

ETHYL CORPORATION
Health and Environment Department

Toxicology and
Regulatory Affairs

1,964 pp

CONTAINS NO CBI

Ethyl Tower
451 Florida Street
Baton Rouge, LA 70801

8EHQ-0989-0648 PLWP
88-870000 15
89-890000 255

September 20, 1989



000653032J

Document Control Officer (TS-790)
Office of Toxic Substances
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Attention: Section 8(e) Coordinator

Dear Sir:

Re: 8EHQ 1286-0648

This is a follow-up to an 8(e) submission (8EHQ 1286-0648) Ethyl Corporation made in December 1986. Pursuant to the Agency's letter dated January 16, 1987, Ethyl Corporation is submitting the enclosed 28-day Oral Toxicity/Reversibility Study in Rats. Preliminary results of this 28-day study was communicated to the Agency in a May 20, 1988 letter.

For your information, a two-year oral feeding study is now in progress. A copy of this study will be made available to the Agency upon completion.

If you have any questions, please call me at (504) 388-7650.

Sincerely,

Louise L. Wen, Ph.D.
Regulatory Affairs Associate

LLW:ab
Enclosure

20 SEP 26 PM 2:51

00002

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

FAX
(717) 586-3450

Repeated Dose Oral Toxicity/Reversibility
Study in Rats - 28 Day

PH 436-ET-001-87

diethyl toluene diamine
(DETD)

APPENDIX I
RAW DATA

CONTAINS NO CBI

2
PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

FAX
(717) 586-3450

Repeated Dose Oral Toxicity/Reversibility
Study in Rats - 28 Day

PH 436-ET-001-87

diethyl toluene diamine
(DETDA)

APPENDIX I
RAW DATA

0 0 0 4

PHARMAKON RESEARCH INTERNATIONAL, II.C.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 43SET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (ppm)

ANIMAL NO	BODY WEIGHT (G)	FULL FEEDER (G)	EMPTY FEEDER (G)	FULL WATER (G)	EMPTY WATER (G)	DOSSAGE (PPM)	OFR TIME	OBSERVATIONS:			
								OBS SV	LOC	OFR TIME	OBS SV LOC OFR TIME
DAY 0 was started on date: 05-19-87											
13	220	668	264	-	668	-	-	N		668	08:03
102	226	668	255	-	668	-	-	N		668	08:04
103	200	668	259	-	668	-	-	N		668	08:05
104	201	668	263	-	668	-	-	N		668	08:06
105	196	668	276	-	668	-	-	N		668	08:09
106	228	668	263	-	668	-	-	N		668	08:11
107	221	668	254	-	668	-	-	N		668	08:11
108	216	668	257	-	668	-	-	N		668	08:12
109	205	668	241	-	668	-	-	N		668	08:12
110	224	668	267	-	668	-	-	N		668	08:13
111	213	668	280	-	668	-	-	N		668	08:13
112	209	668	288	-	668	-	-	N		668	08:14
113	197	668	260	-	668	-	-	N		668	08:14
114	195	668	255	-	668	-	-	N		668	08:15
115	212	668	259	-	668	-	-	N		668	08:16
DAY 1 was started on date: 05-20-87											
113	-	-	-	-	-	-	-	N		668	08:33
102	-	-	-	-	-	-	-	N		668	08:33
103	-	-	-	-	-	-	-	N		668	08:34
104	-	-	-	-	-	-	-	N		668	08:34
105	-	-	-	-	-	-	-	N		668	08:34
106	-	-	-	-	-	-	-	N		668	08:34
107	-	-	-	-	-	-	-	N		668	08:34
108	-	-	-	-	-	-	-	N		668	08:34
109	-	-	-	-	-	-	-	N		668	08:35
110	-	-	-	-	-	-	-	N		668	08:35
111	-	-	-	-	-	-	-	N		668	08:35
112	-	-	-	-	-	-	-	N		668	08:35
113	-	-	-	-	-	-	-	N		668	08:35
114	-	-	-	-	-	-	-	N		668	08:35
115	-	-	-	-	-	-	-	N		668	08:35
DAY 2 was started on date: 05-21-87											
113	-	-	-	-	-	-	-	N		668	08:33
102	-	-	-	-	-	-	-	N		668	08:33
103	-	-	-	-	-	-	-	N		668	08:34
104	-	-	-	-	-	-	-	N		668	08:34
105	-	-	-	-	-	-	-	N		668	08:34
106	-	-	-	-	-	-	-	N		668	08:34

(REPORT CONTINUED)

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 436ET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (PPM)

VIAL NO	BODY WEIGHT		FULL FEEDER		EMPTY FEEDER		FULL WATER		EMPTY WATER		DOSAGE OPR (ppm)	OPR TIME	OBSERVATIONS:						
	(g)	OPR	(g)	OPR	(g)	OPR	(g)	OPR	(g)	OPR			OBS SV LOC OPR TIME						
07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
08	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34	-	-	-	-
09	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35	-	-	-	-
DAY 3 was started on date: 05-22-87																			
13	246	668	291	184	668	-	-	-	-	-	-	-	N	668	08:38	-	-	-	-
02	248	668	300	167	668	-	-	-	-	-	-	-	N	668	08:40	-	-	-	-
03	218	668	288	187	668	-	-	-	-	-	-	-	N	668	08:40	-	-	-	-
04	223	668	294	194	668	-	-	-	-	-	-	-	N	668	08:43	-	-	-	-
06	215	668	276	201	668	-	-	-	-	-	-	-	N	668	08:44	-	-	-	-
06	256	668	287	181	668	-	-	-	-	-	-	-	N	668	08:44	-	-	-	-
07	241	668	301	180	668	-	-	-	-	-	-	-	N	668	08:45	-	-	-	-
08	235	668	296	181	668	-	-	-	-	-	-	-	N	668	08:45	-	-	-	-
09	230	668	293	166	668	-	-	-	-	-	-	-	N	668	08:45	-	-	-	-
10	246	668	295	189	668	-	-	-	-	-	-	-	N	668	08:46	-	-	-	-
11	235	668	290	203	668	-	-	-	-	-	-	-	N	668	08:47	-	-	-	-
12	232	668	282	209	668	-	-	-	-	-	-	-	N	668	08:47	-	-	-	-
13	215	668	281	186	668	-	-	-	-	-	-	-	N	668	08:48	-	-	-	-
14	218	668	286	184	668	-	-	-	-	-	-	-	N	668	08:48	-	-	-	-
15	233	668	286	173	668	-	-	-	-	-	-	-	N	668	08:49	-	-	-	-
DAY 4 was started on date: 05-23-87																			
13	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:21	-	-	-	-
02	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:21	-	-	-	-
03	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:21	-	-	-	-
04	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
05	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
06	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
07	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
08	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
09	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:22	-	-	-	-

(REPORT CONTINUED)

0006

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 436ET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (PPM)

ANIMAL NO	BODY WEIGHT (g)	FULL FEEDER (g)	EMPTY FEEDER (g)	FULL WATER (g)	EMPTY WATER (g)	DRY WASTE (g)	DRY WASTE (g)	DRY WASTE (g)	OBS SV LOC	OBS SV LOC	OBSERVATIONS:			
											OPR	OPR TIME	OPR	OPR TIME
112	-	-	-	-	-	-	-	-	N	GG8	08:23			
115	-	-	-	-	-	-	-	-	N	GG8	08:23			
DAY 5 was started on date: 05-24-87														
116	-	-	-	-	-	-	-	-	N	AM	09:50			
102	-	-	-	-	-	-	-	-	N	AM	09:50			
103	-	-	-	-	-	-	-	-	N	AM	09:50			
104	-	-	-	-	-	-	-	-	N	AM	09:50			
105	-	-	-	-	-	-	-	-	N	AM	09:50			
106	-	-	-	-	-	-	-	-	N	AM	09:50			
107	-	-	-	-	-	-	-	-	N	AM	09:51			
108	-	-	-	-	-	-	-	-	N	AM	09:51			
109	-	-	-	-	-	-	-	-	N	AM	09:51			
110	-	-	-	-	-	-	-	-	N	AM	09:51			
111	-	-	-	-	-	-	-	-	N	AM	09:51			
112	-	-	-	-	-	-	-	-	N	AM	09:51			
113	-	-	-	-	-	-	-	-	N	AM	09:51			
114	-	-	-	-	-	-	-	-	N	AM	09:51			
115	-	-	-	-	-	-	-	-	N	AM	09:51			
DAY 6 was started on date: 05-25-87														
113	-	-	-	-	-	-	-	-	N	GG8	08:48			
102	-	-	-	-	-	-	-	-	N	GG8	08:48			
103	-	-	-	-	-	-	-	-	N	GG8	08:48			
104	-	-	-	-	-	-	-	-	N	GG8	08:48			
105	-	-	-	-	-	-	-	-	N	GG8	08:48			
106	-	-	-	-	-	-	-	-	N	GG8	08:48			
107	-	-	-	-	-	-	-	-	N	GG8	08:48			
108	-	-	-	-	-	-	-	-	N	GG8	08:49			
109	-	-	-	-	-	-	-	-	N	GG8	08:49			
110	-	-	-	-	-	-	-	-	N	GG8	08:49			
111	-	-	-	-	-	-	-	-	N	GG8	08:49			
112	-	-	-	-	-	-	-	-	N	GG8	08:49			
113	-	-	-	-	-	-	-	-	N	GG8	08:49			
114	-	-	-	-	-	-	-	-	N	GG8	08:49			
115	-	-	-	-	-	-	-	-	N	GG8	08:50			
116	-	-	-	-	-	-	-	-	N	GG8	08:50			
DAY 7 was started on date: 05-26-87														
113	284	GG8	283	175	GG8	-	-	-	N	GG8	08:42			
102	292	GG8	291	174	GG8	-	-	-	N	GG8	08:43			
103	250	GG8	291	182	GG8	-	-	-	N	GG8	08:44			

(REPORT CONTINUED)

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 436ET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (ppm)

