



**Report Title: Evaluation of Skin Irritation Potential of Acetic Acid and Octopirox in the Muta Mouse**

**Test Type:** Skin Irritation

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

**Test Substance(s):** G0539.05 – OP in ethanol

**Species:** Muta Mouse

**# of Animals:** 2 mice per group

**Test Conditions:** Male Muta Mice received single dose of Octopirox (7.5, 10 or 15 mg) applied to the shaved skin in 0.1ml ethanol

**Results:** Purpose was a range finding study for a DNA synthesis inhibition study with OP. Dose response for irritation induced by Octopirox was sharp and the Maximum Tolerated Dose was determined to be 7.5 mg. Slight to moderate irritation was caused by 7.5 mg while focal ulceration and severe irritation were induced by single doses of 10 and 15 mg.

**Study #:** B91-0116

**Report Date:** 2/24/92

**QA statement/GLP compliance:** Yes

**Accession #:** 36814

SIRE

BIOLOGICAL SAFETY TEST  
SUMMARY REVIEW

Test Substance: G0539.05 - Octopirox; Glacial Acetic Acid  
Type of Study: Evaluation of Skin Irritation Potential of Acetic Acid and Octopirox  
in the MutaMouse  
Division: H&ESD DRD #: None  
Report Date: February 24, 1992 Study #: B91-0116  
Functional Use: Notebook #: YB-1402

This is a nonregulated study.

It is the responsibility of the divisional toxicologist to ensure that the summary reflects all significant findings of the report. This report has been reviewed for scientific quality and is summarized as follows:

<u>DOSE</u>	<u>GROUP</u>	<u>NUMBER</u>	<u>TEST SUBSTANCE</u>	<u>CONCENTRATION</u>
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See Dosing Information on page 5 of report.

Remarks:

See Summary on page 4 of report.

This report and summary are approved for entry into the WIDS. (Circulate in order.)

Divisional Toxicologist/  
Study Director

R L Binder  
R. L. Binder

Date 2/25/92

Return to QAU, NVL

Dynn Klakau

Date 2/27/92

Entered into WIDS

M. Brown

Date 5-19-92

Microfilming Completed

Date \_\_\_\_\_

#17

**Evaluation of Skin Irritation Potential of Acetic Acid  
and Octopirox in the Muta<sup>TM</sup>Mouse  
B91-0116**

Robert L. Binder, Audrey A. Erickson, Paul J. Reer and Roman E. Frank

Human and Environmental Safety Division  
Miami Valley Laboratories  
The Procter & Gamble Company  
Cincinnati, OH 45239

Report Date 2/24/92

# Procter & Gamble

The Procter & Gamble Company  
Miami Valley Laboratories  
P. O. Box 398707, Cincinnati, Ohio 45239-8707

## QUALITY ASSURANCE STATEMENT

STUDY NUMBER: B91-0116

TEST FACILITY: The Procter & Gamble Company  
Miami Valley Laboratories  
Cincinnati, Ohio 45239

TYPE OF STUDY: Evaluation of Skin Irritation Potential of Acetic  
Acid and octopirox in the MutaMouse

DIVISIONAL REQUEST DOCUMENT: None

TSIN: G0539.05

DATA LOCATION: YB-1402

<u>PORTION(S) OF</u> <u>STUDY AUDITED:</u>	<u>AUDITOR:</u>	<u>DATE</u> <u>AUDITED:</u>	<u>DATE</u> <u>REPORTED</u> <u>TO STUDY</u> <u>DIRECTOR:</u>	<u>DATE</u> <u>REPORTED</u> <u>TO MAN-</u> <u>AGEMENT:</u>
Study Data	L. K. Klahm	3/27/91	4/2/91	5/1/91

In compliance with the Good Laboratory Practices regulations, this study has been audited by the Quality Assurance Unit and the results of those audits have been reported to the appropriate management. The protocol was audited for GLP required elements. The study data accurately reflects the procedures described in the protocol. The reported results accurately reflect the raw data of the study.

