

Report Title: Inhibition Of Epidermal DNA Synthesis In MutaTM Mouse By Octopirox: Time-Course And Dose-Response

Test Type: Genotoxicity Study

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

Test Substance(s): G0539.05, G0539.06 – Octopirox in ethanol

Species: Muta Mouse

of Animals: For dose-response, 10 mice per group were treated.

Test Conditions: Epidermal DNA synthesis in Muta Mouse was determined by measuring the incorporation of ³H-thymidine into DNA. Single doses on shaved skin ranged from 0.075 to 7.5 mg. The dose response was determined two hours after dosing.

Results: Octopirox transiently inhibits epidermal DNA synthesis in Muta Mouse when applied in ethanol. The stimulation of DNA synthesis by Octopirox is also consistent with the observation that it induces hyperplasia in Muta Mouse skin.

Study #: B91-0153

Report Date: 4/27/82

QA statement/GLP compliance: Yes

Accession #: 36907

**INHIBITION OF EPIDERMAL DNA SYNTHESIS IN MUTA™MOUSE BY OCTOPIROX:
TIME-COURSE AND DOSE-RESPONSE**

B91-0153

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Report Date 4/27/92

Procter & Gamble

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QUALITY ASSURANCE STATEMENT

STUDY NUMBER: B91-0153

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

TYPE OF STUDY: Inhibition of Epidermal DNA Synthesis in MutaMouse
by Octopirox: Time-Course and Dose-Response

DIVISIONAL REQUEST DOCUMENT: BY 0856S

TSIN: G0539.05, G0539.06

DATA LOCATION: YB-1402

<u>PORTION(S) OF STUDY AUDITED:</u>	<u>AUDITOR:</u>	<u>DATE AUDITED:</u>	<u>DATE REPORTED TO STUDY DIRECTOR:</u>	<u>DATE REPORTED TO MAN- AGEMENT:</u>
Dosing, 3H-Thymidine Injection, Sacrifice, Dipilation, Collection of Epidermal Cells, Study Data	L. K. Klahm	4/30/91	5/1/91	5/8/91

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.

L. K. Klahm 4/29/92
Quality Assurance Unit - Date

SUMMARY SHEET

Study No. B91-0153

Animal Activity No. AA91-0081

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, TSIN G0539.06)

Storage Conditions: Room temp.

DRD: N/A

Date Study Started: 4/26/91 (First group of mice shaved)

Date Study Completed (in-life): 8/1/91

Report Date: 4/27/92

Study Director: Robert L. Binder

Study Technicians: Audrey A. Erickson
Roman E. Frank

Notebook: YB-1402

Archived at: Miami Valley Laboratories

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I. PURPOSE

These experiments were designed to 1) establish the time of maximal inhibition of DNA synthesis by Octopirox in Muta™Mouse epidermis, and 2) to establish the dose-response for this effect at the time of maximal inhibition.

II. SUMMARY

Epidermal DNA synthesis in Muta™Mouse was determined by measuring the incorporation of ³H-thymidine into DNA. A dose of 7.5 mg of Octopirox applied in 0.1 ml of ethanol, caused a rapid inhibition of epidermal DNA synthesis, with a maximal effect 2 hr after dosing. This was followed by an apparent rebound to higher than control levels of DNA synthesis within 20 hr. All doses of Octopirox tested caused a statistically significant inhibition of epidermal DNA synthesis. The lowest dose (0.075 mg or 11.3 µg/cm²) caused about a 60% inhibition of the incorporation of ³H-thymidine into DNA. Doses from 0.25 to 7.5 mg (37.5 to 1130 µg/cm²) all caused a maximal inhibition of epidermal DNA synthesis of >90%.

III. METHODS

Materials

Octopirox was obtained from Beauty Care Product Development; TSIN G0539.05 was used for the time-course experiments and TSIN G0539.06 was used for the dose-response experiment. Calf thymus DNA was obtained from Sigma Chemical Co. and [(methyl)-³H]-thymidine (5 Ci/mmol) was purchased from Amersham. Other chemicals were of reagent grade or higher quality and their sources are indicated in the study notebook.

