

Report Title: Acute Oral Toxicity (LD50) Study in Rats

Test Type: Acute Oral Toxicity (LD50)

Conducting Laboratory and Location: International Research and Development Corporation

Test Substance(s): #T7121 - 2% Placebo Shampoo at pH 4.5. Undiluted material used for dosing.

Species: Rat

of Animals: 25 male, 25 female

Test Conditions: Dosed orally (gavage) with 5.13, 8.14, 12.92, 20.51, and 32.55 g/kg.

Results: Combined: LD₅₀>10.39 g/kg. See related studies 191-080, 191-081.

Study #: 191-082

Report Date: 03/21/78

Accession #: 20031

International Research and Development Corporation

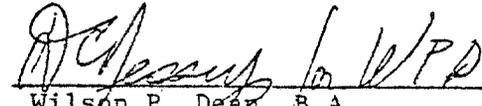
SPONSOR: The Procter and Gamble Company
TEST MATERIAL: T 7121
SUBJECT: Acute Oral Toxicity (LD₅₀) Study
in Rats.

FINAL REPORT
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MAR 23 1978

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Approved by: D. Clifford Jessup, Ph.D
Associate Director of Research

Date: March 21, 1978

International Research and Development Corporation

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APPENDIX 1

LD₅₀ - Record of Individual Dose Level

APPENDIX 2

Acute Oral Toxicity Observation Data

I. SYNOPSIS

The acute oral LD₅₀ and 95% confidence limits of T 7121 in combined male and female rats were found to be 10391 (8269 - 12966) mg/kg.

II. TEST MATERIAL

The test material was received from The Procter and Gamble Company, Cincinnati, Ohio on August 4, 1977. It was identified as "T 7121" and was received as a slightly yellow viscous liquid.

III. METHOD

Twenty-five male and 25 female rats of the Charles River CD strain (obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan) weighing 202 to 262 (pre-fasting body weight) were used for this study. The rats were housed by sex in groups of 5 rats per cage in hanging wire-mesh cages in temperature and humidity controlled quarters. They were maintained in accordance with the recommendations contained in H.E.W. Publication No. 74-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals". The rats were conditioned for a minimum of 7 days prior to study initiation. Water and Purina Laboratory Chow were available ad libitum, except for an overnight period of 18 1/4 to 19 1/4 hours immediately preceding oral administration during which food, but not water, was withheld.

The test material was administered orally by gavage, as received, undiluted, at the following dosage levels to male and female rats: 5126, 8137, 12918, 20507 and 32555 mg/kg.

Five rats of each sex were used at each dosage level. Volumes administered were as follows:

5126 mg/kg level - 5.03 ml/kg
8137 mg/kg level - 8.05 ml/kg
12918 mg/kg level - 12.78 ml/kg
20507 mg/kg level - 20.28 ml/kg
32555 mg/kg level - 32.20 ml/kg

Observations for pharmacotoxic signs and mortality were made at 1/4, 1/2, 1, 2, 4 and 24 hours and daily thereafter for a total of 14 days. Body weights were recorded prior to fasting, immediately preceding dosing and at 14 days. All rats which died on study were subjected to gross necropsy examination as were all survivors at the end of the 14-day observation period.

IV. RESULTS

A. MORTALITY AND LD₅₀ VALUES:

Dose - Mortality Data

Dosage Level mg/kg	Number of Deaths														Total Mortalities				
	Hrs.		Days																
	0-4		1	2		3		4		5		6		7-14		Male	Female	Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M				F
5126																	0/5	0/5	0/10
8137								1	1	1							1/5	2/5	3/10
12918			4	4													4/5	4/5	8/10
20507		1	5	3													5/5	4/5	9/10
32555	2	4	3	1													5/5	5/5	10/10

Acute Oral LD₅₀ and 95% Confidence Limits

Combined Male and Female Rats: 10391 (8269 - 12966) mg/kg.

Statistical Reference

Computations were performed by Mr. R. Bruce, Statistician, Procter and Gamble Company, using the computer program BLISS 17, written by D. J. Finney, University of Edinburgh, Scotland.

B. PHARMACOTOXIC SIGNS:

See attached Appendix 2.