*Lynn K. Klahm 2/25/92*  
Quality Assurance Unit - Date

**SUMMARY SHEET**

**Study No.** B91-0116

**Animal Activity No.** AA91-0061

**Testing Facility:** Biological Testing Facility  
Miami Valley Laboratories  
The Procter and Gamble Co.  
P.O. Box 398707  
Cincinnati, OH 45239

**Test Substance(s):** Octopirox (TSIN G0539.05)  
Glacial Acetic Acid (Mallinckrodt  
lot 2504KENV)

**Storage Conditions:** Octopirox and Acetic Acid, room temp.

**DRD:** N/A

**Date Study Started:** 2/27/91 (Mice shaved)

**Date Study completed (in-life):** 3/13/91

**Study Director:** Robert L. Binder

**Pathologist:** Gary R. Johnson

**Study Technicians:** Audrey A. Erickson  
Roman E. Frank  
Paul J. Reer

**Notebook:** YB-1402

**Archived at:** Miami Valley Laboratories

## CONTENTS

I. PURPOSE.....	4
II. SUMMARY.....	4
III. METHODS.....	4
IV. RESULTS.....	6
V. CONCLUSIONS.....	7
APPENDIX A, Scale for Evaluating Skin Reactions	
APPENDIX B, Summary of Skin Irritation Grading	
APPENDIX C, Pathology Report from G. R. Johnson	
APPENDIX D, Irritation Grading Sheets for Individual Mice	

## I. PURPOSE

The purpose of this study was to range-find doses to be used in a mutation study with acetic acid and a DNA synthesis inhibition study with Octopirox. With acetic acid the intention was to establish the maximum non-ulcerating dose. With Octopirox we intended to establish a dose level that induces moderate irritation.

## II. SUMMARY

Various doses of acetic acid were applied to Muta<sup>TM</sup>Mouse skin once in 0.1 ml of acetone, and skin was evaluated by gross observation and histopathology. The highest dose tested which did not induce ulceration was 20 mg. Focal ulceration was induced by 25 mg of acetic after a delay of 4 to 7 days from the time of dosing.

Similarly, various doses of Octopirox were applied to MutaMouse skin in ethanol. Under these conditions, the dose-response for irritation induced by Octopirox was sharp. Slight to moderate irritation was caused by 7.5 mg (applied once in 0.1 ml of ethanol), while focal ulceration and severe irritation were induced by single doses of either 10 or 15 mg (applied as two applications of 5 and 7.5 mg, respectively). Based on these results, 7.5 mg was determined to be the maximum tolerated dose for use in further studies on Octopirox in MutaMouse.

## III. METHODS

### Materials

Glacial acetic acid was a commercial grade obtained from Mallinckrodt (lot 2504KENV), and Octopirox (TSIN G0539.05) was obtained from Beauty Care Product Development (stock code BX288, lot 6). Other chemicals were of reagent grade and their sources are indicated in the study notebook.

### Animals and Treatments

Male Muta<sup>TM</sup>Mice were received from Hazleton laboratories at 6 weeks of age, and were housed 5/shoebox cage on hardwood chip bedding. A 12 hr light/dark cycle (7:00 am to 7:00 pm) was maintained in the animal room, and Purina Lab Chow and water were available *ad libitum*. Room temperature and humidity were maintained to Biological Testing Facility standards (BTF SOP: ENV 3,4). The mice were carefully shaved during the 7th week of age using a small animal clipper, and only mice in the resting phase of the hair cycle (i.e. animals without obvious hair regrowth within two days of shaving) and without shaving nicks were used. After shaving mice were individually housed to avoid injury to the skin from fighting, and each mouse was individually numbered on its tail with a permanent marker. Mice were not dosed until at least 2 days after shaving.

Test substances were applied once to the shaved dorsal skin in 0.1 ml of acetone (for acetic acid) or ethanol (for Octopirox). The treatment groups are indicated below. Skin was graded for irritation by Paul J. Reer approximately 5 hr after dosing, then every 24 hr for the following 7 days. Within the acetic acid and Octopirox groups the grading of the

mice was done blind, and the numbers assigned to mice (below) were arbitrarily randomized.

