		<u>590</u> --
		Cold to touch Dyspnea Diarrhea Discolored inguinal fur Pale Rough hair coat Necrotic tail Prostrate Death	No signs observed
8-14	--		--
			Alopecia (abdomen/inguinal area)

^a -- = animal died; no data

**ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS**

TABLE 4

Summary of Body Weights (g) - 100 mg/kg group
(5 rats/sex)

MALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	<u>Fasted</u>	<u>Week 1</u>	<u>Week 2</u>	
481	230	291	324	94
482	222	301	347	125
483	230	298	341	111
484	244	319	366	122
485	236	316	353	117
Mean	232	305	346	114
± S.D. ^a	8.2	12.0	15.5	12.3

FEMALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	<u>Fasted</u>	<u>Week 1</u>	<u>Week 2</u>	
486	173	210	223	50
487	166	203	218	52
488	174	206	219	45
489	170	212	220	50
490	183	222	239	56
Mean	173	211	224	51
± S.D.	6.3	7.3	8.7	4.0

^a S.D. = Standard Deviation

**ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS**

TABLE 4 (cont.)

Summary of Body Weights (g) - 500 mg/kg group
(5 rats/sex)

MALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	Fasted	Week 1	Week 2	
491	228	295	344	116
492	222	279	332	110
493	243	313	376	133
494	228	305	332	104
495	227	300	353	126
Mean	230	298	347	118
± S.D. ^a	7.9	12.7	18.3	11.8

FEMALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	Fasted	Week 1	Week 2	
496	174	199	241	67
497	176	214	229	53
498	167	213	229	62
499	184	232	249	65
500	168	202	212	44
Mean	174	212	232	58
± S.D.	6.9	13.0	14.0	9.6

^a S.D. = Standard Deviation

**ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS**

TABLE 4 (cont.)

Summary of Body Weights (g) - 750 mg/kg group
(5 rats/sex)

MALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	Fasted	Week 1	Week 2	
661	216	235	305	89
662	222	283	335	113
663	228	294	338	110
664	246	(257) ^a	-- ^b	-- ^b
665	228	259	323	95
Mean	228	268	325	102
± S.D. ^c	11.2	26.3	15.0	11.6

FEMALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	Fasted	Week 1	Week 2	
666	172	195	222	50
667	179	202	228	49
668	196	(169)	--	--
669	167	180	218	51
670	174	(182)	--	--
Mean	178	192	223	50
± S.D.	11.1	11.2	5.0	1.0

^a () = carcass weight; excluded from the mean

^b -- = animal died; no data

^c S.D. = Standard Deviation

**ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS**

TABLE 4 (cont.)

Summary of Body Weights (g) - 1000 mg/kg group
(5 rats/sex)

MALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	Fasted	Week 1	Week 2	
581	230	(222) ^a	-- ^b	--
582	237	(254)	--	--
583	220	(214)	--	--
584	234	(240)	--	--
585	240	(230)	--	--
Mean	232	--	--	--
± S.D. ^c	7.8	--	--	--

FEMALES				
Animal Number	Body Weight (g)			Cumulative Body Weight Change (g) (Week 2 - Fasted)
	Fasted	Week 1	Week 2	
586	158	(156)	--	--
587	184	(151)	--	--
588	172	(179)	--	--
589	180	206	249	69
590	177	(168)	--	--
Mean	174	206	249	69
± S.D.	10.1	--	--	--

^a () = carcass weight; excluded from the mean

^b -- = animal died; no data

^c S.D. = Standard Deviation

ACUTE ORAL TOXICITY STUDY OF
N-ETHYL-META-TOLUIDINE IN RATS

TABLE 5

Summary of Necropsy Observations
(5 rats/sex/group)

Observation	Incidence							
	100 mg/kg		500 mg/kg		750 mg/kg		1000 mg/kg	
	M ^a	F ^b	M	F	M	F	M	F
No gross lesions	5	5	5	5	4	3	--	1
Lung								
dark/brown	-- ^c	--	--	--	1	1	5	1
mottled	--	--	--	--	--	1	1	1
red/red foci	--	--	--	--	--	--	--	3
Liver								
dark/brown	--	--	--	--	1	2	--	--
pale	--	--	--	--	--	--	--	1
Heart								
dark brown	--	--	--	--	--	1	2	1
Spleen								
dark/brown	--	--	--	--	1	1	5	3
small	--	--	--	--	--	--	--	1
Stomach								
red external surface foci	--	--	--	--	--	--	1	--
Ovary								
yellow	--	--	--	--	--	--	--	1
Urinary Bladder								
red fluid	--	--	--	--	--	--	--	2
Subcutaneous Tissue								
yellow	--	--	--	--	--	--	--	1
Adrenals								
dark brown	--	--	--	--	--	1	--	--
Kidneys								
dark brown	--	--	--	--	--	1	--	1
Thymus								
foci	--	--	--	--	--	--	2	1