VINAL NO	BODY WEIGHT		FULL FEEDER		EMPTY FEEDER		FULL WATER		EMPTY WATER		DOSAGE OPR (ppm)	OPR TIME	OBSERVATIONS:						
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	OBS SV LOC OPR TIME	OBS SV LOC OPR TIME			OBS SV LOC OPR TIME	OBS SV LOC OPR TIME					
104	256	668	290	197	668	-	-	-	-	-	-	-	N	668	08:44				
105	248	668	295	173	668	-	-	-	-	-	-	-	N	668	08:45				
106	297	668	306	169	668	-	-	-	-	-	-	-	N	668	08:46				
107	272	668	270	198	668	-	-	-	-	-	-	-	N	668	08:46				
108	269	668	282	190	668	-	-	-	-	-	-	-	N	668	08:46				
109	259	668	287	190	668	-	-	-	-	-	-	-	N	668	08:47				
110	278	668	315	185	668	-	-	-	-	-	-	-	N	668	08:47				
111	271	668	271	183	668	-	-	-	-	-	-	-	N	668	08:47				
112	272	668	300	174	668	-	-	-	-	-	-	-	N	668	08:57				
113	246	668	297	161	668	-	-	-	-	-	-	-	N	668	08:57				
114	257	668	290	192	668	-	-	-	-	-	-	-	N	668	08:58				
115	272	668	283	164	668	-	-	-	-	-	-	-	N	668	08:58				

DAY 3 was started on date: 05-27-87

103	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:33				
104	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34				
105	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34				
106	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35				
107	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35				
108	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35				
109	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:39				
110	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:39				
111	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:40				
112	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:40				
113	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:40				
114	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:41				
115	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:41				

DAY 9 was started on date: 05-28-87

103	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:32				
102	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:32				
103	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:32				
-	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:32				
104	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:33				
107	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:33				
108	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:33				
109	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34				
110	-	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34				

(REPORT CONTINUED)

0000A

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 436ET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (ppm)

ANIMAL NO	BODY WEIGHT		FULL FEEDER		EMPTY FEEDER		FULL WATER		EMPTY WATER		DOSAGE (ppm)	OBS SV LOC	OBSERVATIONS: OPR TIME			
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)			OBS SV LOC	OPR TIME	OBS SV LOC	OPR TIME
11	-	-	-	-	-	-	-	-	-	-	-	N	668	08:34		
12	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35		
13	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35		
14	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35		
15	-	-	-	-	-	-	-	-	-	-	-	N	668	08:35		
												N	668	08:36		

DAY 10 was started on date: 05-29-87

13	311	668	295	188	668	-	-	-	-	-	-	N	668	08:33
12	318	668	297	199	668	-	-	-	-	-	-	N	668	08:35
103	261	668	255	200	668	-	-	-	-	-	-	N	668	08:36
104	277	668	269	213	668	-	-	-	-	-	-	N	668	08:37
105	267	668	283	221	668	-	-	-	-	-	-	N	668	08:38
106	319	668	263	218	668	-	-	-	-	-	-	N	668	08:40
107	288	668	286	195	668	-	-	-	-	-	-	N	668	08:41
108	290	668	292	200	668	-	-	-	-	-	-	N	668	08:42
109	270	668	298	212	668	-	-	-	-	-	-	N	668	08:42
110	299	668	296	235	668	-	-	-	-	-	-	N	668	08:42
111	296	668	293	191	668	-	-	-	-	-	-	N	668	08:43
112	292	668	272	214	668	-	-	-	-	-	-	N	668	08:44
113	255	668	283	214	668	-	-	-	-	-	-	N	668	08:44
114	275	668	283	207	668	-	-	-	-	-	-	N	668	08:48
115	300	668	295	188	668	-	-	-	-	-	-	N	668	08:52
												N	668	08:53

DAY 11 was started on date: 05-30-87

13	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:31
12	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:31
103	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:32
104	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:32
105	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:32
106	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:32
107	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:33
108	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:33
109	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:33
110	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:34
111	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:34
112	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:34
113	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:34
114	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:35
115	-	-	-	-	-	-	-	-	-	-	-	N	SS	08:35
												N	SS	08:35

DAY 12 was started on date: 05-31-87

(REPORT CONTINUED)

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 436ET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (PPM)

VINA. NO	BODY WEIGHT (G)	FULL FEEDER OPR (G)	EMPTY FEEDER (G)	FULL WATER OPR (G)	EMPTY WATER (G)	DOSAGE OPR (PPM)	OPR TIME	OBSERVATIONS:				
								OBS SV	LOC	OPR TIME	OBS SV LOC OPR TIME	
03	-	-	-	-	-	-	-	N	668	08:31		
02	-	-	-	-	-	-	-	N	668	08:32		
03	-	-	-	-	-	-	-	N	668	08:32		
04	-	-	-	-	-	-	-	N	668	08:32		
05	-	-	-	-	-	-	-	N	668	08:32		
06	-	-	-	-	-	-	-	N	668	08:33		
07	-	-	-	-	-	-	-	N	668	08:33		
08	-	-	-	-	-	-	-	N	668	08:33		
09	-	-	-	-	-	-	-	N	668	08:33		
10	-	-	-	-	-	-	-	N	668	08:34		
11	-	-	-	-	-	-	-	N	668	08:34		
12	-	-	-	-	-	-	-	N	668	08:34		
13	-	-	-	-	-	-	-	N	668	08:34		
14	-	-	-	-	-	-	-	N	668	08:35		
15	-	-	-	-	-	-	-	N	668	08:35		
DAY 13 was started on date: 06-01-87												
03	-	-	-	-	-	-	-	N	668	08:33		
02	-	-	-	-	-	-	-	N	668	08:33		
03	-	-	-	-	-	-	-	N	668	08:33		
04	-	-	-	-	-	-	-	N	668	08:34		
05	-	-	-	-	-	-	-	N	668	08:34		
06	-	-	-	-	-	-	-	N	668	08:34		
07	-	-	-	-	-	-	-	N	668	08:34		
08	-	-	-	-	-	-	-	N	668	08:34		
09	-	-	-	-	-	-	-	N	668	08:35		
10	-	-	-	-	-	-	-	N	668	08:35		
11	-	-	-	-	-	-	-	N	668	08:35		
12	-	-	-	-	-	-	-	N	668	08:35		
13	-	-	-	-	-	-	-	N	668	08:36		
14	-	-	-	-	-	-	-	N	668	08:36		
15	-	-	-	-	-	-	-	N	668	08:36		
DAY 14 was started on date: 06-02-87												
03	342	668	-	179	668	-	-	N	668	08:32		
02	350	668	-	167	668	-	-	N	668	08:33		
03	314	668	278	147	668	-	-	N	668	08:34		
04	305	668	268	172	668	-	-	N	668	08:35		
05	293	668	280	188	668	-	-	N	668	08:35		
06	332	668	281	154	668	-	-	N	668	08:36		
07	316	668	273	187	668	-	-	N	668	08:36		

(REPORT CONTINUED)

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY NUMBER: PH436-ET-001-87
 TEST ARTICLE: DIETHYL TOLUENE DIAMINE
 SPONSOR: ETHYL CORP., BATON ROUGE, LA

INDIVIDUAL ANIMAL DATA

STUDY: 48SET01 GROUP: CONTROL SEX: MALE
 DOSE: 0 (ppm)

ANIMAL NO	BODY WEIGHT (g)	FULL FEEDER (g)	EMPTY FEEDER (g)	FULL WATER (g)	EMPTY WATER (g)	DOSAGE (ppm)	OBS SV LOC	OBSERVATIONS:		
								OFR TIME	OFR TIME	
308	322	668	301	187	668	-	-	-	N	668 08:37
309	303	668	-	200	668	-	-	-	N	668 08:36
410	327	668	271	189	668	-	-	-	N	668 08:38
411	327	668	294	185	668	-	-	-	N	668 08:39
412	325	668	-	152	668	-	-	-	N	668 08:40
413	277	668	277	185	668	-	-	-	N	668 08:41
414	306	668	255	177	668	-	-	-	N	668 08:41
415	324	668	-	173	668	-	-	-	N	668 08:42

DAY 15 was started on date: 06-03-87

403	-	-	-	-	-	-	-	-	TS	668 08:40
404	-	-	-	-	-	-	-	-	TS	668 08:40
405	-	-	-	-	-	-	-	-	N	668 08:40
406	-	-	-	-	-	-	-	-	N	668 08:41
407	-	-	-	-	-	-	-	-	N	668 08:41
408	-	-	-	-	-	-	-	-	N	668 08:41
409	-	-	-	-	-	-	-	-	N	668 08:41
410	-	-	-	-	-	-	-	-	TS	668 08:42
411	-	-	-	-	-	-	-	-	N	668 08:42
412	-	-	-	-	-	-	-	-	N	668 08:43
413	-	-	-	-	-	-	-	-	TS	668 08:43
414	-	-	-	-	-	-	-	-	N	668 08:43
415	-	-	-	-	-	-	-	-	N	668 08:43
									TS	668 08:43

DAY 16 was started on date: 06-04-87

403	-	-	-	-	-	-	-	-	N	668 08:31
404	-	-	-	-	-	-	-	-	N	668 08:31
405	-	-	-	-	-	-	-	-	N	668 08:31
406	-	-	-	-	-	-	-	-	N	668 08:31
407	-	-	-	-	-	-	-	-	N	668 08:31
408	-	-	-	-	-	-	-	-	N	668 08:31
410	-	-	-	-	-	-	-	-	N	668 08:31
411	-	-	-	-	-	-	-	-	N	668 08:31
413	-	-	-	-	-	-	-	-	N	668 08:32
414	-	-	-	-	-	-	-	-	N	668 08:32
									N	668 08:32

DAY 17 was started on date: 06-05-87

403	322	668	281	139	668	-	-	-	N	668 08:32
404	322	668	253	190	668	-	-	-	N	668 08:34

(REPORT CONTINUED)

3

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411
FAX
(717) 586-3450

000132

Repeated Dose Oral Toxicity/Reversibility
Study in Rats - 28 Day

PH 436-ET-001-87

diethyl toluene diamine
(DETD)