A separate data sheet was kept for each animal to record the skin grades which are reproduced in Appendix D. All animals were killed with CO<sub>2</sub> 7 days after the application of the test substances. The treatment area was excised and a strip of skin for histopathological evaluation was cut along the midline, and spread on a piece of blotter paper to prevent curling. Any areas of skin which contained grossly visible lesions were also cut out and spread on blotter paper. All skin samples were fixed in neutral buffered formalin for routine histology (embedding in paraffin and H&E staining). Fixed skin samples were embedded, sectioned and stained by Pathology Associates Incorporated, and histopathological evaluation was by G. R. Johnson.

The experiment was designed so that if it became apparent that the doses used were either too high or too low, additional mice would be dosed with either Octopirox or acetic acid. In fact it became necessary to dose two groups of mice with higher concentrations of Octopirox as described below.

Initially the dose groups were as follows.

<u>Group</u>	<u>Animal Numbers</u>	<u>Treatment</u>
1	2, 5	25 mg acetic acid (all in 0.1 ml acetone)
2	3, 7	20 mg acetic acid
3	1, 8	15 mg acetic acid
4	4, 6	0.1 ml acetone
5	10, 14	7.5 mg Octopirox (all in 0.1 ml ethanol)
6	11, 15	5.0 mg Octopirox
7	9, 13	2.5 mg Octopirox
8	12, 16	0.1 ml ethanol

After 5 days of observation of the above mice, the following additional groups were dosed with Octopirox. Because of the limits of solubility of Octopirox, two half doses in 0.1 ml of ethanol had to be applied for both groups 9 and 10.

<u>Group</u>	<u>Animal Numbers</u>	<u>Treatment</u>
9	17, 18	10 mg Octopirox
10	19, 20	15 mg Octopirox

Samples submitted for histology were identified by the group # and animal #. Additionally the following lesions were collected, which were also identified by group #, animal # and the designation L for lesion.

<u>Group</u>	<u>Animal Number</u>	<u>Gross Description of Lesion</u>
1	2	Ulcerated area
1	5	Ulcerated area
2	3	Reddened scaly area
10	20	Ulcerated area

After initial histopathological evaluation of the samples, tissue block 1-5L was recut because the ulcerated area apparently was missed. An ulcer was found in the recut sample.

#### IV. RESULTS

The scale used for grading skin irritation is in Appendix A. A summary of skin irritation grading results is presented in Appendix B, and histopathological observations are summarized in the report from G. R. Johnson in Appendix C. The detailed individual skin grading sheets are included in Appendix D.

##### Acetic Acid

The highest dose of acetic acid (25 mg) induced focal ulceration and focal areas of severe irritation, as indicated by severe erythema. The irritant effect of this high dose was delayed and did not become apparent until 4 to 5 days after dosing. Ulcers grossly visible at the time of killing were verified histologically (Appendix C, samples 1-2L, 1-5LR). Additionally, histopathological evaluation indicated that the high dose of acetic acid induced a variety of effects characteristic of skin irritation including moderate hyperplasia in both mice in nonlesional areas, and minimal to moderate inflammation (see Table 1, Appendix C).

The two mice treated with 20 mg of acetic acid differed in their responses. In mouse 2-7 no grossly or microscopically visible irritant responses were observed. In contrast, microscopic examination of skin from mouse 2-3 revealed slight to moderate hyperplasia, slight hyperkeratosis, minimal to slight inflammation, as well as other indications of irritation. Additionally, slight desquamation was observed grossly, which is consistent with slight hyperkeratosis. In general in this study the severity of irritation observed grossly during skin grading was well correlated with microscopic examination of treated skin. One exception was the lesional area which was sampled from mouse 2-3 (see above). Notes recorded at the time of necropsy indicated that the lesion was a reddened scaly area, which was verified microscopically. However, no notations about this area were made during skin grading.

Mouse 3-1, dosed with 15 mg of acetic acid, displayed only very minimal signs of irritation either grossly or after microscopic examination of treated skin. No microscopic or gross changes were noted in the skin of the second mouse (3-8) treated with this dose of acetic acid.