Animals and Dosing

Male mice of the Muta™Mouse strain were received from Hazleton Research Laboratories at approximately 6 to 7 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 L-42 in the Biological Testing Facility (BTF), and Purina Lab Chow and water were available *ad libitum*. Room temperature and humidity were maintained to BTF standards (BTF SOP: ENV 3,4). The mice were carefully shaved 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. The resting phase of the hair cycle occurs in many mouse strains during 7 - 9 weeks of age. Based on prior experience with female albino mice, we initially shaved the male mice used here at the beginning of the 7th week of age (1st time-course experiment). However, when shaved at this time several mice had hair regrowth. Subsequently we shaved mice during the 8th week of age and did not have a problem with hair regrowth. After shaving mice were individually housed, and treatments were not begin until at least 2 days after shaving.

The Muta™Mouse strain has 3 coat colors: black, brown and golden brown, and mice of different colors were distributed as uniformly as possible among the various treatment groups. Mice were uniquely identified with the group and animal numbers, which were written on the tail with a permanent marker. Cages were also labelled with this information.

Animal treatments

Based on a preliminary range-finding dermal irritation study (B91-0116), the maximum dose of Octopirox evaluated was 7.5 mg. Octopirox doses were applied to the shaved area in 0.1 ml of ethanol using a micropipettor, and control mice were treated with ethanol. The dose solutions were

dripped over the shaved area to achieve uniform coverage, while avoiding the border so that the dose was not wicked into the surrounding hair

Time-course Experiments

Two experiments were conducted to establish the time of maximal inhibition of epidermal DNA synthesis after a single topical treatment with 7.5 mg of Octopirox. In the first experiment mice were killed at the following times after treatment: 2, 4, 6, 8 and 20 hr (all ± 10 min). At each time point there were 4 Octopirox-treated and 4 ethanol control animals. All mice were injected i.p. with 1 $\mu\text{Ci/g}$ body weight of ^3H -thymidine 1 hr (± 5 min) before sacrifice as indicated in Appendix A. The dosing of animals was staggered 5 min apart to allow injection and sacrifice at the correct times. Because of an error in sample labelling only 3 mice were evaluated at certain time points as indicated in Table 1.

In the second time-course experiment, mice were killed 1, 2, 3 and 4 hr after treatment. Again at each time point there were 4 Octopirox-treated and 4 ethanol control mice, with the exception of the 3 hr control group, where 1 mouse died immediately upon injection of the ^3H -thymidine. Because of the need for the earlier time point, mice in the second experiment were injected with ^3H -thymidine as described above at 0.5 hr rather than 1 hr before killing. Data from a preliminary experiment using CD-1 mice (Notebook VE-1418, pg 50) indicated that there is little difference in the level of a ^3H -thymidine incorporation into epidermal DNA when mice are killed 0.5 to 1 hr after i.p. injection of the labelled nucleotide.

Dose-response experiment

Groups of 10 mice were treated with the indicated doses of Octopirox, applied once to the shaved dorsal skin in 0.1 ml of ethanol as described above. The dosing was staggered so that mice were killed 2 hr after treatment, which was determined to be the time at which DNA synthesis inhibition was maximal. All mice will be injected i.p. with 1 $\mu\text{Ci/g}$ body weight of ^3H -thymidine 1 hr (± 5 min) before sacrifice.

Because of the large number of mice used, this experiment was conducted over 3 days. On each of the first 2 days, 4 mice from each group were treated and killed. On the third day, the final 2 mice from each group were treated and killed.

Experimental groups were as follows:

<u>GROUP</u>	<u>DOSE</u> (mg applied in 0.1 ml of ethanol)
1	0
2	0.075
3	0.25
4	0.75
5	2.50
6	7.50

The data from one mouse in Group 2 was not used because low acid soluble counts in the epidermal extract indicated that there was a problem with the injection of ^3H -thymidine.

Estimation of area of treated skin.