RECEIVED

HEALTH & ENVIRONMENT
INFORMATION CENTER

Submitted to

Ethyl Corporation
Baton Rouge, Louisiana

Gary G. Bolus
Gary G. Bolus, B.A., LAT
Study Director

8/30/89
Date

Richard K. Matthews
Test Management Facility

8/30/89
Date

0729

Table of Contents

	Page
SUMMARY.....	1
STUDY DESCRIPTION.....	4
TEST ARTICLES.....	6
TEST SYSTEM.....	7
METHODS.....	9
CLINICAL EXAMINATION.....	10
PATHOLOGY.....	11
RESULTS.....	12
Body Weights.....	12
Daily Body Weight Gains.....	13
Daily Food Consumption.....	13
Clinical Signs.....	14
Mortality.....	14
Necropsy.....	14
Absolute Organ Weights.....	15
Relative Organ to Body Weights.....	15
Relative Organ to Brain Weights.....	16
Clinical Chemistry.....	17
Hematology.....	19
Ophthalmologic Examinations.....	20
Histopathology.....	21
CONCLUSION.....	24
TABLES.....	25
Table 1 - Summary of Body Weights.....	25
Table 2 - Summary of Daily Body Weight Gains.....	31
Table 3 - Summary of Daily Food Consumption.....	35
Table 4 - Individual Observations.....	41
Table 5 - Incidence of the Gross Findings.....	69
Table 6 - Absolute Organ Weights.....	114
Table 7 - Relative Organ to Body Weights.....	126
Table 8 - Relative Organ to Brain Weights.....	138
Table 9 - Summary of Clinical Chemistry.....	150
Table 10 - Summary of Hematology.....	168
QUALITY ASSURANCE STATEMENT.....	174
COMPLIANCE STATEMENT.....	175
APPENDIX I Raw Data	
APPENDIX II Pathology	
APPENDIX III Analytical	

000734

Repeated Dose Oral Toxicity/Reversibility Study in Rats - 28 Day

PH 436-ET-001-87
Diethyl toluene diamine (DETDA)

SUMMARY

The test articles, diethyl toluene diamine (DETDA), Lot #2-87 and Run 544, Drum 6131, were incorporated into standard certified commercial laboratory rat diet and fed ad libitum to seven groups of Sprague Dawley rats (3 treatment groups per test article) seven days per week for a period of 14 or 28 days. Each of the seven dose groups, containing fifteen males and fifteen females per group, were fed the diet mixture at dose levels of 0 (control) ppm, 50 (low) ppm, 125 (mid) ppm or 320 (high) ppm of each DETDA material. At the end of the 14 and 28 day treatment intervals, five randomly selected rats per sex per treatment group were necropsied. The remaining rats received untreated control diet for 28 additional days and served as the recovery/reversibility group.

There were no pharmacotoxic signs exhibited by the females in any treatment group during the study. The majority of the high dose DETDA Run 544 treated males exhibited several pharmacotoxic signs. The onset of the majority of these observations occurred on Day 35 and continued until study termination. These observations included decreased activity, abnormal stance, abnormal gait, poor grooming, decreased body tone, piloerection and excessive defecation and scab formation. None of the rats died on test.

Statistically significant reductions in the group mean male body weights were observed in the high dose DETDA treated groups during the majority of evaluated intervals, when compared to the controls. In the period following DETDA treatment (Days 28-56), the high dose male Run 544 group continued to lose weight, while animals treated with Lot 2-87 at the same dose gained weight. Female body weights were significantly reduced in both high dose DETDA groups at three intervals during the study, when compared to the controls.

Comparisons of the male and female group mean body weights by dose groups between DETDA materials indicated a significant lower 320 ppm Run 544 value male weights versus the Lot 2-87 group during Days 49-through 56. No significant differences were noted for the females.

The daily body weight gains were significantly reduced at several intervals for the males in both the Run 544 and Lot 2-87 treatment groups at each level, when compared to the controls. Female body weight gains were less severely affected however, significance was indicated when compared to the controls.

Statistically significant differences were also noted when the daily gains of the different DETDA treatment groups were compared. Trend-related significant differences were observed for the high dose males. A significantly lower value was noted for the male high dose Run 544 group on Day 56, when compared to the Lot 2-87 treated rats. The total mean daily gain was also significantly reduced in the high dose Run 544 group. There were no such trend-related differences observed for the females.

The daily food consumption for the males was significantly reduced in both high dose DETDA groups at several time periods, when compared to the controls.

There were no significant differences noted in food consumption of the males or females when groups treated with the different DETDA Lots were compared to each other by dose level.

000735

Summary Continued

Ocular examinations were performed on all animals prior to the scheduled sacrifices. Lesions noted at 14 and 28 days of DETDA treatment were considered inconsequential of either the Lot 2-87 or Run 544, Drum 6131. The pretermination ocular examination was performed on Day 56 of the study. Two high dose Run 544 male rats exhibited multifocal anterior cataracts. These lesions suggest a possible ocular toxicologic effect of the test compound.

The majority of necropsy observations recorded at either the 14 or 28 interim sacrifices were non-specific in nature. These observations included enlarged mandibular lymph nodes, scabs on the skin, lung and liver abnormalities, enlarged mesenteric nodes, kidneys exhibiting cortical cyst or dilated pelvic areas, parovarian uterine cysts, slight uterine hydrometra and a red area on the tip of the heart. Observations recorded at the terminal sacrifice (Day 56) included similar observations of the lungs, livers and kidneys. In addition, changes which were specific to the high dose Run 544 male group included reduced adipose tissue, rough hair coat, small salivary glands, small spleens, poorly visible, dark and small pancreata, small testes, seminal vesicles, epididymides, prostrate and gastrointestinal tract lesions. There were no similar DETDA related changes noted at necropsy for the females.

There were no statistically significant differences observed in the absolute male or female organ weights at the Day 14 or Day 28 necropsy. At the Day 56 terminal necropsy, the high dose Run 544 male body weights, liver and testes weights were significantly reduced, when compared to the control group. No significant differences were noted for the females at Day 56.

Comparisons by dosage group between the DETDA materials indicated the high dose male body weights, absolute liver and testes weights in the Run 544 group were significantly lower than the Lot 2-87 group at the terminal (Day 56) necropsy.

A statistically significant increase in the high dose male 2-87 relative brain and testes to body weights was observed at the Day 14 necropsy, when compared to the controls. The mid dose Run 544 male relative testes weights were also significantly larger. There were no significant organ weight changes noted for the females, when the dosage groups were compared to the controls at the Day 14 interim sacrifice. The male relative liver weights were significantly larger in both high dose DETDA treated groups at the Day 28 necropsy. Significant increases were noted in both high dose DETDA treated female relative kidney weights. In addition, the female high dose Run 544 brain and liver weights were significantly larger, when compared to the control group.

There were no significant differences observed for either the male or female relative organ to body weights, when comparisons were made between the different DETDA materials by dose level at the Day 14 or Day 28 interim sacrifice.

At terminal necropsy (Day 56), the high dose Run 544 males displayed significantly larger relative brain, kidney and adrenal to body weights as compared to the controls. Females in the high dose 2-87 group exhibited significantly larger relative kidney weights, when compared to the controls.

When comparisons between DETDA materials were analyzed, significantly lower relative brain and kidney to body weights were recorded in the Run 544 high dose treated males versus the 2-87 males. No significant differences were noted for the females.

000730

Summary Continued

No statistically significant differences were observed for either the male or female relative organ to brain weights at the Day 14 or Day 28 interim sacrifices when compared to the control group. At the Day 56 necropsy, the percent liver to brain weight was significantly reduced in the high dose Run 544 treated males.

There were no significant differences noted for the relative organ to brain weights, when the different DETDA materials were compared by treatment level to each other at any of the scheduled sacrifices.

Evaluation of the blood chemistry parameters revealed several significant differences between controls and DETDA treatment groups of the same dose at each scheduled sacrifice. The majority of the significant differences were noted in the Run 544 mid and high dose male and female dosage groups at the Day 28 interim sacrifice and the Day 56 terminal sacrifice. There were however, significant differences between controls and low dose DETDA groups at each sacrifice.

Hematological evaluations indicated the low dose Lot 2-87 male platelet counts were significantly lower than the controls at the Day 14 interim necropsy. At Day 28, significant hematological differences from controls were high dose male and mid dose female platelet counts.

Comparisons of DETDA materials at the Day 28 interim sacrifices indicated the platelet counts for each Run 544 DETDA treated female were significantly larger than the Lot 2-87 females.

At the terminal necropsy (Day 56), the high dose Run 544 males exhibited a significant increase in hemoglobin and decrease in leucocyte and platelet counts, comparing controls alone.

When comparisons were made between DETDA formulations, the hemoglobin values were significantly larger and the leucocyte counts smaller in the high dose Run 544 males.

Histopathological evaluation of the tissues from the rats exposed to DETDA 2-87 and DETDA Run 544 Drum 6131 for twenty-eight days resulted in compound-related degenerative acinar changes in the pancreas at levels of 125 ppm and 320 ppm in males and 320 ppm in females. A similar pancreatic acinar degeneration was not present at the 50 ppm dosage for either compound. Male rats were more severely affected and at an earlier time than the female rats. The pancreatic effects of DETDA 2-87 almost recovered by the end of the 28 day recovery period. However, the changes in the males exposed degeneration of the islet cells of the pancreas and secondary changes of cachexia and diabetes.

Based upon the results of the Repeated Dose Oral Toxicity/Reversibility Study in Rats with diethyl toluene diamine (DETDA), Lot 2-87 appears to have exerted an effect, which was less toxic, than the Run 544, Drum 6131 preparation after exposure for 28 consecutive days.

000737

Repeated Dose Oral Toxicity/Reversibility Study in Rats - 28 Day
Diethyl toluene diamine (DETD)

PH 436-ET-001-87

Sponsor: Ethyl Corporation
451 Florida
Baton Rouge, LA 70801

Testing Facility: Pharmakon Research International, Inc.
Waverly, PA 18471

Study No.: PH 436-ET-001-87

Purpose of the Study: To compare the palatability and toxicity of two commercial preparations of diethyl toluene diamine (DETD) incorporated into standard certified laboratory rat diet when fed to rats ad libitum for a period of 14 or 28 days and also after 28 days of recovery on a standard diet.

Ownership of the Study: The sponsor owns the study. All raw data, wet tissue, analysis and reports are the property of the sponsor.

Study Monitor: Marcia Hardy, D.V.M., Ph.D., Ethyl Corporation

Study Director: Gary G. Bolus, B.A., LAT, Pharmakon Research International, Inc.

Study Ophthalmologist: Thomas J. Kern, D.V.M., DACVO, Cornell University

Study Pathologist: Larry Ackerman, V.M.D., Ph.D., Experimental Pathology Laboratories

Technical Performance: Dennis J. Margitich, B.S., RLAT, Victor T. Mallory, B.S., RLAT, Karen H. Lantzsch, RLAT, Gary G. Bolus, B.A., LAT and Susan V. Mecca, B.S., LAT, Patricia Giglio, B.S., Nira Madison and Susan Ayers

Q.A.U. Responsible Personnel: Douglas B. Hay, Ph.D. and Leslie Pinnell, M.S.