C. BODY WEIGHTS:

All surviving rats exhibited normal body weight gains with the exception of 2 males at the 5126 mg/kg level, 2 males at the 8137 mg/kg, 1 male at the 12918 mg/kg level and 1 female at the 20507 mg/kg level all of which exhibited less than normal body weight gains and 1 male and 2 females at the 5126 mg/kg level which showed body weight losses.

D. NECROPSY FINDINGS:

Necropsy observations were noted as indicated on pages 5 and 6.

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1. Rats which died during the study period:

Gross Necropsy Observations:	Dosage Level mg/kg Number Exhibiting Sign/Number Necropsied							
	8137		12918		20507		32555	
	Male	Female	Male	Female	Male	Female	Male	Female
Partially cannibalized		1/2		2/4				
Wet abdomen			3/4	4/4	2/5	3/4	3/5	
Yellow material around anogenital region					2/5			1/5
Lungs, congestion	1/1	2/2						
Thymus, hemorrhage							1/5	
Stomach, distension	1/1					1/4		2/5
Stomach, gas filled	1/1							
Stomach, fluid filled					1/5	1/4	2/5	4/5
Stomach, glandular muco- sa, scattered erosions		1/2						
Stomach, non-glandular mucosa, thickened		1/2						
Stomach, non-glandular mucosa, ulcerated		1/2						
Stomach mucosa, conges- tion						1/4	1/5	3/5
Small intestine, red fluid filled								1/5
Intestines, distension			4/4	4/4	4/5	2/4	3/5	2/5
Intestines, fluid filled			4/4	4/4	4/5	3/4	3/5	3/5
Intestinal mucosa, con- gestion						1/4		
Kidneys, yellow foci				1/4				
Cecum, filled with green- ish brown fluid					1/5			

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2. Rats which were sacrificed following 14 days of observation:

Gross Necropsy Observations:	Dosage Level mg/kg Number Exhibiting Sign/Number Necropsied							
	5126		8137		12918		20507	
	Male	Female	Male	Female	Male	Female	Male	Female
No gross lesions			2/4		1/1			1/1
Lungs, congestion	2/5	1/5						
Lungs, consolidation		1/5						
Lungs, dark grey foci			1/4			1/1		
Stomach, fluid filled						1/1		
Stomach, non-glandular mucosa, thickened	5/5	5/5	1/4	2/3				
Kidneys, yellowish sed- iment in renal pelvis and atrophic		1/5						
Uterus, hydrometra				1/3				

191-082

APPENDIX 1

IRDC = A.327

LD50 - RECORD OF INDIVIDUAL DOSE LEVEL

Dose level g/kg bw 5.126 g/kg P&G Code T-7121
Number of animals dosed Male 5 Female 5

All data are based on sample as received.

Sample Preparation: _____ g of sample was mixed with _____ (g) (ml) solvent _____ to make a dosing solution of _____ % _____ (w/w) _____ (w/v).

Specific Gravity: 10 ml of sample weighed 10.20 g at 24 °C, S.G. = 1.020 g/ml.

Individual Animal Data

Date	Animal #	1	2	3	4	5	1	2	3	4	5
	Sex	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
9-21-77	Prefasted Wt. g	260	258	259	262	261	230	232	233	233	230
9-22-77	Fasted Wt. g	243	242	239	245	240	212	220	232	221	218
9-22-77	Dose/Rat ml	1.22	1.22	1.20	1.23	1.21	1.07	1.11	1.12	1.11	1.09
	Time of Death										
	14 Day Wt. g	321	339	350	378	365	237	249	231	211	254

7 DAY WT

Avg. prefasted weight 243.6 g DP 10/13/77
Avg. prefasted weight of survivors 243.6 g
Avg. 14 day weight of survivors 257.5 g
Total Dead: Male 0 Female 0
Animals Received: CR 9/15

5.126 g/kg : 5.03 mg/kg
1.020 g/ml

Worker's Signature Steve Blaza
Corroborating Witness G. Row

Date 9-22-77
Date 9/77/77

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APPENDIX 1

IRDC - H 327

LD50 - RECORD OF INDIVIDUAL DOSE LEVEL

Dose level g/kg bw 8.137 P&G Code T 7121

Number of animals dosed Male 5 Female 5

All data are based on sample as received.