To allow the doses used in the dose-response experiment to be expressed on a per cm^2 basis, the area treated was estimated in the following way. Separate groups of mice were killed with CO_2 , then 0.1 ml of ethanol or 7.5 mg of Octopirox in ethanol was applied to shaved skin exactly as done above. The border of the treated area was marked with a permanent ink marker, and then the treated skin was excised and laid flat. The marked area was traced onto a piece of clear plastic film, and the areas were determined by G. M. Ridder using image analysis techniques (report in Appendix D).

Epidermal DNA Synthesis

Epidermal DNA synthesis *in vivo* was determined by measuring the incorporation of ^3H -thymidine into DNA by a modification of the methods of Bairo *et al.* (1971) and Smart *et al.* (1986), as described in detail in Appendix A. DNA was determined by the method of Burton (1968) as described in Appendix B.

Statistics

For statistical analysis of data, analysis of variance was used, providing that Barlett's test of homogeneity was not significant. Otherwise, Wilcoxon's rank sum test was used (Snedecor and Cochran, 1967). Linear regression analysis was performed, using dose as the independent variable (Draper and Smith, 1981). This analysis included a check for lack of fit. All statistical tests were conducted at a 5%, two-sided risk level. Significance at the 1% and 0.1% levels are also reported, where appropriate. A summary of statistical analyses is in Appendix C.

IV. RESULTS

The results of the time-course experiments are summarized in Table 1 and Figure 1. From the first experiment it can be seen that a single topical application of 7.5 mg of Octopirox in ethanol caused a rapid decrease in epidermal synthesis in MutaTMMouse, followed by a recovery and rebound to higher than control activity within 20 hr of dosing. In this experiment a maximal inhibition of ^3H -thymidine incorporation into DNA (expressed as $\text{dpm}/\mu\text{g}$ DNA) was observed at 2 hr after dosing, at which time DNA synthesis was inhibited 92%. Because of the extent of inter-animal variability, the effect of Octopirox was significant only at 2 and 4 hr after dosing. However, the measured levels of DNA synthesis remained below control activities until at least 8 hr after dosing. At 20 hr after dosing the rate of DNA synthesis was 1.7-times the concurrent control level, but this difference was not statistically significant.

To define more precisely the time of maximal inhibition of epidermal DNA synthesis, a second experiment was conducted, with more closely spaced sampling times between 1 and 4 hr after dosing. In this experiment epidermal DNA synthesis was inhibited 78% within 1 hr of dosing Octopirox. Again, the maximal effect was observed 2 hr after dosing (88% inhibition), followed by a recovery in the rate of DNA synthesis during the next 2 hr. In both the first and second experiments it can be seen that Octopirox was without effect on acid soluble dpm/mg of epidermis. This indicates that the inhibition of ^3H -thymidine incorporation into DNA was not the result of inhibited uptake of the nucleotide, and supports the conclusion that Octopirox caused an inhibition in DNA synthesis. Also, the general consistency of the normalized acid soluble counts indicates the reproducibility of the technique used to inject the ^3H -thymidine.

The dose-response for the inhibition of epidermal DNA synthesis in MutaTMMouse is shown in Table 2 and Figure 2. In order to allow the dose to be expressed as $\mu\text{g}/\text{cm}^2$, the areas of skin covered by 0.1 ml of ethanol or 7.5 mg of Octopirox in ethanol were determined in groups of three mice, as

described in Methods. The measured areas were 6.79 ± 0.65 and 6.54 ± 0.74 cm², for the ethanol and 7.5 mg Octopirox groups, respectively (data \pm S.D.). These results indicate that the high dose Octopirox solution spread over essentially the same area as ethanol alone. Therefore, an overall mean value of 6.66 cm² was used to estimate dose on a μ g/cm² basis for all dose groups.

The dose-response for the inhibition of epidermal DNA synthesis by Octopirox was determined 2 hr after dosing, the time of maximal inhibition by the high dose. All doses of Octopirox tested caused a statistically significant inhibition of epidermal DNA synthesis. At the lowest dose (0.075 mg or 11.3 μ g/cm²) incorporation of ³H-thymidine into DNA was inhibited 60.4%. A maximal inhibition of about 91% was achieved by the next higher dose (0.25 mg or 37.5 μ g/cm²). All the higher doses caused a similar inhibition of DNA synthesis of >90%. Consistent with the data from the time-course experiments, Octopirox was without effect on acid soluble counts over the entire dose-range.

