

Report Title: 14-Day Range-Finding Study in Rats

Test Type: Reproduction Toxicity

Conducting Laboratory and Location: P&G, Miami Valley Laboratories, Biological Testing Facility, Cincinnati, OH 45241

Test Substance(s): #G0539.01 – Octopirox in diet

Species: Rat

of Animals: 5 rats per group

Test Conditions: 6 groups adult female Sprague-Dawley rats; Octopirox was fed in the daily diet at doses of 0, 50, 100, 250, 500, or 1000 mg/kg/day for 14 consecutive days.

Results: The maximum tolerated dose was determined to be 500 mg/kg.

Study #: B85-0179

Report Date: 06/12/85

QA Statement/GLP Compliance: Yes

Accession #: 30604

THE PROCTER & GAMBLE COMPANY
Miami Valley Laboratories
P. O. Box 39175
Cincinnati, Ohio 45247

14-DAY RANGE FINDING STUDY IN RATS

B85-0179

BYCR0393

G0539.01

June 12, 1985

G0539.01

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THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

The following study was reviewed by the Quality Assurance Unit:

TEST FACILITY: The Procter & Gamble Company
 Miami Valley Laboratories
 Cincinnati, Ohio 45247

STUDY NUMBER: B85-0179

DIVISIONAL REQUEST DOCUMENT: BYCR0393

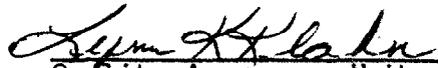
TSIN: G0539.01

TYPE OF STUDY: 14-Day Range Finding Study in Rats

FINAL REPORT REVIEWED BY: M. P. Bauer

DATE OF REVIEW: May 29, 1985

This is a pilot/investigational study. Only the final study report was reviewed for inaccuracies. The results reflect the raw data of the study.

 6/12/85
Quality Assurance Unit - Date

qaufm
4/85

Summary

Six groups of adult female Sprague-Dawley rats were fed G0539.01 in their diet for 14 days at doses of 0, 50, 100, 250, 500, or 1000 mg/kg/day. During the first few days of the treatments, the rats dosed with the three highest doses did not eat normal amounts, but resumed normal feed consumption by and during the second week. Despite the mild palatability problem at 250 mg/kg, these rats gained slightly more body weight than controls during the 14-day period. The rats dosed with 500 mg/kg gained about 20% less than the controls, while those dosed with 1000 mg/kg lost weight (5g). In addition, 4 of 5 rats given 1000 mg/kg/day of G0539.01 exhibited symptoms of anemia during the last few days of the study. Therefore, the 500 mg/kg dose is a maximum tolerated dose for future studies.

Objective

The purpose of this study was to determine a dose that would be mildly toxic to the adult female rat to use as the maximum tolerated dose (MTD) in a reproduction/teratology study. The rat has been historically used for these studies because of its short gestation period and large litter sizes. The oral route was chosen to maximize systemic exposure, and the diet was selected as the vehicle because of the practicality of this method for the long-term treatment in a multi-generation study. The study was done at:

The Procter and Gamble Company
Miami Valley Laboratories
P.O. Box 39175
Cincinnati, Ohio 45247

Study No: B85-0179
DRD No: BYCRO393
TSIN: G0539.01
Notebook No: YE-831
Study Director: G. A. Nolen
Divisional Toxicologist: J. E. Weaver
In-Life Initiation: 4/18/85
In-Life Completion: 5/8/85

Methods and Materials

Fifty-four adult female Sprague-Dawley rats (~200g) were obtained from Charles River Breeding Laboratories, Portage, Michigan and after a 6-day acclimation period, six groups of five rats each were randomly selected and distributed into the groups so that the mean body weights of the groups were similar. The rats were ear-tagged with Monel tags bearing unique numbers. They were housed in individual, stainless steel hanging wire cages and were fed Purina Laboratory chow and tap water ad libitum.

G0539.01 was mixed with ground Purina chow at levels to provide daily doses of 0, 50, 100, 250, 500 or 1000 mg/kg for 14 consecutive days. At the sacrifice, the animals in the high-dose group were subjected to a visual inspection of the internal organs. Otherwise, there were no known deviations from the protocol, attached as Appendix A, which provides the other details of the study.