Dates of Performance: May 19, 1987 through July 15, 1987
Necropsy Dates: June 3, 1987, June 17, 1987 and July 15, 1987

Good Laboratory Practices Statement: This study was conducted in compliance with the Good Laboratory Practices Regulations as stated in the EPA Good Laboratory Practice Standards (Subpart I, Part

000738

792, Chapter I of Title 40, Code of Federal Regulations], as well as the Organisation for Economic Co-operation and Development (OECD) Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on 12 May, 1981.

Records
Maintained:

All raw data, final reports, documentation, protocol and amendments are maintained in the Pharmakon Research Archives.
Body weights (initial, twice weekly and final)
Food consumption, twice weekly
Animal Health Certificate (Purchase Order)
Schedule of events
Water and food analysis
Feed Lot Number
Formalin Lot No. and Supplier
Temperature and humidity recordings
Test article preparation
Samples of test article
Identification sample of ear markings
Pharmacological and toxicological signs
Necropsy findings
Organ Weights
Hematology reports
Ophthalmologic findings
Clinical biochemistry reports
Histopathological reports

Notebook
Reference:

Notebook #1158, 1158A, 1158B, 1158C

Statistical
Analysis:

Evaluation of equality of means was made by the one way analysis of variance using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine significant differences from control means. Tukey's test was used to determine significant differences between the DEIDA lots at the same dose levels.

Computer Analysis:

All raw data were summarized and statistically evaluated using LABCAT modules designed by Innovative Programming Associates, Inc., One Airport Place, Princeton, NJ 08540 or SYSTAT, a PC based statistical package designed by SYSTAT, Inc., Evanston, IL 60201.

Raw Data:

Standard Pharmakon Research Notebook
Computer generated Pharmakon Research Study Forms
All raw data

Archive Retention:

All raw data, samples of the test articles, study file and the final report will be maintained in the Pharmakon Research Archives. Wet tissue, paraffin blocks and slides will be maintained at Experimental Pathology Laboratories, P.O. Box 474, Herndon, VA 22070.

0 7 3 5

TEST ARTICLES

000739

Compound
Description: diethyl toluene diamine (DETDA) -- amber liquid

Run Number: 544

Drum Number: 6131

Amount Submitted: 10 gallons

Date Received: March 27, 1986

Special Handling
Instructions: Standard precautions

Compound
Description: diethyl toluene diamine (DETDA) -- amber liquid

Lot Number: 2-87

Amount Submitted: 2 gallons

Date Received: January 30, 1987

Special Handling
Instructions: Standard precautions

Test Article
Preparation: Preparation of the test article for administration to the rats consisted of incorporating the test article in the diet utilizing a liquid-solids blender, (Twin-Shell Intensifier Blender, Patterson-Kelly Company, East Stroudsburg, Pennsylvania) according to the Standard Operating Procedure PH-036A on file at Pharmakon Research International, Inc.

Diet Mixture
Samples Samples of diet mixtures (approximately 200 grams each) were placed into Nalgene^R jars for shipment to the analytical chemistry laboratory at Ethyl Corporation, 8000 GSRI Avenue, Baton Rouge, Louisiana, 70820. The container was labeled with the following information:

1. Study number
2. Date of sampling
3. Dose level of compound in the feed
4. Test article or code for test article

The samples were packed in dry ice in a styrofoam container. An additional set of all samples was retained and stored at -20°C until the results of the analysis were received.

000740

Homogeneity: Tests were conducted by the sponsor prior to initiation of the study to verify the homogeneity of the test article mixture in the feed. A total of three samples per mixing level (top left, top right and bottom), 18 samples for six dose levels, were collected and submitted to the sponsor each time the diet was prepared. Untreated samples were submitted for purposes of comparison.

Stability in Diet: The stability in the diet was determined previously by the sponsor to be 43 days.

Stability Under Test Conditions: The stability under test conditions was determined by the sponsor to be 4 days. The diet was renewed twice a week.

Authenticity and Purity of Test Article: The purity, identity, strength and stability of the test articles are the responsibility of the sponsor. There was no apparent change in the physical state of the test articles during administration.

Stability of Test Article under Conditions of Storage: Samples of the test articles were sent to the sponsor on Day 0 (May 19, 1987) and October 26, 1987 to determine stability under storage conditions (under Nitrogen at room temperature).

Test Article/Diet Mixtures Verification: Samples of the test article/diet mixtures and untreated diet were submitted to the sponsor on May 5, May 6, May 18 and May 20, 1987 for analysis of test article concentration in diet mixtures. The results of these analyses are attached on Appendix III.

TEST SYSTEM

Species: Rat

Strain: Sprague Dawley

Supplier (Source): Charles River Laboratories, Wilmington, Massachusetts

Sex: Male and female

Age at Initiation: Forty-eight (48) days old

No. on Study: Two hundred and ten (210) animals (105 males and 105 females).

0797

Method and
Justification for
Randomization:

000741
Treatment groups were housed by vertical cage positioning. Randomization was carried out by use of a random number table. Before technical initiation all rats were weighed, ranked according to body weight and assigned to treatment groups using a table of random numbers so that each treatment group would have a similar distribution according to body weight. An Analysis of Variance was performed to insure that no statistical significance was present between groups.

Acclimation
Period:

The rats were acclimated twelve days. During this conditioning period, the rats were weighed and observed for any pharmacotoxic signs of disease or inadequate weight gain.

System of
Identification:

Individual cages were marked with a rat number. Rats were individually identified by ear tags (Gey Band). The first cage card for each group and sex contained a legend of compound and dose level as well as rat number.

HUSBANDRY

Research Facility
Registration:

U.S.D.A. Registration No. 23-107 under the Animal Welfare Act 74: SC 2131 et seq.

Animal Rooms:

Separate isolation by test system.
Light cycle - 12 hours light, 12 hours dark.
Temperature/Humidity - Every attempt was made to maintain a temperature of 22°C ± 3°C and a humidity of 30 to 70%.

Housing:

Rats were housed individually in stainless steel 1/2" wire mesh cages. Size in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Resources, National Research Council.

Sanitization:

Waste material was removed two times a week. Cages and feeders were sanitized every two weeks.

Food:

Purina Certified Rodent Meal^R, ad libitum. Feeders were designed to reduce soiling, bridging, and scattering.

Food Analysis:

Certified food was used and the analysis from the supplier maintained in the central files.

Water Analysis:

Availability - fresh tap water, ad libitum. Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

000742

onset, degree and duration. Cageside observations included, but were not limited to, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behavior patterns. Mortality checks were made daily and recorded. Twice weekly measurements were made of food consumption, the diet was replenished with fresh freezer stored diet, and the animals were weighed.

CLINICAL EXAMINATIONS

Clinical Laboratory Studies:

Blood was drawn by percutaneous cardiocentesis. All animals were fasted overnight prior to the time of sacrifice. The following determinations were made on ten rats (5/sex) prior to initiation. In addition, the following hematology and clinical chemistry parameters were made on all animals sacrificed approximately 24 hours after 14, 28 and 56 days.

Hematology:

Hemoglobin
Hematocrit
Erythrocyte count
Total and differential leucocyte counts
Platelet count

Clinical Biochemistry:

Calcium
Phosphorus
Chloride
Sodium
Potassium
Fasting Glucose
Serum alanine aminotransferase
Serum aspartate aminotransferase
Serum amylase
Serum lipase
Gamma glutamyl transpeptidase
Urea nitrogen
Albumen
Blood creatinine
Total bilirubin
Total serum protein measurements
Serum insulin

Ophthalmologic:

Ophthalmological examinations using an ophthalmoscope were made prior to the administration of the test substance on Days 14 and 28 of treatment and at the end of recovery (Day 56) on all animals.

0 7 3 9

000743

PATHOLOGY

Gross Necropsy:

All animals were subjected to necropsy, which included examination of the external surface of the body, all orifices, the cranial, thoracic, abdominal and pelvic cavities and their contents. The following organs were weighed:

liver	gonads
adrenal glands	brain
kidneys	

The following organs and tissues from the animals sacrificed after 14 and 28 days of treatment and after the 28 day recovery period were preserved in a suitable medium for future histopathological examination:

All gross lesions
Brain-including sections of medulla/pons
Cerebellar cortex and cerebral cortex
Pituitary
Thyroid/parathyroid
Thymus
Lungs
Trachea
Heart
Sternum with bone marrow
Salivary glands
Liver
Spleen
Kidneys/adrenals
Pancreas
Gonads
Uterus
Accessory genital organs (epididymis, prostate,
and, if present, seminal vesicles)
Aorta
Skin
Esophagus
Nasal turbinates
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Rectum
Urinary bladder
Representative lymph node (mesenteric)
Mammary gland
Thigh musculature

0 7 4 0

000744

Peripheral nerve
Eyes
Femur-including articular surface
Spinal cord at three levels - cervical, midthoracic
and lumbar
Exorbital lachrymal glands

Histopathology:

A full histopathologic evaluation of the following organs and tissues were performed in all control and 320 ppm dosed animals sacrificed approximately 24 hours after 14 and 28 days of treatment and after the 28 day recovery period.

All gross lesions
Lungs
Liver
Adrenal glands
Kidneys
Gonads
Salivary gland
Eyes
Pancreas
Spleen
Mesenteric lymph node
Thymus
Sternum with bone marrow
Adipose tissue deposition (whenever possible)

In addition, the sternum with bone marrow, eyes, kidney, liver, mesenteric lymph node, pancreas, salivary gland, spleen, thymus, gross lesions and adipose tissue deposition was evaluated in low and mid dose groups.

RESULTS

Body Weights:

The group mean body weight data are presented in Table 1. Individual body weight data are attached in Appendix I. Statistically significant reductions in the mean male body weights were observed in the high dose (320 ppm) group on study Days 7 through 56 in the Lot 2-87 treated rats and Days 7, 14 and 21 through 56 in the Run 544 treated rats, when compared to the controls. In addition, the male body weights in the mid dose (.25 ppm) were also significantly reduced on Days 7 and 14 in the Run 544 treated rats. In the recovery period following DETDA treatment (Days 28-56), the high dose Run 544 group continued to lose weight, while animals treated with Lot 2-87 at the same dose gained weight.

0 7 4 0

745

METHODS

Rationale for Test System: Rats have historically been used for establishing safety criteria for human exposure.

Compound Preparation: Chemically treated diet was prepared on May 5, 1987, May 6, 1987, May 18, 1987 and May 20, 1987.