^aM = Male

^bF = Female

^c-- = zero incidence

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TABLE 6

Calculation of LD₅₀

LD50

STUDY NUMBER	TEST ARTICLE NEM	SPECIES Rat	SEX BOTH
ANIMALS/SEX/GROUP		5	
TOTAL ANIMALS USED		30	
TOTAL ANIMALS USED	6.7-93.3%	20	

CALCULATIONS

Y-INTERCEPT	-25.63585
SLOPE	10.57936
16% CONCENTRATION	632.8322
50% CONCENTRATION	786.7056
84% CONCENTRATION	977.9934
T-VALUE	4.3
CORRELATION COEFFICIENT	.9867642

DATA SUMMARY

LEVEL	DOSE	LOG DOSE	# DEAD	% DEAD	PROBIT
1	500.00	2.70	0	0.0	3.04
2	750.00	2.88	3	30.0	4.48
3	1000.00	3.00	9	90.0	6.28

THE LD50 FOR COMBINED M&F IS 786.71 mg/kg
THE 95% CONFIDENCE LIMITS 585.17 TO 1057.65 mg/kg

CALCULATION METHOD--MILLER AND TAINTER
(PROC. SOC. EXP. BIO MED., 57:261-264, 1944)

VII. APPENDIX

APPENDIX 1

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Study No.

PROTOCOL

1. Title:
Acute Oral Toxicity Study of N-Ethylmeta-Toluidine in Rats.
2. Sponsor:
ChemFirst, Inc.
1001 Industrial Road
Pascagoula, MS 39581-3237
3. Testing Facility:
4. Objective:
The objective of the study is to evaluate the toxicity (LD_{50}) of the test substance following administration of oral dosing.
5. Duration:
The minimum duration of the study will be 14 days.
6. Proposed Study Dates:
 - a. Treatment Initiation: April 2, 1997
 - b. Biophase Termination: April 25, 1997
 - c. Draft Report Completion: June 13, 1997
7. Protocol Approval:
 - a. Study Director: _____
 - b. Section Head: (_____
 - c. Sponsor Representative: _____
8. This protocol complies with specific requirements of the Sponsor.

9. Test Substance:

- a. **Identification:** The test substance is N-Ethyl-meta-Toluidine (NEM) (CAS No. 102-27-2). It is a clear, colorless liquid.
- b. **Handling Precautions:** When working with the test substance, study personnel must wear eye protection, 2 pairs of gloves (latex over polyethylene) and organic vapor respirator.
- c. **Assay:** The Sponsor will perform all necessary analyses on the test substance.
- d. **Storage:** The test substance will be stored at room temperature (approximately 22°C).
- e. **Dispensation:** An appropriate syringe will be used to dispense individual doses of the test substance.
- f. **Disposition:** Reserve samples will be retained by the Sponsor. All quantities of test substance which are dispensed will be documented. At the time of the acceptance of the report by the Sponsor, arrangements will be made for the return of residual test substance to the Sponsor. will not be required to retain any samples.
- g. **Preparation:** For the initial dose group, the test substance will be prepared as a 10% (w/v) solution in corn oil and administered at a dosing volume of 10 ml/kg body weight. For subsequent dose groups, the test substance will be prepared in corn oil.

10. Test System:

- a. **Model:** Male and female Sprague-Dawley rats (CrI:CDRBR) ordered from Charles River Laboratories, Inc., Wilmington, MA will be used in this study. The animals will be approximately 36-43 days of age and will weigh approximately 125-150 grams on arrival.
- b. **Selection of Test System and Route of Administration Justification:** The Sprague-Dawley rat is a model widely used in acute toxicity testing. A significant body of experience with this animal exists against which its reaction to the test substance can be evaluated. Oral dosing corresponds to a potential route of human exposure.
- c. **Housing:** Animals will be housed individually in stainless steel wire cages suspended over excrement pans.
- d. **Cleaning and Sanitation:** Animal cages and rooms will be cleaned and sanitized prior to placing animals in them, and periodically thereafter in accordance with accepted animal care practices and relevant standard operating procedures. Absorbent animal cage boards will be used in all excrement pans below the stainless steel mesh floor to absorb liquids.
- e. **Food:** Certified Purina Rodent Chow 5002 (PMI Feeds, Inc., St. Louis, MO) will be provided ad libitum, except for a period of approximately 18 hours immediately preceding test substance administration and 3 to 4 hours after dosing. No known contaminants are expected to be present in the basal diet that would interfere with the test substance or test system and would confound the interpretation of the study.

- f. Water: water will be provided *ad libitum* by means of an automatic watering system. Water supply is periodically monitored for bacterial contamination and chemical composition (i.e., electrolytes, metals, etc.).
- g. Animal Identification: Animals selected for the study will receive a permanent identification number tag which will be inserted through the pinna of the right ear. Individual cage cards will also be provided. The identifying numbers assigned will be unique within the animal room used.
- h. Environmental Control: Animal rooms will be lighted with fluorescent lights and maintained on a 12-hour light/12-hour dark cycle. Room temperature and humidity will be regulated to avoid extreme fluctuations.