Results and Discussion

Table 1 shows the overall results of the study. The rats at 500 mg/kg of G0539.01 gained about 20% less than the controls, while those fed 1000 mg/kg lost about 5g of body weight during the 14 days. In addition, the latter exhibited very pale eyes during the last few days of the study, indicative of anemia in the albino rat. These animals were examined internally at sacrifice, and they had pale internal organs, and thin, watery blood, further indicating an anemic-type-blood disorder.

As the data indicate, the feed consumption was depressed in the groups at the three highest doses of G0539.01. Table 2 shows the data for the individual animals and for the first two days of dosing, and the two weekly periods. The diet was quite unpalatable at the three highest doses for the first 2-3 days in a dose responsive manner. However, this was transitory, and by mid-week all of the groups were consuming near normal amounts of feed, even the high-dose group. Thus, it appears that G0539.01 is quite toxic to female rats at the 1000 mg/kg dose, while the 500 mg/kg dose is only mildly toxic. Therefore, the 500 mg/kg/day dose would be a maximum tolerated dose (MTD) for future reproductive/teratology studies.

G. A. Nolen
G. A. Nolen
Study Director

TABLE 1
14-DAY RANGE FINDING STUDY OF G0539.01

		GAINS IN BODY WEIGHT FOR 14 DAYS					
		g					
Group		1	2	3	4	5	6
G0539.01, mg/kg/day		0	50	100	250	500	1000
		11	7	15	28	3	1
		-6	18	20	14	17	-5
		30	15	16	-6	4	-3
		7	19	16	4	8	-10
		3	5	-3	9	5	-10
Means		9.0	12.8	12.8	9.8	7.4	-5.4

		CUMULATIVE FEED CONSUMPTION					
		g					
		230	240	207	248	198	206
		229	261	224	207	174	182
		219	230	239	182	196	200
		229	205	250	201	184	197
		223	205	214	210	243	178
Means		226.0	228.2	226.8	209.6	199.0	192.6
Means g/day		16.2	16.3	16.2	15.0	14.2	13.8

14-DAY RANGE FINDING STUDY OF G0539.01
INDIVIDUAL ANIMAL DATA AT VARIOUS PERIODS

8

G0539.01, mg/kg/day	Rat No.	Initial Wt.	Wt. At 1 Week	Final Wt.	Feed Cons. Days 1&2	Feed Cons. Days 1-7	Feed Cons. Days 8-14	Week 1 Intake Of G0539.01 mg/kg/day	Week 2 Intake Of G0539.01 mg/kg/day	14-Day Intake Of G0539.01 mg/kg/day
0	1512	227	236	238	28	112	118	---	---	---
	1520	225	229	219	29	114	115	---	---	---
	1541	219	233	249	33	104	115	---	---	---
	1549	230	229	237	33	112	117	---	---	---
	1553	221	224	224	30	109	114	---	---	---
Means		224.4	230.2	233.4	30.6	110.2	115.8			
50	1510	228	233	235	33	123	117	51	50	50.5
	1522	226	238	244	39	133	128	55	53	54.0
	1524	230	241	245	32	113	117	46	48	47.0
	1527	220	229	239	29	101	104	43	44	47.0
	1528	216	219	221	28	100	105	47	48	46.0
Means		224.0	232.0	236.8	32.2	114.0	114.2	48.3	48.6	48.9
100	1502	223	234	238	33	107	100	87	84	85.5
	1504	232	238	252	30	105	119	82	94	88.0
	1508	229	233	245	35	116	123	92	100	96.0
	1518	219	239	235	36	129	121	104	103	103.5
	1542	215	214	212	31	106	108	93	102	97.5
Means		223.6	231.6	236.4	33.0	112.6	114.2	91.6	96.6	94.1
250	1513	230	240	258	30	114	134	283	349	316.0
	1533	221	215	235	20	81	126	217	360	288.5
	1538	232	233	226	24	76	106	195	315	255.0
	1550	216	220	220	28	99	102	271	311	291.0
	1554	227	227	236	25	96	114	262	324	293.5
Means		225.2	227.0	235.0	25.4	93.2	116.4	245.6	331.8	288.7
500	1505	224	229	227	19	88	110	500	581	540.5
	1516	228	236	245	17	67	107	359	537	448.0
	1517	231	236	235	22	84	112	470	592	531.0
	1539	214	218	222	18	70	114	418	646	532.0
	1543	220	217	225	28	112	131	650	735	692.5
Means		223.4	227.2	230.8	20.8	84.2	114.8	479.4	618.2	548.8
1000	1507	216	209	217	21	87	119	984	1285	1134.5
	1509	228	214	223	15	69	113	765	1167	966.0
	1521	226	219	223	22	82	118	873	1194	1033.5
	1529	219	205	209	23	87	110	984	1209	1096.5
	1544	231	224	221	17	73	105	784	1080	932.0
Means		224.0	214.2	218.6	19.6	79.6	113.0	878.0	1187.0	1032.5