Rationale for Dose Selection: Dose levels were selected on the basis of previous 90 Day subchronic toxicity feeding data.

Dose Administration

Group*	No. of Animals	Test Article Lot #	Concentration	
			ppm	Estimated (mg/kg/rat/day)
I	30 (15M, 15F)	-	0	0
II	30 (15M, 15F)	2-87	50	5
III	30 (15M, 15F)	2-87	125	12.5
IV	30 (15M, 15F)	2-87	320	32
V	30 (15M, 15F)	Run 544, Drum 6131	50	5
VI	30 (15M, 15F)	Run 544, Drum 6131	125	12.5
VII	30 (15M, 15F)	Run 544, Drum 6131	320	32

*Approximately 24 hours after treatment for 14 and 28 days, five animals/sex/group were sacrificed. The remaining animals in each treatment group were administered untreated control diet (study Days 29-56) and sacrificed on Day 57.

Route of Administration: Oral, in diet

Rationale for Route of Administration: To determine the toxicity of the test articles by oral ingestion.

Frequency and Duration of Administration: Daily for 14 or 28 days. In addition, the remaining rats from each treatment group continued on test, untreated, on study Days 29-56.

Length of Study: Fifty-six (56) days

Methods of Study Performance: The animals were fed the diet/compound mixtures of one of two DETDA lot numbers 7 days/week for 14 or 28 days. Approximately 24 hours after 14 and 28 days of treatment, five animals/sex/group, selected by a random number table, were sacrificed. The remaining animals received untreated control diet for an additional 28 days. These animals were sacrificed to determine the recovery/reversibility of any treatment effects following Day 56. Signs of toxicity were recorded as they were observed, including the time

000746

The mean male body weights of the Run 544, Drum 6131, 320 ppm group were significantly lower when compared to the Lot 2-87 group on Days 49 through 56 (termination).

Female body weights were significantly reduced in both high dose DETDA groups on Day 14, mid dose on Day 24 and high dose on Day 42 in the Lot 2-87 groups, when compared to the controls.

No significant differences were noted for the females, when the different test formulations were compared to each other by dose level.

Daily Body Weight Gains:

The summary of the daily group mean body weight gains are set forth in Table 2. The individual data are attached in Appendix I. The daily body weight gains were significantly reduced in the mean Run 544 treated males in the low dose group (50 ppm) on Days 14 and 28, mid dose (125 ppm) on Days 7 and 14 and high dose (320 ppm) on Days 3, 14, 24 through 26, 35, 42 and 56, when compared to the control group. Daily body weight gains were significantly larger in the Run 544 low and mid dose group males on Day 10. The total gain was also significantly reduced in the high dose Run 544 treated males.

Statistically significant differences were also noted, when the daily gains between the different male DETDA treatment groups were compared. The Run 544 male mid dose group was significantly lower than the Lot 2-87 group on Day 7. On Day 10, however, Lot 2-87, low dose treated males gained significantly less than the Run 544 animals. The inverse was noted on Day 14 for the low dose group. A significantly lower value was noted for the male high dose Run 544 group on Day 56 when compared to the Lot 2-87 treated rats. The total mean daily gain was also significantly reduced in the high dose Run 544 group.

Females treated with Lot 2-87 were statistically reduced on Day 24 in the mid dose group and Days 14 and 28 in the high dose group when compared to the control group. Statistically significant suppression of body weight gain was exhibited in the low dose on Day 14, mid dose Day 3 and high dose group on Days 3, 14, 21 and 28 in the Run 544 treated rats, when compared to the controls.

The Run 544 low dose female body weight gains were significantly lower on Day 14, when compared to the Lot 2-87 group and significantly larger on Day 31.

Daily Food Consumption:

The group mean daily food consumption determinations are presented in Table 3. The individual food

0 7 4

747

consumption data are attached in Appendix I. The daily food consumption for the males was significantly reduced in both high dose DETDA groups on Days 3 through 14, 21 and 24, when compared to the controls. Reduced feed consumption was also observed in the Lot 2-87 high dose males on Day 17. Increased consumption was observed in the Run 544 males on Day 56.

There were no significant differences noted in males food consumption, when the different DETDA Lots were compared to each other by dose level.

High dose DETDA treated females exhibited significantly reduced food consumption in the 2-87 and Run 544 groups on Days 10 through 14, 21 and 28. The food consumption for the Lot 2-87 high dose treated females was also significantly reduced on Days 7 and 24. High dose Run 544 females also exhibited significantly reduced food consumption on Days 3 and 29.

There were no significant differences noted in female's food consumption, when the different DETDA Lots were compared to each other by dose level.

Clinical Signs:

Pharmacotoxic signs were observed and recorded for the individual animals and presented in Table 4. There were no signs exhibited by the females in any treatment group during the study. Signs exhibited by the males in the 2-87 treatment groups included chromodacryorrhea in three animals during Days 4-7. The majority of the high dose DETDA Run 544 treated males exhibited several pharmacotoxic signs. The onset of these observations occurred on Day 35 and continued until study termination. These observations included decreased activity, abnormal stance, abnormal gait, poor grooming, decreased body tone, piloerection and excessive defecation. One high dose Run 544 male exhibited scab formation on Days 10 through 20. One high dose DETDA, Lot 2-87 treated male exhibited decreased body tone on Days 42 through 50.

Mortality:

None of the rats died on test. Scheduled sacrifices are presented in Table 4.

Necropsy:

The incidence of the gross necropsy findings are summarized in Table 5. The individual observations recorded at necropsy and the individual necropsy sheets are attached in Appendix I. The majority of observations noted at either the Day 14 or Day 23 interim sacrifices were non-specific, low in incidence and without dose-dependent relationship. Similar observations, such as enlarged lymph nodes and dark

11744

000748

red lung lobes were observed in the majority of the treatment groups. Other gross changes included enlarged adrenals, kidneys exhibiting dilated pelves or cortical cysts, parovarian uterine cysts, hydrometra, skin scab. lobular liver lobes, prominent Peyer patches and adhered lung lobes. None of the above recorded Day 14 or Day 28 necropsy observations were observed dose-dependently or DETDA specific. There were, however, terminal necropsy findings (Day 56) which occurred with greater frequency in the high dose Run 544 treated males. These changes were reduced adipose tissue, rough hair coat, small salivary glands, small spleens, poorly visible, dark and small pancreata, small testes, seminal vesicles, epididymides, prostrate and gastrointestinal tract lesions. There were no similar DETDA related changes noted at necropsy for the females.

Absolute Organ Weights:

The absolute organ weight data are presented in Table 6. The individual organ weight raw data may be found in Appendix I. There were no statistically significant differences observed in the absolute male or female organ weights at the Day 14 or Day 28 necropsy, when compared to the control group. At the Day 56 terminal necropsy. the high dose Run 544 male body weights, liver and testes weights were significantly reduced.

Statistical comparisons made by groups treated with the DETDA lots indicated the high dose male body weights, liver and testes weights in the Run 544 group were significantly lower than the Lot 2-87 group at the terminal (Day 56) necropsy.

No significant differences were found between female organ weights at the Day 14, 28 or 56 necropsy.

Relative Organ to Body Weights:

The relative organ to body weights are presented in Table 7. The individual organ to body weight data are attached in Appendix I. A statistically significant increase in the high dose male 2-87 brain and testes weights was observed at the Day 14 necropsy when compare. to the controls. The mid dose Run 544 male testes weights were also significantly larger. There were no significant organ weight changes noted for the females, when the dosage groups were compared to the controls.

There were no significant differences observed for either the male or female relative organ to body weights, when comparisons were made between groups treated with the DETDA lots at the same dose level.

0 7 4 5

000749

The male relative liver weights were significantly larger in both high dose DETDA treated groups at the Day 28 necropsy, when compared to the control group. Significant increases were noted in both high dose DETDA treated female kidney weights. In addition, the female high dose Run 544 brain and liver weights were statistically larger, when compared to the control group.

There were no significant differences observed for either the male or female relative organ weights, when comparisons were made between the different DETDA materials by dose level at the Day 28 sacrifice.

At terminal necropsy (Day 56) the high dose Run 544 males displayed significantly larger relative brain, kidney and adrenal weights as compared to the controls.

When comparisons between DETDA materials were analyzed, significantly larger relative brain and kidney weights were recorded in the Run 544 high dose treated males versus the 2-87 males.

Females in the high dose 2-87 group exhibited significantly larger kidney weights, when compared to the controls at the Day 56 necropsy. The relative liver weights were also significantly larger in the Run 544 treated females, when compared to the control group.

There were no significant differences in the relative female organ weights, when comparisons were made between the DETDA formulations at the same treatment levels.

Relative Organ to
Brain Weights:

The relative organ to brain weight data are presented in Table 8. No statistically significant differences were observed for either the male or female relative organ to brain weights at the Day 14 or Day 28 interim sacrifices, when compared to the control group.

At the Day 56 necropsy, the percent liver to brain weight was significantly reduced in the high dose Run 544 treated males. No additionally significant data was apparent for either the males or females, when comparisons were made to the control group.

There were no significant differences noted for the relative organ to brain weights, when the different DETDA materials were compared by treatment level to each other at the Day 56 sacrifice.

750
000748

Clinical Chemistry:

Results of the blood chemistry evaluations at the scheduled sacrifices are summarized in Table 9. The individual data are presented in Appendix I. Statistically significant lower values were observed in total protein, albumin, phosphorus, creatinine and chloride in the mid and high dose male Run 544 groups, when compared to the controls at the Day 14 sacrifice. Male mid dose blood urea nitrogen and amylase values were also significantly reduced in the Run 544 treatment groups. A significant increase was observed in the calcium levels in the mid and high dose Run 544 dosed males.

Significant decreases were noted in bilirubin in the low and mid dose 2-87 male groups and the mid and high dose sodium groups, when compared to the controls. Total protein values were significantly larger in each 2-87 DETDA treatment groups, when compared to the controls.

The values for blood urea nitrogen, serum amylase, chloride, creatinine, total protein, albumin and phosphorus were significantly lower for the Run 544 males, when compared to the 2-87 males in the mid and high dose. The sodium and calcium values in the mid and high dose Run 544 males were significantly larger.

Significant differences were also noted for the females at the Day 14 interim sacrifice. Albumin, phosphorus, lipase and creatinine were significantly reduced in the mid and high dose Run 544 dose groups, when compared to the control group. The low dose creatinine and glucose Run 544 values were significantly lower, as were the low and mid dose chloride values. Low dose calcium levels for the Run 544 females were significantly larger than the control. Significant differences were noted for the 2-87 treated females, including decreases in: low, mid and high dose creatinine, low and high dose chloride and mid dose glucose. Increases were observed in the high dose calcium determinants of females treated with 2-87.