11. Methods:

- a. Quarantine: The animals purchased for use in this study will be held in quarantine for at least one week prior to assignment to test groups. During the quarantine period, survival checks will be performed at least daily. At the end of the quarantine period, the rats will receive a thorough physical examination to ensure their suitability for use as test animals.
- b. Assignment to Groups: Animals will be assigned to groups using a constrained random process such that all groups tested will be comparable in pretest body weight and all other relevant parameters, but assignment of individual animals to groups will be random.
- c. Dose Levels: An initial group of five male and five female rats will receive 1000 mg of test substance per kilogram of body weight, and will be observed for a period of 14 days thereafter. At least two additional test groups (five animals/sex/group) will be used to determine the LD₅₀. Dose levels for the additional groups will be based upon the mortality seen at the 1000 mg/kg level. In no case will the highest dose level exceed 5 g/kg body weight.
- d. Test Substance Administration: Following a fasting period of approximately 18 hours, each animal on test will receive an appropriate single dose of the test substance by gavage via an appropriately sized intubation feeding needle. All animals will be dosed at a constant dosing volume of 10 ml/kg body weight.
- e. Final Disposition of Animals: All animals surviving to the end of the 14-day observation period will be euthanized by carbon dioxide asphyxiation and subjected to a gross necropsy.

12. Observations:

- a. Mortality and Reactions: All study animals will be observed closely and frequently during the first four hours following treatment, periodically through the remainder of the first day, and at least once daily (including weekends and holidays) during the subsequent 14-day observation period for obvious signs of toxicity, including death. Animals found dead will be removed for gross necropsy and the death recorded in the study notebook. All signs of altered behavior, changes in coat condition, unusual discharge of body fluid, lesions, or other relevant observations will be recorded.
- b. Body Weight: All study animals will be weighed immediately prior to the administration of the test dose, weekly thereafter, and at death or the termination of the study.

Study No.

- c. Necropsy: A gross necropsy will be performed on all test animals which die during the study or are sacrificed at study termination. The necropsy will include examination of all body surfaces and orifices. In addition, the external appearance of the brain, heart, lungs, spleen, liver, kidneys, gastrointestinal tract, urinary bladder and gonads will be examined. The stomach will be opened, and the rest of the gastrointestinal tract and the urinary bladder opened and examined if external lesions are present. Tissues will not be retained unless the Study Director should specify otherwise.

13. Results:

Mortality, clinical and gross necropsy observations, and body weight data will be tabulated, as appropriate, and the findings presented in a formal written report. When possible, the LD₅₀ will be calculated using the method of Miller & Tainter (Proc. Soc. Exp. Bio Med., 57: 261-264, 1944), along with a 95% confidence interval.

14. Data Notebooks:

- a. Contents: All original data will be maintained in notebooks and will include, but not necessarily be limited to, the following:

- (1) The original signed protocol and all amendments/deviations
- (2) Study authorization by Sponsor
- (3) Test substance information
- (4) Animal receiving records
- (5) Randomization procedures
- (6) Dosage calculations
- (7) Body weights
- (8) Daily observations and mortality records
- (9) Environmental records (room temperature and humidity readings)
- (10) Necropsy records
- (11) Animal caretaker records

- b. Storage: All original data and the original final report will be retained in the Archives for one year after the submission of the signed final report. At that time, the Sponsor will be contacted in order to determine the final disposition of the raw data and will be responsible for all costs associated with continued storage of the raw data in the Archives or for the shipment of these materials to a new storage facility. Quality Assurance Unit will maintain a complete record of the disposition of all archival material.

15. Final Reports:

One copy of a draft report will be submitted to the Sponsor for review. After receipt and review of the Sponsor's comments, appropriate changes will be made and two copies of a signed Final Report will be provided to the Sponsor.

16. Personnel:

Curricula vitae, training records and job descriptions for all personnel involved in the conduct of the study are on file.

17. Regulatory Compliance:

- a. Test Guidelines: This study will be conducted in compliance with requirements of the following testing guideline:

U.S. EPA TSCA *Health Effects Testing Guidelines*, 40 CFR 798.1175, "Acute Oral Toxicity," 1992.

b.

18.

19. Animal Welfare Compliance Statement:

This study will comply with all applicable sections of the Animal Welfare Act (Title 9, Code of Federal Regulations), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH Office of Protection from Research Risks, 1986), and the Guide for the Care and Use of Laboratory Animals (1996). The Sponsor's signature on the study protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments, and that no known acceptable non-animal alternatives were available. Wherever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress, and pain to animals. All methods to be used are described in this study protocol or in written laboratory standard operating procedures.

20. Changes of the Protocol:

No changes of the protocol will be made without the consent of the Sponsor. All changes to the protocol will be signed and dated by the Study Director and maintained with the protocol. In the event that the Sponsor authorizes a protocol change verbally, the change will be honored and will be followed up with written verification.

21. Humane Treatment Statement:

The Institutional Animal Care and Use Committee _____ has reviewed this protocol and deems its study design appropriate to meet the objectives of the study, while minimizing both pain and distress to the test animals. If anesthetic, analgesic, or tranquilizer drugs can be used, they are the proper type for the given species. If euthanasia is to be performed, the method is proper for the given species.

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