Sample Preparation: g of sample was mixed with (g) (ml) solvent to make a dosing solution of % (w/w) (w/v).

Specific Gravity: 10 ml of sample weighed 10.11 g at 22 °C, S.G. = 1.011 g/ml.

Individual Animal Data

		1	2	3	4	5	1	2	3	4	5
Date	Animal #	66323	66324	66325	66326	66327	66328	66329	66330	66331	66332
	Sex	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
<u>2:00 pm</u>	Prefasted Wt. g	219	210	215	211	212	202	211	207	209	210
	Fasted Wt. g	208	197	207	200	202	190	197	196	194	200
	Dose/Rat ml	1.67	1.59	1.63	1.61	1.63	1.53	1.59	1.58	1.50	1.6
	Time of Death				DAY 4		DAY 4		DAY 3		
	14 Day Wt. g	250	216	285		285		230		245	226
	7 DAY Wt. g	249	211	259		239		226		235	220

Avg. prefasted weight 211 g
 Avg. prefasted weight of survivors 212 g
 Avg. 14 day weight of survivors 253.6 g
 Total Dead: Male 1 Female 2
 Animals Received: C.R. 8/29

$$\frac{8.137 \text{ g/kg}}{1.011 \text{ g/ml}} = 8.05 \text{ ml/kg}$$

Worker's Signature Gene Harris
 Corroborating Witness G. Kowig

Date 9/2/77 9/3/77
 Date 9/2/77 9/3/77

LD50 - RECORD OF INDIVIDUAL DOSE LEVEL

Dose level g/kg bw 12.918 P&G Code T-7121
Number of animals dosed Male 5 Female 5

All data are based on sample as received.

Sample Preparation: — g of sample was mixed with — (g) (ml) solvent — to make a dosing solution of — % — (w/w) — (w/v).

Specific Gravity: 10 ml of sample weighed 10.11 g at 22 °C, S.G. = 1.011 g/ml.

Individual Animal Data

		1	2	3	4	5	1	2	3	4	5
Date	Animal #	66333	66334	66335	66336	66337	66338	66339	66340	66341	66342
	Sex	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
<u>2:00 pm</u>	Prefasted Wt. g	214	213	212	208	207	210	212	214	215	21
	Fasted Wt. g	202	200	200	195	197	200	199	199	199	106
	Dose/Rat ml	2.58	2.57	2.57	2.49	2.52	2.57	2.54	2.54	2.54	2.5
<u>DAY</u>	Time of Death		1	1	1	1	1	1	1		1
	14 Day Wt. g	228								245	
	7 DAY Wt. g	229								231	

Avg. prefasted weight 211.6 g
Avg. prefasted weight of survivors 214.5 g
Avg. 14 day weight of survivors 236.5 g
Total Dead: Male 4 Female 4
Animals Received: C.R. 8/29

$$\frac{12.918 \text{ g/kg}}{1.011 \text{ g/ml}} = 12.78 \text{ mg/ml}$$

Worker's Signature Gene Harris
Corroborating Witness G. Rowig

Date 9/2/77 9/5/77
Date 9/2/77 9/2/77

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APPENDIX 1

IRDC # A 327

LD50 - RECORD OF INDIVIDUAL DOSE LEVEL

Dose level g/kg bw 20.507 P&G Code T 7121
Number of animals dosed Male 5 Female 5

All data are based on sample as received.

Sample Preparation: g of sample was mixed with (g) (ml)
solvent to make a dosing solution of
 % (w/w) (v/v).

Specific Gravity: 10 ml of sample weighed 10.11 g at 22 °C,
S.G. = 1.011 g/ml.