Analyses of the data between the DETDA lot treatments, revealed significantly lower values for chloride, amylase, total protein, albumin and phosphorus in the mid dose Run 544 females group. Significantly larger mid and high dose insulin values were also noted in those females treated with Run 544 DETDA.

There were several blood chemistry changes recorded for the males and females at the Day 28 interim

0 7 4 7

sacrifice which were significantly different from the controls. Changes noted for the males included creatinine values that were significantly lower in each of the DETDA treated groups. Total protein, albumin, phosphorus and GGPT determinants were significantly lower than the control values in each of the Run 544 treated groups. Blood urea nitrogen values were significantly lower in the Run 544 mid and high dose groups, whereas the SGOT and chloride parameters were significantly lower in the latter dose group. Increases which were statistically significant included high dose Run 544 male sodium, insulin and amylase values. Significant alterations from controls were also recorded in the DETDA 2-87 groups. Statistically significant decreases were recorded for the males in creatinine (all groups) and mid dose SGPT, total protein, albumin, phosphorus and high dose chloride values. GGPT decreases were noted in the low and high dose groups.

Comparisons between chemical chemistry parameters of groups treated with different DETDA materials at the Day 28 sacrifice indicated a significantly larger sodium value for the Run 544 high dose males and mid dose phosphorus versus the 2-87 males.

Statistically significant decreases were observed in each of the Run 544 female treated levels in total protein, albumin and blood urea nitrogen values at the Day 28 interval, when compared to the control. Sodium values of the low and mid dose groups were significantly lower. Serum lipase values were significantly higher and potassium lower in the low dose Run 544 groups when compared to the controls. The sodium values were significantly decreased in each of the 2-87 treated levels. The mid and high dose potassium levels were significantly lower, when compared to the controls. The mid dose serum lipase and GGPT values were significantly increased.

Comparisons between DETDA materials indicated the mid and high dose blood urea nitrogen values were significantly lower in the Run 544 females. The high dose Run 544 females' potassium and sodium values were significantly larger and the high dose total protein values lower, when the DETDA lots were compared by dose level.

At the Day 56 terminal sacrifice, significant alterations in the blood chemistry parameters were observed for both males and females. Statistically significant increases were noted in blood urea

752
~~000750~~

nitrogen, SGOT and GGPT parameters for the high dose Run 544 males, when compared to the controls. Mid and high dose Run 544 male decreases were recorded in the calcium values, as were low and mid dose sodium and mid dose phosphorus. A significant increase was recorded in the low dose chloride of Run 544 treated males. Changes recorded for the 2-87 males included significantly increased creatinine values at each treatment level, phosphorus at the mid and high dose, and chloride at the mid dose when compared to the controls.

Comparisons of the DETDA-treated males by material and dose level indicated significantly larger blood urea nitrogen, SGOT and GGPT values in the Run 544 high dose group. Sodium values were significantly lower in the low and mid dose group. Amylase and albumin values in the high dose Run 544 groups were significantly reduced. Phosphorus and chloride were significantly lower in the mid dose in the Run 544 groups.

Differences were also noted in the clinical chemistry parameters of DETDA-treated females sacrificed at Day 56. High dose Run 544 females exhibited significant decreases in mid dose and high dose blood urea nitrogen and high dose creatinine, when compared to the controls. A significant increase was observed in the high dose calcium and total protein values. The mid dose lipase values were also significantly higher than the controls. Females treated with Lot 2-87 exhibited significantly larger mid and high dose calcium and chloride values, mid dose total protein and high dose sodium values.

Comparisons between the Run 544 and Lot 2-87 treated females indicated statistically significant lower creatinine and blood urea nitrogen values for the high dose Run 544 females. A significant low dose albumin value was noted for the Run 544 females.

Hematology:

The results from the hematologic evaluation are summarized in Table 10. The individual data are presented in Appendix I. The low dose male Lot 2-87 platelet counts were significantly lower at the Day 14 necropsy, when compared to the controls. No other significant hematological alterations in male or females parameters were noted at Day 14 sacrifice, when comparisons were made to the controls or between the DETDA treatment groups.

0749?

753
~~000751~~

The high dose male and mid dose female platelet counts were significantly lower at the Day 28 necropsy in the Run 544 DETDA groups, when comparisons were made to the control group.

Comparisons of DETDA materials at Day 28 indicated the platelet counts for each DETDA-treated level of the Run 544 females were significantly larger, when compared to the Lot 2-87 treated animals.

High dose males in the Run 544 treated group exhibited a statistically significant increase in hemoglobin values at the Day 56 sacrifice, when compared to controls. Significant decreases in the male leucocyte and platelet counts were noted at this level.

The hemoglobin values for the high dose Run 544 males were significantly larger and the leucocyte values significantly smaller than the Lot 2-87 males at Day 56.

Ophthalmologic Examination:

All rats in each dose group were examined prior to study initiation and prior to the scheduled sacrifices utilizing the indirect ophthalmoscopic procedure. The examination identified adnexa, conjunctiva, sclera, cornea, anterior chamber, lens and posterior segment (vitreous, retina and optic disc). Eighteen rats exhibited abnormal ocular defects prior to study initiation. These animals were subsequently replaced with normal animals. On Day 14 of the study (prior to the interim necropsy), seven ocular lesions were noted. Striate retinopathy of the right eye was detected in one control male. Focal keratitis and striate retinopathy (both in the right eyes) were recorded from a low dose 2-87 male and mid dose 2-87 female, respectively. Three lesions were noted in the high dose 2-87 group. A hyaloid remnant was recorded in the right eye of one male whereas, hyphema and striate retinopathy was exhibited in the left eye of a male and female, respectively.

One lesion was detected in the Run 544 Drum 6131 treated rats. Striate retinopathy was detected in the left eye of a mid dose male. The presumed cause for these lesions were previous or current SDA virus infection.

On study Day 28, the rats remaining on test were examined. Ten lesions were detected. Two control males displayed retinopathy in the right eye. Patent hyaloid remnant was observed in the left eye of one low dose 2-87 male and striate retinopathy in the left

0798

754
000752

eye in one mid dose male and high dose female also in the 2-87 groups. Striate retinopathy was detected in the right eye in one low dose Run 544 female and one mid dose male. This mid dose male also displayed hyphema (small pupil) in the left eye. In addition, one male in this treatment level exhibited patent hyaloid remnant of both eyes. Focal corneal opacity was detected in the left eye of one high dose Run 544, male.

On study Day 56, the pretermination ocular examination was performed on the seventy remaining animals. Five lesions were noted. Two control males and one low dose Run 544, female exhibited right eye striate retinopathy. Multifocal anterior cataracts were detected in two high dose Run 544, males. The appearance of the cataracts suggests a possible ocular effect of the test material. Cataracts may result from direct toxic effect on lens cells, indirect lens cells, or primary metabolic abnormalities which affect other body systems. The retinopathy is most likely associated with previous SDA virus infection.

Histopathology:

Untreated Controls

Evaluation of the pancreas at 14 days demonstrated no microscopic changes in two males and two females. In the remaining rats, there was a minimal multifocal acinar degeneration present in two males and two females and a slight multifocal acinar degeneration in one male and one female. No significant microscopic changes were present in any of the other tissues evaluated.

Evaluation of the pancreas at 28 days showed no microscopic changes in the pancreas of one male and two females. In the remaining rats, there was a minimal to slight multifocal acinar degeneration in four males and two females. Three female rats had minimal to moderate multifocal or diffuse acinar basophilia. No significant microscopic changes were present in any of the other tissues evaluated.

At Day 56, no microscopic changes were present in the pancreas of three males and one female. Minimal multifocal acinar degeneration was present in one male and one female. Minimal to slight multifocal to diffuse acinar basophilia was present in one male and four females. No significant microscopic changes were present in any other tissues evaluated.

0754

755
000753

DETDA 2-87 (Groups II, III, and IV)

In the pancreas of the rats receiving 320 ppm (Group IV) and sacrificed at 14 days, all five males had a moderate to moderately severe multifocal acinar degeneration. A slight focal acinar atrophy was present in one of these males (Number 7495). Male Number 7500 had a slight lymphoid depletion of the spleen, minimal involution of the thymus, and atrophy of the adipose tissues. A minimal to slight multifocal acinar degeneration (similar to the untreated controls) was present in four of the five females of Group IV. A moderate multifocal acinar degeneration was present in the fifth female. No significant microscopic changes were present in the other tissues evaluated at 14 days from the rats receiving 50, 125 or 320 ppm of DETDA 2-87.

In the pancreas of the rats receiving 320 ppm and sacrificed at 28 days, a moderate to moderately severe multifocal acinar degeneration was present in four of five males and all five females. In the fifth male, this multifocal acinar degeneration was only slight. These changes were accompanied in all ten rats by a minimal to moderate diffuse atrophy of the acinar cells. The salivary glands of three males and three females had minimal to moderate multifocal acinar degeneration. Adipose tissue atrophy was present in one female receiving 320 ppm of DETDA 2-87. One male (Number 7468) and one female (Number 7480) receiving 1.5 ppm had moderate multifocal pancreatic acinar degeneration. Female Number 7480 also had a slight diffuse atrophy of the pancreas. No significant microscopic changes were present in the other tissues evaluated at 28 days from the rats receiving 50, 125 or 320 ppm of DETDA 2-87.

In the pancreas of the rats and sacrificed at Day 56 (following a 28 Day recovery period), there was a moderate multifocal acinar degeneration in two of the five males and one of the five females. Minimal diffuse atrophy of acinar cells was present in two males and three females. Minimal to slight multifocal basophilia of acinar cells was present in three males and one female. Male Number 7492 had a moderate vacuolation of islet cells and an atrophy of the adipose tissues. Incidental findings also present in male Number 7492 were a moderate multifocal granulomatous hepatitis of the liver and a diverticulum in the small intestine. A moderate multifocal acinar degeneration of the pancreas was present in three males receiving 125 ppm of DETDA

756
000754

2-87. No significant microscopic changes were present in any of the other tissues evaluated at 56 days from the rats receiving 50, 125 or 320 ppm of DETDA 2-87.