INTERDEPARTMENTAL CORRESPONDENCE

From: D.K. Hysell

Date: 5-13-85

To: G.A. Nolen

Subject: Animal Health Evaluation Report (HER85-019, R85-0315, B85-0179)

Five female rats (1507, 1509, 1521, 1529, 1544, were submitted for necropsy on 5-8-85. The following gross necropsy observations were made by J.K. Maurer.

1507

1.No significant lesions.

1509

- 1.Generalized paleness in carcass, organs and tissues.
- 2.Watery blood.
- 3.Pale bone marrow.
- 4.No other significant lesion.

1521

- 1.Generalized paleness in carcass, organs and tissues.
- 2.Watery blood.
- 3.Pale bone marrow.
- 4.No other significant lesion.

1529

- 1.Generalized paleness in carcass, organs and tissues.
- 2.Watery blood.
- 3.Pale bone marrow.
- 4.No other significant lesion.

1544

- 1.Generalized paleness in carcass, organs and tissues.
- 2.Watery blood.
- 3.Pale bone marrow.
- 4.No other significant lesion.

Gross Necropsy Diagnosis: 1507 - Cause of death undetermined.
1509, 1521, 1529 and 1544 were anemia.



D.K. Hysell

I N T E R D E P A R T M E N T A L C O R R E S P O N D E N C E

FROM: Operations Section

DATE: April 2, 1985

TO: G. A. Nolen

SUBJECT: STUDY PLACEMENT AUTHORIZATION

This is to authorize you to carry out the following study according to the attached protocol.

Notice: This study is expected to be submitted to the following regulatory agency: FDA. The stipulations of the protocol are to be implemented in complete conformance with Good Laboratory Practices Regulations (21 CFR, Part 58) for nonclinical laboratory studies.

Test: 14-day Range Finding
Study in Rats

Protocol No.: Special Protocol dated 3/7/85

Test Substance No.: G0539.01

Doc. Req. No.: BYCRO393

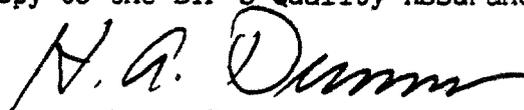
Physical Form: Powder

Eighty percent of the cost of this study should be cross-charged to Beauty Care Division and the remaining 20% to Detlef Müller at ETC.

Matters involving the scientific aspects of the work can be handled directly with the Divisional Toxicologist. All unused samples are to be returned to the Divisional Toxicologist at the following address:

Mr. J. E. Weaver
SWTC - Room No. 2D23
Telephone No. 8-55-2302

Complete both copies of the attached protocol by adding your study number and proposed start and completion dates. The Study Director should define the start and completion dates on the protocol and sign and date both copies. Retain one and return one copy to the BTF's Quality Assurance Unit.



H. A. Derner
Human & Environmental Safety Division

Attachments

cc: Quality Assurance Unit
J. E. Weaver

SUBJECT

NONCLINICAL STUDY - REGULATORY
STATUS

ATTENTION

Notifications pertaining to:

DLD # BYCR0393
TSIN G0539 01

1. Studies requested on the above document:

- are expected to be submitted to the following regulatory agencies as a GLP regulated study: FDA

- are expected to be submitted to the following regulatory agencies but is not a GLP regulated study: _____

Metabolism, Pharmacological Screen, Other: _____

- are not expected to be submitted to a regulatory agency. (Boxes #3 and #4 below need not be checked).