Individual Animal Data

Date	Animal #	1					2				
		66343	66344	66345	66346	66347	66348	66349	66350	66351	66352
	Sex	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
<u>9/7/77</u>	Prefasted Wt. g	240	231	230	235	238	239	229	235	236	233
<u>9/8/77</u>	Fasted Wt. g	225	217	218	221	219	226	215	223	219	218
<u>9/8/77</u>	Dose/Rat ml	4.56	4.40	4.42	4.48	4.44	4.58	4.36	4.52	7.05	7.0
<u>DAY</u>	Time of Death	1	1	1	1	1	1	1	1		4.75 9.2
	14 Day Wt. g									235	222
	7 Day Wt. g										

Avg. prefasted weight 234.6 g
Avg. prefasted weight of survivors 236 g
Avg. 14 day weight of survivors 235 g
Total Dead: Male 5 Female 5
Animals Received: C.R. 8/29

$\frac{20.507 \text{ g/kg}}{1.011 \text{ g/ml}} = 20.28 \text{ ml/kg}$

Worker's Signature Gene Harris
Corroborating Witness B. Row

Date 9/2/77 9/5/77
Date 9/2/77 9/8/77

LD50 - RECORD OF INDIVIDUAL DOSE LEVEL

Dose level g/kg bw 32.555 P&G Code T-7121
Number of animals dosed Male 5 Female 5

All data are based on sample as received.

Sample Preparation: — g of sample was mixed with — (g) (ml) solvent — to make a dosing solution of — % — (w/w) — (w/v).

Specific Gravity: 10 ml of sample weighed 10.11 g at 22 °C, S.G. = 1.011 g/ml.

Individual Animal Data

Date	Animal #	1					2				
		66353	66354	66355	66356	66357	66358	66359	66360	66361	66362
	Sex	♂	♂	♂	♂	♂	♀	♀	♀	♀	♀
<u>9/7/77</u>	Prefasted Wt. g	230	229	234	236	229	234	254	253	250	252
<u>9/8/77</u>	Fasted Wt. g	217	215	224	222	216	220	239	240	239	235
<u>9/8/77</u>	Dose/Rat ml	6.99	6.92	7.21	7.15	6.96	7.08	7.70	7.73	7.63	7.70
DAY	Time of Death	4 HR 9/2/77	9/9/77 1	9/9/77 1	4 HR 9/3/77	9/9/77 1	9/9/77 1	4 HR 9/3/77	4 HR 9/3/77	4 HR 9/3/77	4 HR 9/3/77
	14 Day Wt. g										

2:00 pm

Avg. prefasted weight 240 g
Avg. prefasted weight of survivors — g
Avg. 14 day weight of survivors — g
Total Dead: Male 5 Female 5
Animals Received: C.K. 8/29

$$\frac{32.555 \text{ g/kg}}{1.011 \text{ g/ml}} = 32.20 \text{ ml/kg}$$

Worker's Signature Gene Harris
Corroborating Witness [Signature]

Date 9/2/77 9/3/77
Date 9/2/77 9/3/77

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ACUTE ORAL TOXICITY OBSERVATION DATA

No. of Males/Females Affected - Blank Square = Normal

P&G Code T 7121 Dose Level g/kg 20.507 Dose Time 8:55 AM Dose Date 9/8/77

SYMPTOM:	Time	Hours				Days																
		1/4	1/2	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Motor activity Increase																						
Motor activity decrease		8(4)	9(4)	10(5)	10(5)	9(4)	1(1)	1(1)	1(1)	2(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)		
Respiratory rate Increase																						
Respiratory rate decrease		1(0)	1(0)	3(1)	3(1)	9(4)	1(1)	1(1)	1(1)	1(1)	0											
Fine body tremors						1(1)																
Coarse body tremors																						
Bleaching																						
Cyanosis																						
Gaspng																						
Abnormal Gripping																						
Diarrhea			1(1)	3(1)	6(3)	8(3)																
Pilo Erection		1(1)		3(1)																		
Other: SALIVATION		6(3)	4(2)	5(2)	5(2)	5(2)																
ATAXIA		3(2)	5(2)	6(3)	6(3)	9(4)	1(1)		0					1(1)	1(1)							
URINATION			6(3)	4(1)	7(3)	7(4)	1(1)		1(1)	1(1)	0											
Dec. Limb Tone						1(1)																
Prostration																						
DEATHS						1(1)	8(3)															
Worker's Initials		WAM	JM	JM	JM	JM	GH	PH	GH	SP	WAM	GH	JM	SP	JM	WAM	SP	JM	WAM	SP	JM	WAM

Special Notes: (Including Necropsy Observations)

LD50 - RECORD OF INDIVIDUAL DOSE LEVEL.