DETDA Run 544 Drum 6131 (Groups V, VI and VII)

In the pancreas of the rats receiving 320 ppm (Group VII) and sacrificed at 14 days, there was a moderate multifocal acinar degeneration in three males and one female. No other significant microscopic changes were present in any of the tissues evaluated at 14 days from the rats receiving 50, 125, or 320 ppm of DETDA Run 544 Drum 6131.

In the pancreas of the rats receiving 320 ppm and sacrificed at 28 days, there was a moderate multifocal acinar degeneration in four males and three females, and moderately severe degeneration in two females. These changes were accompanied by minimal to moderate diffuse acinar atrophy in all ten animals. Multifocal acinar basophilia was present to a minimal degree in one female and to a moderate degree in one male. A minimal to slight multifocal acinar degeneration of the salivary gland was present in two males and four females. Atrophy of the adipose tissue was observed in one female. One male receiving 50 ppm and one male receiving 125 ppm had moderate multifocal acinar degeneration and slight diffuse atrophy of the pancreas. No other significant microscopic changes were present in any of the tissues evaluated at 28 days from the rats receiving 50, 125 or 320 ppm of DETDA Run 544.

In the females at all three dose levels and sacrificed at Day 56, there was very little pancreatic change, merely a minimal to slight multifocal acinar degeneration similar to the untreated controls. However, in the males receiving 320 ppm (Group VII), a moderate to moderately severe diffuse acinar atrophy was present in four of the five males. This was accompanied by a slight to moderate diffuse basophilia of the acinar cells and a slight to moderately severe vacuolation of the islet cells. Concurrent in these four rats, there were bilateral cataractous changes in the eyes. In the fifth high dose male rat, the pancreas had a moderate multifocal acinar cell degeneration, a slight diffuse atrophy of the acinar cells, and a minimal vacuolation of islet cells. No cataractous changes were present in the eyes of this rat. In three of the five high dose males, there was a moderate to moderately severe lymphoid depletion of the spleen, an absence or severe involution of the

0759

757
000755

thymus, and an atrophy of the adipose tissues. These rats also had a reduction in the number of bone marrow cells and a lymphoid depletion in the mesenteric lymph nodes. Minimal to moderate focal atrophy of the salivary gland was present in two of these three male rats. Vacuolation of the tubular cells of the kidney was present in two of the five high dose males. One of these high dose males had atrophy of the liver and atrophy of the reproductive organs (testes, prostate and seminal vesicles).

Two males receiving 125 ppm of DETDA Run 544 had moderate multifocal degeneration of the pancreatic acinar cells and two had a slight diffuse atrophy of the pancreas. No significant changes were present in the other tissues evaluated from the males or females receiving 50, 125 or 320 ppm of DETDA Run 544 Drum 6131.

Dose time compound-related histomorphologic changes occurred in the pancreas of male and female rats receiving both formulations of DETDA, when administered orally for 28 days at the mid and high dose levels of 125 and 320 ppm, respectively. Similar effects were not evident at the low dose of 50 ppm for either DETDA 2-87 or DETDA Run 544. The pancreatic changes were characterized principally by an increased incidence and severity of acinar cell degeneration. Following the 28 Day Recovery Period, three of five male rats receiving 320 ppm of DETDA Run 544 demonstrated a progression of these changes to include moderate to moderately severe islet cell vacuolation and the secondary effects of pancreatic insufficiency.

A variety of spontaneous and incidental findings occurred in various organs and tissues. These lesions were of the usual number and type commonly diagnosed in rats of this age and strain and occurred for the most part at comparable incidences and severity in the untreated control and treated rats.

A detailed evaluation of the microscopic changes are presented in Appendix II from Experimental Pathology Laboratories, Inc.

CONCLUSION

Based primarily on the severity and progressive treatment-related histopathological changes of the rats with the Run 544, Drum 6131 material and secondly the correlated alterations on chemical chemistry, it appears that the DETDA lot 2-87 formulation was less toxic than the Run 544, Drum 6131 preparation.

0 7 5 9

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY: PH436-ET-001-87; DETDA
 SPONSOR: ETHYL CORP., BATON ROUGE, LA
 TABLE 1

758
 000755

SUMMARY OF BODY WEIGHTS (Grams)

STUDY: 436ET01

SEX: MALE

PERIOD	DOSE: (ppm) GROUP:	0	50	125	320	50	125	320
		CONTROL	LOT 2-87	LOT 2-87	LOT 2-87	RUN 544	RUN 544	RUN 544
DAY 0	MEAN	211	204	208	203	202	202	206
	S.D.	11.4	11.4	10.7	9.9	8.9	11.9	12.2
	N	15	15	15	15	15	15	15
DAY 3	MEAN	233	229	233	221	227	226	223
	S.D.	13.0	15.2	12.4	11.0	10.5	9.1	11.6
	N	15	15	15	15	15	15	15
DAY 7	MEAN	260	260	265	250**	259	252*	254*
	S.D.	18.1	18.1	17.3	13.6	8.9	15.5	15.6
	N	15	15	15	15	15	15	15
DAY 10	MEAN	290	281	286	270**	266	279	275
	S.D.	18.2	19.3	18.4	12.2	12.1	16.2	14.9
	N	15	15	15	15	15	15	15
DAY 14	MEAN	318	311	310	287**	309	295**	293**
	S.D.	19.2	21.5	21.2	13.6	13.5	17.7	14.0
	N	15	15	15	15	15	15	15
DAY 17	MEAN	327	321	329	297**	330	316	308
	S.D.	22.2	21.2	19.6	17.1	12.8	19.0	14.7
	N	10	10	10	10	10	10	10
DAY 21	MEAN	345	345	350	316**	351	335	321*
	S.D.	22.8	22.0	21.8	18.0	15.7	20.4	16.8
	N	10	10	10	10	10	10	10
DAY 24	MEAN	364	363	370	329**	369	350	332*
	S.D.	29.1	25.8	23.6	18.9	17.1	22.3	16.2
	N	10	10	10	10	10	10	10

* P less than .05

** P less than .01

Analysis of Variance using DUNNETT'S Procedure

(REPORT CONTINUED)

0 7 5 5

757
~~009757~~

PHARMAKON RESEARCH INTERNATIONAL, INC.
 REPEATED DOSE ORAL TOX/REVERSIBILITY
 STUDY: PH436-ET-001-87; DETDA
 SPONSOR: ETHYL CORP., BATON ROUGE, LA
 TABLE 1

SUMMARY OF BODY WEIGHTS (Grams)

		STUDY: 436ET01				SEX: MALE			
PERIOD	DOSE: (ppm)	0	50	125	320	50	125	320	
	GROUP:	CONTROL	LOT 2-87	LOT 2-87	LOT 2-87	RUN 544	RUN 544	RUN 544	
DAY 28	MEAN	376	381	386	336**	389	365	337**	
	S.D.	31.1	28.5	26.0	18.4	19.5	24.6	20.7	
	N	10	10	10	10	10	10	10	
DAY 31	MEAN	405	392	404	343**	406	383	344**	
	S.D.	25.8	25.7	27.2	19.1	28.7	27.6	13.5	
	N	5	5	5	5	5	5	5	
DAY 34	MEAN	416	409	417	350**	411	397	325**	
	S.D.	22.4	16.7	10.0	22.5	28.5	35.4	34.0	
	N	5	5	5	5	5	5	5	
DAY 38	MEAN	426	417	428	360**	429	405	323**	
	S.D.	14.1	25.7	6.7	27.2	39.7	40.0	34.0	
	N	5	5	5	5	5	5	5	
DAY 42	MEAN	440	419	432	359**	430	413	307**	
	S.D.	16.6	24.4	7.3	33.4	36.5	47.2	43.9	
	N	5	5	5	5	5	5	5	
DAY 45	MEAN	458	441	460	377**	449	427	307**	
	S.D.	28.7	25.8	9.3	37.3	46.3	42.7	54.2	
	N	5	5	5	5	5	5	5	
DAY 49	MEAN	463	449	461	372**	453	441	286** ^a	
	S.D.	32.7	30.4	2.3	51.1	20.0	44.8	67.7	
	N	5	5	5	5	5	5	5	
DAY 52	MEAN	481	463	482	392**	477	456	311** ^a	
	S.D.	32.0	27.4	12.1	49.8	33.9	46.2	56.2	
	N	5	5	5	5	5	5	5	

* P less than .05

Analysis of Variance using DUNNETT'S Procedure

** P less than .01

(REPORT CONTINUED)

a - denotes significance between DETDA Lots at same dose level (p less than .05)

0 7 5 6

EPL

1857

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

PHARMAKON STUDY NUMBER PH 436-ET-001-87

REPEATED DOSE ORAL TOXICITY/
REVERSIBILITY STUDY IN RATS - 28 DAY

PATHOLOGY REPORT

Submitted to:

Pharmakon Research International, Inc.
Waverly, PA 18471

November 3, 1987

1 0 5 3

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

TABLE OF CONTENTS

	<u>Page</u>
PATHOLOGY SUMMARY	1
QUALITY ASSURANCE REPORT CERTIFICATION.	10

14 DAY SACRIFICE

SUMMARY INCIDENCE TABLES

Males	I-1
Females	I-11

HISTOPATHOLOGY INCIDENCE TABLES

Males	II-1
Females	II-10

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

Males	III-1
Females	III-5

28 DAY SACRIFICE

SUMMARY INCIDENCE TABLES

Males	IV-1
Females	IV-11

HISTOPATHOLOGY INCIDENCE TABLES

Males	V-1
Females	V-10

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

Males	VI-1
Females	VI-7

TABLE OF CONTENTS (CONTINUED)

Page

56 DAY SACRIFICE

SUMMARY INCIDENCE TABLES

Males	VII-1
Females	VII-17

HISTOPATHOLOGY INCIDENCE TABLES

Males	VIII-1
Females	VIII-15

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

Males	IX-1
Females	IX-10

1360

PATHOLOGY SUMMARY

1056

PHARMAKON STUDY NUMBER PH 436-ET-001-87

REPEATED DOSE ORAL TOXICITY/REVERSIBILITY
STUDY IN RATS - 28 DAY

PATHOLOGY SUMMARY

Microscopic examinations were performed on selected tissues from 210 Sprague-Dawley rats used in a twenty-eight day repeated dose oral toxicity and reversibility study. The purpose of this study was to determine the toxicity of two formulations of Diethyl Toluene Diamine (DETDA 2-87 and DETDA Run 544 Drum 6131) when administered orally for up to twenty-eight consecutive days with necropsies performed on selected animals after fourteen, twenty-eight, and fifty-six (recovery) days. The animals used in this study are outlined in the following table:

<u>Group</u>	<u>Number of Animals</u>	<u>Test Article</u>	<u>Dose (ppm)</u>
I	30 (15 Male, 15 Female)	Untreated	0
II	30 (15 Male, 15 Female)	DETDA 2-87	50
III	30 (15 Male, 15 Female)	DETDA 2-87	125
IV	30 (15 Male, 15 Female)	DETDA 2-87	320
V	30 (15 Male, 15 Female)	DETDA Run 544 Drum 6131	50
VI	30 (15 Male, 15 Female)	DETDA Run 544 Drum 6131	125
VII	30 (15 Male, 15 Female)	DETDA Run 544 Drum 6131	320

According to protocol, the following hematoxylin and eosin stained tissues were evaluated from all rats in Groups I, IV, and VII: adrenals, eyes, gonads (ovaries and testes), kidneys, liver, lung, mesenteric lymph node, pancreas, salivary gland, spleen, sternum (bone and bone marrow), thymus, and all gross lesions. Adipose tissue

deposition was evaluated wherever possible as an indication of the nutritional state of the animal.