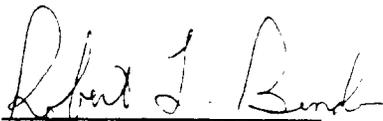
V. CONCLUSIONS

The data presented here demonstrate that Octopirox transiently inhibits epidermal DNA synthesis in MutaTMMouse when applied in ethanol. The pattern observed, with a rapid inhibition followed by an apparent rebound to higher than control levels of DNA synthesis, is similar to that seen with other agents that inhibit epidermal DNA synthesis in mice, such as hydroxyurea (Suss and Maurer, 1968) and acetic acid (Slaga *et al.*, 1975). The increase in DNA synthesis above control levels observed 20 hr after dosing was not statistically significant, but a similar effect was induced by Octopirox in CD-1 mice (Binder *et al.*, 1992). The stimulation of DNA synthesis by Octopirox is also consistent with the observation that it induces hyperplasia in MutaTMMouse skin (Study B91-0116).

The highest dose of Octopirox tested (7.5 mg) was found previously to be the highest concentration that could be applied to MutaTMMouse skin once, without inducing ulceration (Study B91-0116), and was considered a maximum tolerated dose. The data presented here demonstrate that this dose caused a maximal inhibition of DNA synthesis. In fact a 30-fold lower concentration, also produced a similar inhibition.

VI. ACKNOWLEDGEMENTS

We acknowledge G. M. Ridder for his skillful evaluation of treatment areas by image analysis, and A. Merritt and R. D. Bruce for statistical analysis.



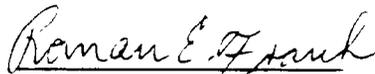
Robert L. Binder

4/27/92

Date



Audrey A. Erickson



Roman E. Frank

VII. REFERENCES

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Suss, R. and Maurer, H. R. Reduced binding of carcinogenic hydrocarbons to DNA of mouse skin during inhibition of DNA synthesis. *Nature* 217: 752-753 (1968).

Table 1. Time-Course for Inhibition of Epidermal DNA Synthesis by 7.5 mg of Octopirox Topically Applied in 0.1 ml of Ethanol^a

Hours After Dosing	dpm/ μ g DNA		Acid Soluble dpm/mg Tissue	
	Control	Octopirox	Control	Octopirox
<i>First Experiment</i>				
2	200 \pm 5 (3)	16 \pm 1 (3) ^b	1099 \pm 40 (3)	1176 \pm 26 (3)
4	147 \pm 17	55 \pm 23 ^c	1227 \pm 41	1279 \pm 43
6	121 \pm 37	60 \pm 13	1153 \pm 113	1156 \pm 54
8	180 \pm 9 (3)	124 \pm 21 (3)	1313 \pm 54 (3)	1352 \pm 31 (3)
20	101 \pm 32	173 \pm 13 (3)	1201 \pm 102	1265 \pm 24 (3)
<i>Second Experiment</i>				
1	162 \pm 27	36 \pm 14 ^c	1337 \pm 49	1182 \pm 115
2	207 \pm 37	18 \pm 3 ^c	1196 \pm 152	1187 \pm 44
3	85 \pm 44 (3)	28 \pm 2	1257 \pm 194 (3)	1374 \pm 36
4	94 \pm 47	56 \pm 1	1271 \pm 40	1361 \pm 83

^a N = 4, except where indicated otherwise in parenthesis. All values \pm S.E.

^b Significantly different from respective control value, $p < 0.001$.

^c Significantly different from respective control value, $p < 0.05$.