2. - The test substance has been characterized and results are shown on the test substance characterization report which accompanies the DLD.

3. - The method of synthesis fabrication or derivation of the test related substances has been documented. (Required for regulated studies).

4. - Stability testing has been done or will be done on the test substance. (Required for regulated studies).

Sponsor's Divisional Toxicologist: _____

Date: 3-7-85

bg:MLQAUZ

14-Day Range Finding Study in Rats

Test Substance Identification Number (TSIN) G0539.01

Divisional Request Document (DRD) Number BYCR0393

Sponsor:

The Procter & Gamble Company
Cincinnati, Ohio

Testing Facility:

The Procter & Gamble Company
Miami Valley Laboratories - BTF
Cincinnati, OH 45247

Study Number:

B85-0179

Purpose:

To establish doses that will be used in a subsequent study to determine the teratogenic potential of the test substance in rats.

Study Duration:

About 4 weeks

Records to be Maintained:

All records that would be required to reconstruct the study and demonstrate adherence to the protocol.

Test Substance:

<u>TSIN:</u>	<u>DRD Number:</u>	<u>Description</u>			<u>Expiration Date</u>
		<u>Color</u>	<u>Physical Form</u>		
G0539.01	BYCRO393	White	Powder		12/7/85

Purity (% Active):

nominally 100%

Storage Conditions: (Check One)

- Room temperature Refrigerator Freezer
 Other Ambient (50°F-90°F)

Hazards: (Check One)

- None known. Take ordinary precautions in handling.
 Other. Specify Irritant.

Special Instructions: (Check One)

- None
 Avoid undue skin & eye contact. Flush with water if undue contact occurs.

Route of Administration
of Test Substance and
Reason for Choice:

The oral route has been chosen because it is an anticipated route of human exposure.

Test System:

Species

Rat

Strain

Sprague-Dawley, COBS^R CD^R

Source

Charles River Breeding Laboratories, Portage, Michigan, or other source approved by Testing Facility and Sponsor.

Justification for Selection of Test System:

The rat is one rodent commonly used for the evaluation of teratogenicity and is acceptable in the guidelines of the FDA, EPA, and OECD.

Age and Weight:

Females - 80-120 days old; sexually mature virgins weighing a minimum of 220 g at study initiation (acclimation).

Housing:

Individually in suspended SS wire-mesh cages.

Quarantine

Minimum of 5 days.

Identification:

Metal ear tags

Animal Care:

Follow the approved Standard Operating Procedures of the Test Facility.

Diet:

A single lot of Purina Rodent Chow #5001, or other source approved by Sponsor and Study Director and water from a municipal water supply will be available ad libitum.

Diet and Water Analysis:

None. There are no known contaminants expected which would interfere with the study.

As follows: _____

Animal Groups and
Dose Levels:

The study will consist of 5 test groups and 1 control group, each consisting of 5 females. The control group will receive the vehicle on a comparable regimen. Individual dosages will be based on the most recent body weights. All animals, if treated by gavage, will be dosed at a constant volume, e.g. 10 ml/kg at weight, unless other specific instructions are supplied.

Dose Preparation:

Test Groups: (Check appropriate box)

- Dose test substance undiluted
- Dose as a ___ % (w/w) solution/suspension of test substance in _____
- Dose as a ___ % (w/v) solution/suspension of test substance in _____
- Dose per special instructions (see page 2) or add additional instructions to Appendix 1.

Pre-dose Requirements
and Group Assignments:

Animal conditioning
and Selection

Upon receipt, the animals will be inspected for disease and if accepted for the study will be housed individually and fed Purina Chow or feed described previously and water ad libitum during acclimation. The animals will be observed daily for any overt signs of disease and will be given a detailed physical examination just prior to study initiation. All animals with evidence of disease or other abnormalities will be discarded following the approved Standard Operating Procedures of the Test Facility.

Observations:

Prior to test substance administration, the rats will be observed daily for mortality and overt changes in appearance and behavior.

Test Substance
Administration:

The test substance will be administered orally daily for 14 consecutive days. Administer by gavage.
 Administer in the diet. Other _____

DOSE AT: 50, 100, 250, 500 and 1000 mg/kg/day.