##### Octopirox

In contrast to acetic acid, Octopirox produced a more graded skin irritant response in MutaMice. The most severe reaction to Octopirox in the high dose group (15 mg) was observed grossly between days 2 to 4 after dosing, so by the time of necropsy, when samples for histology were collected, the irritation was subsiding. The 15 mg dose of Octopirox induced focal ulceration in both mice tested (10-19 and 10-20). The 10 mg dose also induced a small area of slight ulceration in one mouse (9-18). The ulcer on mouse 10-20 persisted until the time of necropsy, and was verified histologically (Appendix C, Table 1, 10-20L). Ulcers on mice 10-19 and 9-18 had healed by 3 to 4 days

after dosing. In addition to ulceration, the 10 and 15 mg doses of Octopirox induced severe erythema and slight to moderate desquamation; the 15 mg dose also induced slight edema, observed grossly. Microscopic evaluation revealed that the 15 mg dose of Octopirox induced changes indicative of irritation including slight to moderate hyperplasia, minimal crusting and minimal to slight inflammation in nonlesional areas 7 days after dosing. In the ulcer observed microscopically, hyperplasia was severe, crusting was moderate and inflammation was marked. Only minimal microscopic changes were noted in one mouse at the 10 mg dose (9-17).

No ulceration was induced by the 7.5 mg dose of Octopirox. This dose did induce slight erythema in one mouse (5-10), and slight to moderate desquamation in mice 5-10 and 5-14, respectively. No microscopic changes were noted in the skin of mouse 5-10 at the time of necropsy, 7 days after dosing. In the skin of mouse 5-14, 7.5 mg of Octopirox induced irritant effects including moderate hyperplasia, slight hyperkeratosis and minimal inflammation. The gross irritant responses induced by the lower doses of Octopirox (2.5 and 5 mg) were similar to those seen with 7.5 mg, and consisted of slight to moderate erythema and slight to moderate desquamation. Only minimal microscopic changes were noted in one mouse (6-15) at the 5 mg dosage.

#### V. CONCLUSIONS

In summary, 25 mg of acetic acid, applied to MutaMouse skin once in 0.1 ml of acetone, induced focal ulceration, and 20 mg was the highest dose of acetic acid tested which did not induce ulceration.

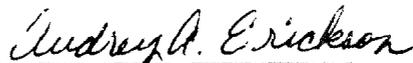
The dose-response for irritation induced in MutaMouse skin by a single topical dose of Octopirox in ethanol was sharp. Both the 10 mg and 15 mg doses induced ulceration and severe irritation. The highest dose tested, which did not induce ulceration was 7.5 mg. This dose did cause slight to moderate irritation, based on gross and microscopic examination of treated skin. Considering these findings, 7.5 mg was selected as the maximum dose of Octopirox for further studies in MutaMouse.



Robert L. Binder

2/24/92

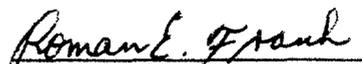
Date



Audrey A. Erickson



Paul J. Reer



Roman E. Frank

SCALE FOR EVALUATING SKIN REACTIONS<sup>+</sup>

Erythema (not including eschar area)

- 0 - None
- 1 - Slight (barely perceptible)
- 2 - Moderate (well defined)
- 3 - Severe (beet red)

Edema (not including eschar area)

- 0 - None
- 1 - Slight (barely perceptible to well defined by definite raising)
- 2 - Moderate (raised approximately 1 mm)
- 3 - Severe (raised more than 1 mm)

Atonia (not including eschar area)

- 0 - Normal
- 1 - Slight (impairment of elasticity)
- 2 - Moderate (slow return to normal)
- 3 - Marked (no elasticity)

Desquamation (not including eschar area)

- 0 - None
- 1 - Slight (slight scaling without evidence of peeling)
- 2 - Moderate (large flakes with sloughing)
- 3 - Marked (pronounced flaking with denuded areas)

Fissuring (not including eschar area)

- 0 - None
- 1 - Slight (definite cracks in epidermis)
- 2 - Moderate (cracks in dermis)
- 3 - Marked (cracks with bleeding)

<sup>++</sup>Eschar

- N - No
- Y - Yes

<sup>++</sup>Exfoliation (sloughing of the eschar tissue)

- N - No
- Y - Yes

<sup>+</sup> Grades assigned should be based on the most severely affected area except when area is judged to be < 20% of the treatment site. Severe reactions occurring on < 20% of a site should be described in a footnote.