Dose level g/kg bw _____ P&G Code _____

Number of animals dosed Male _____ Female _____

All data are based on sample as received.

Sample Preparation: _____ g of sample was mixed with _____ (g) (ml)
solvent _____ to make a dosing solution of
_____ % _____ (w/w) _____ (w/v).

Specific Gravity: _____ ml of sample weighed _____ g at _____ °C,
S.G. = _____ g/ml.

Individual Animal Data

Date	Animal #																		
	Sex																		
	Prefasted Wt. g																		
	Fasted Wt. g																		
	Dose/Rat ml																		
	Time of Death																		
	14 Day Wt. g																		

Avg. prefasted weight _____ g
Avg. prefasted weight of survivors _____ g
Avg. 14 day weight of survivors _____ g
Total Dead: Male _____ Female _____

Worker's Signature _____ Date _____

Corroborating Witness _____ Date _____

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

Lab Project No. 191-082 Sheet 1 Date 8/5/77 Authorized by *J. C. [Signature]* Dr. Jey

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<u>Compound</u>	<u>Identification Number</u>	<u>IRDC No.</u>
I-7121		A-327

TITLE: ACUTE TOXICITY STUDIES IN RATS AND RABBITS

Conduct in accordance with the attached protocol.

Procter & Gamble
Standard Procedure / for Toxicological Evaluation

Acute Oral Toxicity - Rats

Date:

Issue #2

Purpose:

To measure the acute oral toxicity of a substance in order that it might be compared to more familiar materials.

Animals:

Rats, Charles River Caesarean-derived, 190-270 g, conditioned to the environment for a minimum of 7 days. Maintain the animals according to standards outlined in the Guide For the Care and Use of Laboratory Animals, DHEW No. (NIH-74-23), 1974.

Procedure:

Place 10 animals in each group, divided equally by sex. Determine prefasted and fasted body weights. Fast the animals for 18-20 hours before administering the test material.

Dissolve or suspend the test material in an appropriate vehicle at the required concentrations and record quantities mixed. Deliver the test material into the stomach of the animal from a syringe fitted with a size 8 catheter as a stomach tube or from a syringe fitted with a 13-gauge animal-feeding needle.

Choose 4 dosage levels, with the lowest being approximately the highest no-death dosage level observed in range-finding studies, or being estimated from prior results with similar compounds. Unless otherwise specified, choose higher dosage levels according to a geometric progression of 1.4 (i.e. the second dose equals the first dose $\times [1.4]^2$, the third dose equals the first dose $\times [1.4]^3$, etc.). Administer each dosage level to one group of 10 animals. Adjust the dose for each animal according to fasted weight to give the specified quantities of material per unit of body weight. Administer additional dose levels, as needed, so that the total number of dead is sufficient to calculate an LD₅₀ value. Immediately after dosing, return the animal to ad libitum feeding. Record all of the above information on the "LD₅₀ Record of Individual Dose Level" sheet (Appendix I).

Observations:

Observe the animals and their behavior at intervals of 15, 30, 60, 120 and 240 minutes after dosing and daily thereafter for 14 days. Use the "Toxicity Observation Data" sheet (Appendix 2) to record symptoms and number of animals involved. Unusual observations or symptoms not listed on the "Toxicity Observation Data" sheet should be noted where appropriate (under "Symptoms" or "Special Notes"). Necropsy all animals that die during the course of the study. At 14 days, weigh all surviving animals and record weights. Necropsy surviving animals and examine them grossly for abnormalities.

Acute Oral Toxicity - Rats (P&G Procedure) (cont'd)

Report:

Report all data recorded on the Record of Individual Dose Level and the Acute Oral Toxicity Observation Data sheets. Report the LD₅₀ and 95% confidence limits of the test material as calculated by the Probit Method*. File the final report within 3 weeks after completion of the experiment.

Principal Investigator: M. J. Winrow

Date 7/28/77