Observations and Measurements:

Daily Observations

The animals will be observed twice daily for mortality and clinical signs of toxicity during the treatment period.

Body Weights

Body weights will be recorded at initiation, Day 7, Day 14 and other _____

Food Consumption

The food consumed will be measured once each week, or other _____

Deaths

Animals that die on the test will be discarded. All animals surviving at Day 14 will be sacrificed and discarded.

Protocol Changes

If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Sponsor. As soon as practical, this change and the reason for it, should be documented in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist. This document is then to be attached to the protocol as an addendum.

Report

A comprehensive report will be prepared upon completion of the study.

This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

Sponsor: The Procter & Gamble Co., Cincinnati, Ohio

Date Approved by Sponsor's Divisional Toxicologist James E. Weaver, Divisional Toxicologist
James E. Weaver
3-7-85

Proposed Start Date: 4/24/85
Defined as BEGINNING OF RANGE-FINDING STUDY - 1ST TREATMENT DAY

Proposed Completion Date: 5/13/85
Defined as LAST DAY OF TREATMENT AND SACRIFICE.

Proposed Report Date: 6/1/85
Defined as DRMFT SUBMITTED TO QAU

Study Director: S. A. Nolan
Date: April 4, 1985

Study Cost: \$1 4950⁰⁰/₁₀₀

Protocol - Appendix 1
Instructions for the Preparation and Handling of
Test Substance - Vehicle Mixtures

1. Test substance - Vehicle mixture should be prepared:

[] Daily Weekly [] See instructions below.

Storage: Room temperature [] Refrigerator [] Other

2. Mixtures that are not true solutions should be mixed with the use of a magnetic stirring bar during dosing procedures. Mixtures involving the diet, give special instructions below.

3. A homogeneity determination [] will will not be required. If a homogeneity determination is required, sample the mixture from the top, middle, and bottom of the mixing container, and return samples, appropriately labelled, to the Divisional Toxicologist. Amount of each sample _____

A concentration and/or stability analysis of the mixture [] will will not be required. If required, amount of sample is _____.

4. Shipping Instructions:

Ship: [] ambient temperature [] frozen [] other _____

Ship to: _____

5. Special Instructions:

**TEST SUBSTANCE CHARACTERIZATION REPORT
(TSCR)**

For Tox Office
Use Only:
DRD # BYCR0393
TSIN 160539.01

Characterization, Microbial and Properties Information:

	<u>Date Submitted</u>	<u>Submitter Code (if exists) or Lab Notebook #</u>	<u>Component or Property</u>	<u>(√)</u>	<u>Measured Value</u>	<u>Limits</u>	<u>Testing Lab or Data Source</u>
1	<u>2/27/85</u>	<u>JDM 0108</u>	<u>MCT</u>	<u>√</u>	<u>Pass</u>	<u>Must Pass</u>	<u>Micro</u>
2	<u>11/8/84</u>	<u>HC-0173-46</u>	<u>% Octopirox</u>	<u> </u>	<u>100.26%</u>	<u>98% Min.</u>	<u>1B21</u>
3	<u>12/05/84</u>	<u>84312004</u>	<u>Assay</u>	<u> </u>	<u>99.4</u>	<u>97% Min.</u>	<u>Analytical</u>
4	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
5	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
6	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
7	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
8	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
9	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
10	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
11	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
12	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
13	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
14	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
15	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

12. Approvals:

The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product should be obtained and evaluated by Products Research.

a. Process Development: *JDM* (Signature) John Melanson (Name) 3/4/85 (Date)

b. Products Research: *T. Johnston* (Signature) (Name) 2/27/85 (Date)

 finished product samples will be retained by Quality Assurance.

c. GMP-Quality Assur.: *T. Johnston* (Signature) *T. Johnston* (Name) 3/4/85 (Date)

13. The characterization tests requested are appropriate and the test substance is acceptable for: acute animal test; subchronic animal test; chronic animal test; human safety test; [] in vitro test; [] environmental safety test.

James E. Weaver (Toxicologist's Signature) (Name) 3/25/85 (Date)

TSCR Distribution: Original - Tox Office; Copies - Toxicologist, GMP/QA, Products REsearch and Process Dev.