<sup>++</sup> Discontinue scoring on portion of test site with eschar/exfoliation.

APPENDIX B.

Summary of Skin Irritation Grading

<u>TREATMENT</u>	<u>GROUP AND ANIMAL NUMBERS</u>	<u>MAXIMUM SCORES<sup>A</sup></u>			<u>ULCERATION<sup>B</sup></u>
		<u>ERYTHEMA</u>	<u>DESQUAMATION</u>	<u>EDEMA</u>	
25 mg Acetic Acid	1-2, 1-5	1 <sup>c</sup> , 3	0, 0	0, 0	Y, Y
20 mg Acetic Acid	2-3, 2-7	0, 0	1, 0	0, 0	N, N
15 mg Acetic Acid	3-1, 3-8	1, 0	0, 0	0, 0	N, N
Acetone Control	4-4, 4-6	0, 0	0, 0	0, 0	N, N
15 mg Octopirox	10-19, 10-20	3, 3	1, 2	1, 1	Y, Y
10 mg Octopirox	9-17, 9-18	3, 3	1, 1	0, 0	N, Y
7.5 mg Octopirox	5-10, 5-14	1, 0	1, 2	0, 0	N, N
5.0 mg Octopirox	6-11, 6-15	1, 0	1, 2	0, 0	N, N
2.5 mg Octopirox	7-9, 7-13	2, 0	0, 1	0, 0	N, N
Ethanol Control	8-12, 8-16	0, 0	0, 0	0, 0	N, N

<sup>A</sup> Individual animal scores are as indicated in the scale in Appendix A. Maximum scores may be for only parts of the treated areas. In some cases (usually higher doses) the score was noted on several days. No atonia, fissuring, eschar or exfoliation were observed.

<sup>B</sup> Y = yes, N = no.

<sup>C</sup> Small areas of severe irritation were not graded for erythema.



Skin tissues were submitted to Pathology Associates Incorporated/or routine histologic processing and staining with hematoxylin eosin. These stained skin sections were observed and evaluated blind by Gary R. Johnson, Veterinary Pathologist.

### Results

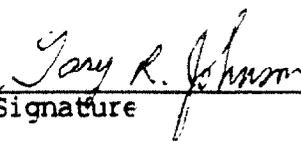
Please refer to Table 1 for results of the histopathologic evaluation.

Twenty-five mg acetic acid (Group 1) applied once was the only dose of acetic acid that produced histologic evidence of ulceration. Twenty mg acetic acid (Group 2) was the highest dose tested that did not show histologic evidence of ulceration.

Histologic Lesions listed in Table 1 were considered as an irritant response of the skin to treatment. As such, any or all of the lesions listed could serve as an indicator of irritation. Mouse skin treated with 15 mg of Octopirox showed the most evidence of irritation. Overall, this response approximated one of moderate severity although there were differences among lesion types and between the two treated subjects in terms of lesion severity. Ten mg of Octopirox produced no or only slight evidence of irritation.

### Discussion

The lesions noted in this histologic evaluation are considered typical for irritant responses. Epidermal hyperplasia is perhaps the lesion most easily induced as it is known to occur with simple physical stroking of the skin. Crust, microulcers, edema and degeneration/necrosis are lesions generally associated with more pronounced irritation. Crust and ulcers in particular denote the loss of a functional epidermal barrier. The other lesions indicate possible perturbation of barrier function. In this context 25 mg acetic acid and 15 mg Octopirox may be perceived as destroying normal barrier function. To a lesser extent this may be true for 20 mg acetic acid (i.e. crust). Other dose of test materials were associated with no or only minimal irritant responses based on the skin sections evaluated microscopically.

  
\_\_\_\_\_  
Signature

**APPENDIX D**

**Irritation Grading Sheets for Individual Animals**

(Note: The sheets are arranged in numerical order by animal number; see the text for coding.)