Table 2. Dose-response for the Inhibition of Epidermal DNA Synthesis by Octopirox Topically Applied in 0.1 ml of Ethanol

Octopirox Dose		dpm/ μ g DNA ^a	% Inhibition	Acid Soluble dpm ^a mg tissue
mg	μ g/cm ²			
0	0	144 \pm 20	0	1181 \pm 62
0.075	11.3	57 \pm 9 ^b	60	1274 \pm 37
0.25	37.5	13 \pm 2 ^c	91	1187 \pm 40
0.75	113	8 \pm 1 ^c	94	1163 \pm 35
2.50	375	12 \pm 5 ^c	92	1136 \pm 25
7.50	1130	14 \pm 3 ^c	90	1175 \pm 67

^aN = 10, except for 0.075 mg Octopirox where N = 9. Data \pm S.E.

^b Significantly different than the control group, $p < 0.01$.

^c Significantly different than the control group, $p < 0.001$.

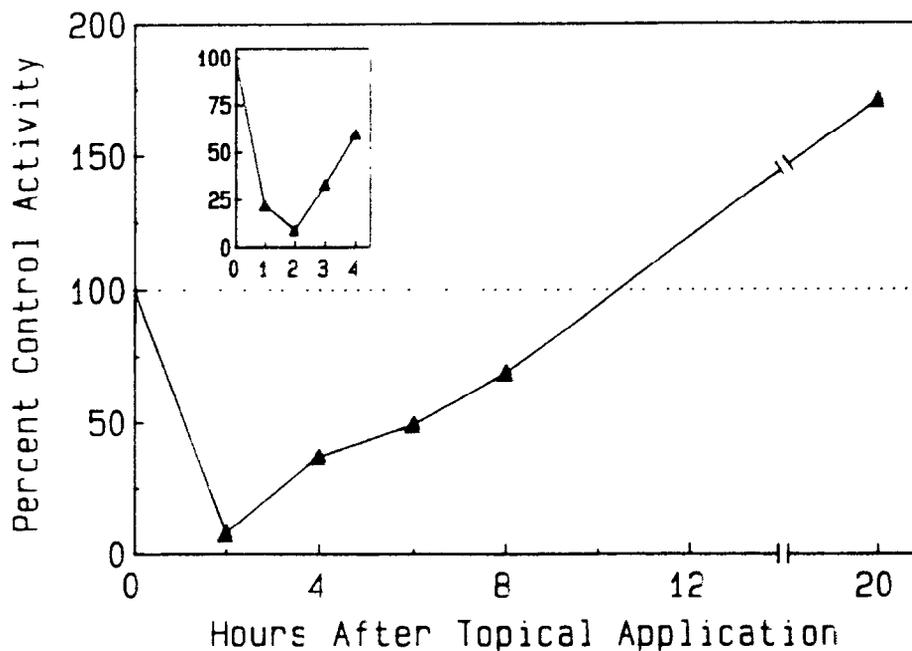


Figure 1. Inhibition of epidermal DNA synthesis in MutaTMMouse by a single topical dose of 7.5 mg of Octopirox in 0.1 ml of ethanol. At each time point the activity in the Octopirox-treated mice is expressed as the percent activity in the concurrent ethanol control mice. The data in the main figure are from the first experiment and the inset represents the results of the second experiment described in Table 1.