Based on the microscopic findings found in the high dose rats in Groups IV and VII, the following tissues were evaluated from the rats in Groups II, III, V, and VI: bone marrow (sternum), eyes, kidneys, liver, mesenteric lymph node, pancreas, salivary gland, spleen, thymus, and all gross lesions. Adipose tissue deposition was also evaluated.

Hematoxylin and eosin stained slides of the required tissues were prepared by Experimental Pathology Laboratories, Inc. Microscopic findings for each tissue examined from each animal are listed in the Histopathology Incidence Table. Inflammatory, degenerative, and hyperplastic changes were graded one to five depending upon severity. Nongradable changes, such as cysts and developmental changes, were designated as present (P) in the Histopathology Incidence Tables. All lesions are summarized by treatment groups and sex in the Summary Incidence Tables together with the total number of animals for each group for which tissues were examined. A tabulation of the gross lesions observed at the time of necropsy with the corresponding microscopic change, if applicable, is given in the Correlation of Gross and Microscopic Findings Tables. The description of the gross findings on these tables was transcribed from the Individual Animal Worksheets prepared at the time the necropsies were performed.

The following tissues were evaluated in a blind fashion from the fourteen, twenty-eight, and fifty-six day sacrifices: pancreas, spleen, thymus, adipose tissue, and salivary glands.

RESULTS IN GENERAL

Dose/time compound-related histomorphologic changes occurred in the pancreas of male and female rats receiving both formulations of DETDA when administered orally for twenty-eight days at the mid and high dose levels of 125 and 320 ppm, respectively. Similar effects were not evident at the low dose of 50 ppm for either DETDA 2-87 or DETDA Run 544 Drum 6131. The pancreatic changes were characterized principally by an increased incidence and severity of acinar cell degeneration. Following the twenty-eight day recovery period, three of five male rats receiving 320 ppm of DETDA Run 544 Drum 6131 demonstrated a progression of these changes to include moderate to moderately severe islet cell vacuolation and the secondary effects of pancreatic insufficiency.

A variety of spontaneous and incidental findings occurred in various organs and tissues. These lesions were of the usual number and type commonly diagnosed in rats of this age and strain and occurred for the most part at comparable incidences and severity in the untreated control and treated rats.

RESULTS OF UNTREATED CONTROLS (Group I)Untreated Controls - 14 Days

Evaluation of the pancreas demonstrated no microscopic changes in two males and two females. In the remaining rats, there was a minimal multifocal acinar degeneration present in two males and two females and a slight multifocal acinar degeneration in one male and one female. No significant microscopic changes were present in any of the other tissues evaluated.

Untreated Controls - 28 Days

Evaluation of the pancreas at twenty-eight days showed no microscopic changes in the pancreas of one male and two females. In the remaining rats, there was a minimal to slight multifocal acinar degeneration in four males and two females. Three female rats had minimal to moderate multifocal or diffuse acinar basophilia. No significant microscopic changes were present in any of the other tissues evaluated.

Untreated Controls - 56 Day Recovery

No microscopic changes were present in the pancreas of three males and one female. Minimal multifocal acinar degeneration was present in one male and one female. Minimal to slight multifocal to diffuse acinar basophilia was present in one male and four females. No significant microscopic changes were present in any other tissues evaluated.

RESULTS OF DETDA 2-87 (Groups II, III, and IV)DETD 2-87 - 14 Days

In the pancreas of the rats receiving 320 ppm (Group IV), all five males had a moderate to moderately severe multifocal acinar degeneration. A slight focal acinar atrophy was present in one of these males (Number 7495). Male Number 7500 had a slight lymphoid depletion of the spleen, minimal involution of the thymus, and atrophy of the adipose tissues. A minimal to slight multifocal acinar degeneration (similar to the untreated controls) was present in four of the five females of Group IV. A moderate multifocal acinar degeneration was present in the fifth female. No significant microscopic changes were present in the other tissues evaluated at fourteen days from the rats receiving 50, 125, or 320 ppm of DETDA 2-87.

DETD 2-87 - 28 Days

In the pancreas of the rats receiving 320 ppm, a moderate to moderately severe multifocal acinar degeneration was present in four of five males and all five females. In the fifth male, this multifocal acinar degeneration was only slight. These changes were accompanied in all ten rats by a minimal to moderate diffuse atrophy of the acinar cells. The salivary glands of three males and three females had minimal to moderate multifocal acinar degeneration. Adipose tissue atrophy was present in one female receiving 320 ppm of DETDA 2-87. One male (Number 7468) and one female (Number 7480) receiving 125 ppm had moderate multifocal pancreatic acinar degeneration. Female Number 7480 also had a

slight diffuse atrophy of the pancreas. No significant microscopic changes were present in the other tissues evaluated at twenty-eight days from the rats receiving 50, 125, or 320 ppm of DETDA 2-87.

DETDA 2-87 - 56 Day Recovery

In the pancreas of the rats following the recovery period, there was a moderate multifocal acinar degeneration in two of the five males and one of the five females. A minimal diffuse atrophy of acinar cells was present in two males and three females. A minimal to slight multifocal basophilia of acinar cells was present in three males and one female. Male Number 7492 had a moderate vacuolation of islet cells and an atrophy of the adipose tissues. Incidental findings also present in male Number 7492 were a moderate multifocal granulomatous hepatitis of the liver and a diverticulum in the small intestine. A moderate multifocal acinar degeneration of the pancreas was present in three males receiving 125 ppm of DETDA 2-87. No significant microscopic changes were present in any of the other tissues evaluated at fifty-six days from the rats receiving 50, 125, or 320 ppm of DETDA 2-87.

RESULTS OF DETDA RUN 544 DRUM 6131 (Groups V, VI, and VII)

DETDA Run 544 Drum 6131 - 14 Days

In the pancreas of the rats receiving 320 ppm (Group VII), there was a moderate multifocal acinar degeneration in three males and one female. No other significant microscopic changes were present in any

of the tissues evaluated at fourteen days from the rats receiving 50, 125, or 320 ppm of DETDA Run 544 Drum 6131.

DETTA Run 544 Drum 6131 - 28 Days

In the pancreas of the rats receiving 320 ppm, there was a moderate multifocal acinar degeneration in four males and three females, and moderately severe degeneration in two females. These changes were accompanied by minimal to moderate diffuse acinar atrophy in all ten animals. Multifocal acinar basophilia was present to a minimal degree in one female and to a moderate degree in one male. A minimal to slight multifocal acinar degeneration of the salivary gland was present in two males and four females. Atrophy of the adipose tissue was observed in one female. One male receiving 50 ppm and one male receiving 125 ppm had moderate multifocal acinar degeneration and slight diffuse atrophy of the pancreas. No other significant microscopic changes were present in any of the tissues evaluated at twenty-eight days from the rats receiving 50, 125, or 320 ppm of DETDA Run 544 Drum 6131.

DETTA Run 544 Drum 6131 - 56 Day Recovery

In the females at all three dose levels at fifty-six days, there was very little pancreatic change, merely a minimal to slight multifocal acinar degeneration similar to the untreated controls. However, in the males receiving 320 ppm (Group VII), a moderate to moderately severe diffuse acinar atrophy was present in four of the five males. This was accompanied by a slight to moderate diffuse basophilia of the acinar cells and a slight to moderately severe vacuolation of the

islet cells. Concurrent in these four rats, there were bilateral cataractous changes in the eyes. In the fifth high dose male rat, the pancreas had a moderate multifocal acinar cell degeneration, a slight diffuse atrophy of the acinar cells, and a minimal vacuolation of islet cells. No cataractous changes were present in the eyes of this rat. In three of the five high dose males, there was a moderate to moderately severe lymphoid depletion of the spleen, an absence or severe involution of the thymus, and an atrophy of the adipose tissues. These rats also had a reduction in the number of bone marrow cells and a lymphoid depletion in the mesenteric lymph nodes. Minimal to moderate focal atrophy of the salivary gland was present in two of these three male rats. Vacuolation of the tubular cells of the kidney was present in two of the five high dose males. One of these high dose males had atrophy of the liver and atrophy of the reproductive organs (testes, prostate, and seminal vesicles).

Two males receiving 125 ppm of DETDA Run 544 Drum 6131 had moderate multifocal degeneration of the pancreatic acinar cells and two had a slight diffuse atrophy of the pancreas. No significant changes were present in the other tissues evaluated from the males or females receiving 50, 125, or 320 ppm of DETDA Run 544 Drum 6131.

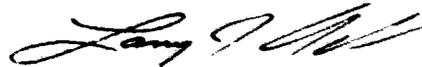
CONCLUSION

Exposure to DETDA 2-87 and DETDA Run 544 Drum 6131 for twenty-eight days resulted in compound-related degenerative acinar changes in

067
EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

the pancreas at levels of 125 ppm and 320 ppm in males and 320 ppm in females. A similar pancreatic acinar degeneration was not present at the 50 ppm dosage for either compound. Male rats were more severely affected and at an earlier time than the female rats. The pancreatic effects of DETDA 2-87 had fairly well resolved following the twenty-eight day recovery period but the changes in the males exposed to 320 ppm of DETDA Run 544 Drum 6131 appeared to progress and included degeneration of the islet cells of the pancreas and secondary changes of cachexia and diabetes.



LARRY J. ACKERMAN, V.M.D.
Pathologist

November 3, 1987

LJA/wk