ANIMAL #: 2

STUDY #: B91-0116

DATE	3/1	3/2	3/3	3/4	3/5	3/6	3/7	3/8												
1991																				
ANIMAL WEIGHT, g																				
PLACEMENT # MV																				
TOTAL DOSE, ml																				
CLIPPED (Y or N)*																				
OBSERVATIONS -- code for evaluation of skin reactions is in protocol																				
ERYTHEMA	1	0	0	0	①	②	①	①												
DEMA	0	0	0	0	0	0	0	0												
TONIA	0	0	0	0	0	0	0	0												
DESQUAMATION	0	0	0	0	0	0	0	0												
CRACKING	0	0	0	0	0	0	0	0												
ESCHAR	N	N	N	N	N	No	No	No												
EXFOLIATION	N	N	N	N	N	No	No	No												
WORKER'S INITIALS	PR	PR	PE	PR	PE	PE	PR	PR												

\* Y = yes; N = no

① Ulcerated area on R+ flank with scabbing.

② 5% severe ulcerated area on R+ flank with scabbing. Another 10% is severely irritated with rash appearance, Edema around scabbed area.

③ Small area on left front flank.





ANIMAL #: 5

STUDY #: 691-0116

DATE	3/1	3/2	3/3	3/4	3/5	3/6	3/7	3/8												
1991																				
ANIMAL WEIGHT, R																				
CLANCE # MV																				
TOTAL DOSE, ml																				
IPPED (Y or N)*																				
OBSERVATIONS -- <sup>Wrong Score PR 3/7/91</sup> code for evaluation of skin reactions is in protocol																				
ERYTHEMA	0	0	①	①	①	②	③	④												
EDMA	0	0	0	0	0	0	0	0												
ONIA	0	0	0	0	0	0	0	0												
SQUAMATION	0	0	0	0	0	0	0	0												
SSURING	0	0	0	0	0	0	0	0												
CHAR	N	N	N	N	N	N	N	N												
EPOLIATION	N	N	N	N	N	N	N	N												
WORKER'S INITIALS	PR																			

Y = yes; N = no

- ① very small area on back that appears to be anti from rubbing on cage or small pimple. Right side just off midline.
- ② ~50% of test site has a rash type irritated area. Redness of grade 3.
- ③ type irritation
- ④ small ulcerated area Lt. rear.





























Study Number: 871-0116  
Study Director: R. L. Binder

Evaluation of Skin Irritation Potential of Acetic Acid  
and Octopirox in the Mutamouse

Purpose:

To range-find the maximum doses which will be used in a mutation study with acetic acid and a DNA synthesis inhibition study with Octopirox. With acetic acid it is our intention to establish the maximum nonulcerating dose. With Octopirox we will establish a dose that induces a moderate level of irritation.

Methods:

Male Mutamice will be received from Hazelton Laboratories at 6 weeks of age, and will be housed 5/mc/3000 cage on hardwood chip bedding. A 12 hr light/dark cycle (7:00 am to 7:00 pm) will be maintained in the animal room, and Purina Lab Chow and water will be available ad libitum. Room temperature and humidity will be maintained to ATF standards (ATF SOP: ENV 3.4). The mice will be carefully shaved during the 7th week of age using a small animal clipper, and only mice in the resting phase of the hair cycle (i.e. animals without obvious hair regrowth within two days of shaving) and without shaving nicks will be used. After shaving mice will be individually housed to avoid injury to the skin from fighting. Treatments will not begin until at least 2 days after shaving.

Test substances will be applied once to the shaved dorsal skin in 0.1 ml of acetone (for acetic acid) or ethanol (for Octopirox). The treatment groups are indicated below. Skin will be graded for irritation by Paul Reer approximately 3 hr (+/- 15 min) after dosing, then every 24 hr (+/- 1 hr) for the following 7 days. Within the acetic acid and Octopirox groups the grading of the mice will be blind, and the numbers assigned to mice (below) were arbitrarily randomized. Each mouse will be individually numbered on its tail with a permanent marker using the code indicated below, and will be housed individually in a labelled cage.

Gloves will be worn when handling the animals for grading, and will be changed between each animal. A separate data sheet will be kept for each animal to record the skin grades which are described below. If an animal develops ulceration at the treatment site, it will be sacrificed as soon as this condition is observed. All animals will be sacrificed with CO<sub>2</sub>. When animals are sacrificed, a strip of skin from the treatment area will be spread on blotter paper and fixed in neutral buffered formalin for routine histology (embedding in paraffin and H&E staining). The remaining skin from the treatment site and the livers of all animals will be frozen in liquid nitrogen and stored at -80° for possible mutation analysis. Colon and small intestine will be collected by David Wilcox for in-vitro kinase analysis and possible latter mutation analysis.

N=2

Group	Animal Numbers	Treatment
1	2, 5	25 mg acetic acid (all in 0.1 ml acetone)
2	3, 7	20 mg acetic acid
3	6, 1	15 mg acetic acid
4	4, 8	0.1 ml acetone
5	10, 14	7.5 mg Octopirox (all in 0.1 ml ethanol)
6	11, 13	5.0 mg Octopirox
7	9, 13	2.5 mg Octopirox
8	12, 16	0.1 ml ethanol

R. L. Binder 2/22/91

if it becomes apparent that the doses used were either too high or too low, additional mice may be dosed with either octoparox or acetic acid, and graded as above. These mice will be assigned additional group and animal numbers.

Samples submitted for histology will be identified by the group # and animal #.

The following markings will be used to identify individual animals.

- Dots for 1 - 4 •
- Dash for 5 -
- X for 10 X

Appropriate combinations will be used for other numbers. For example, 15 would be:

X-•

R. J. Binder 2/22/92

Study: B91-0116  
Sponsor: The Procter & Gamble Company  
Study Director: R. L. Binder  
Regulatory Status: Investigative

In addition to the tissues collected for histological evaluation, skin and liver samples were collected from the animals 1 - 16 for possible mutation analysis by E. D. Thompson. These specimens are being stored in the ultralow freezer in room D-3 BTF and any data obtained will be recorded in notebook YE-1500. They will be retained until consumed during analysis or until deemed no longer useful.

In this study, the purpose of treating mice with acetic acid was to range-find doses for a subsequent skin mutation assay with this compound. Similarly, the purpose of treating mice with Octopirox was to range-find doses for latter studies of the inhibition of epidermal DNA synthesis and for a subsequent skin mutation assay. If additional specimens are needed for skin mutation analysis, the skin samples collected here might be used for that purpose, and if that is the case the results will be reported to the Study Director and referenced in this notebook.

The liver specimens may be used in initial efforts by E. D. Thompson to set-up a liver mutation assay in-house (notebook reference YE-1500). Topically, applied acetic acid and Octopirox should have no effect on the rate of liver mutations, and the results of any liver analyses that may be done do not bear on the interpretation of this study, and will not be reported to the Study Director.

Additionally, colon samples were collected from animals 1 - 6 for possible thymidine kinase measurements and initial efforts at establishing a colon mutation assay by David Wilcox. These samples were taken to evaluate the utility of the Muta<sup>tm</sup> Mouse model, and the results are completely unrelated to this study, and will not be reported back to the Study Director. The only reason these samples and the liver samples were taken from this group of Muta<sup>tm</sup> Mice was because of the extremely high cost of this transgenic strain, and concerns around reducing animal use.

Histological specimens from this study will be retained at the archives of Pathology Associates Incorporated. Retention will be as follows: histological specimens on slides, indefinitely; histological specimens in parafin blocks, 15 years; wet tissues, 5 years. Additionally, the specimen retention and disposal SOP from Pathology Associates Incorporated is included on the following pages.

Study Personnel Were:

R. L. Binder (RLB)  
A. A. Erickson (AAE)  
R. E. Frank (REF)  
P. Reer (PR)

R L Binder  
4/24/91

**PROTOCOL AMENDMENT**

Histopathological evaluation of skin samples was to be done by G. R. Johnson. This was not indicated in the original protocol.

*R I Bender*  
*2/8/92*