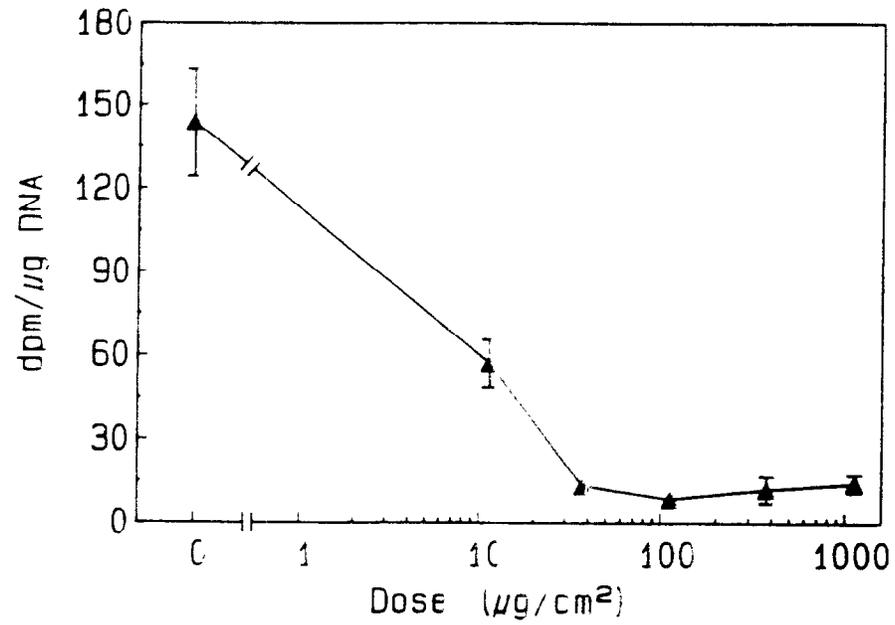


Figure 2. Dose-response for the inhibition of epidermal DNA synthesis in Muta™ Mouse by single topical doses of Octopirox in ethanol. N = 9 or 10; error bars indicate S.E.

APPENDIX A

STANDARD PROCEDURE FOR MEASURING EPIDERMAL DNA SYNTHESIS

- 1) All mice will be injected i.p. with 1 $\mu\text{Ci/g}$ body weight of ^3H -thymidine (5 Ci/mmol) either 30 min or 1 hr (± 5 min) before sacrifice, depending on the experimental design. The ^3H -thymidine will be prepared in isotonic saline at 0.8 $\mu\text{Ci}/\mu\text{l}$, and will be injected using 50 μl Hamilton gastight syringes with 25 gauge needles.
- 2) The depilatory, NEET (Whitehall Laboratories), will be applied to the treated skin about 4 min before the time of sacrifice, and the mice will be killed with CO_2 , then the NEET will be removed by washing with deionized water. The skin will be dried with paper towels, and the central portion of the treated area will be excised to ensure that only dosed skin is sampled. Epidermis will be isolated by scraping with a razor blade (Binder *et al.* Carcinogenesis 10: 2351 - 2357, 1989).
- 3) The epidermal scrapings from each mouse will be frozen in liquid nitrogen on the razor blade used for scraping, then weighed in a tared 1.5 ml microcentrifuge tube. The epidermal samples will be stored in a -80°C freezer until analysis.
- 4) The epidermal scrapings from each individual mouse will be homogenized in 2.5 ml of ice-cold 0.4 N perchloric acid (PCA) using a Polytron with a chilled PT10 generator (2 intervals of 15 sec at setting 6) in 16 X 125 mm plastic tubes. The homogenates will be immediately transferred to 12 X 75 mm polypropylene tubes, and kept on ice until the samples from all mice have been homogenized. All homogenates will be allowed to remain on ice for at least 15 min before centrifugation.
- 5) Tubes will be spun at 2500 rpm at 4°C for 10 min in a Sorvall GSA rotor (about 900 X g) to pellet the precipitate.
- 6) The supernatant fractions will be carefully decanted and saved to estimate the soluble ^3H -thymidine pool. During the digestion of the acid precipitable fractions (below), the supernatant fractions will be centrifuged at 7500 rpm for 10 min to eliminate any contamination with acid precipitable material, then one 1.0 ml aliquots of the supernatants will be counted in 10 ml of Beckman Readysafe LSC cocktail. Quantification of ^3H dpm will be as indicated below.
- 7) The pellets will be washed in 2 ml of ice-cold 0.2 N PCA, by vortexing until they are well broken-up. Samples will be centrifuged as in 5) above. The supernatant fluids will be discarded.
- 8) The pellets will then be washed twice in 2 ml of ice-cold absolute ethanol as in 5) above.
- 9) After the final wash, the ethanol will be removed, and 2 ml of 0.5 N PCA will be added to each tube. The pellets will be resuspended by vortexing, taking care not to leave large pieces on the walls of the tubes. The tubes will be tightly capped, then heated in a waterbath at 90°C for 20 minutes, then vortexed again, and placed on ice for 10 min. The hydrolyzed DNA will be separated from protein and RNA by centrifuging for 10 min at 7500 rpm in a GSA rotor, the supernatant fractions will be centrifuged again for 10 min at 7500 rpm.
- 10) About 1.5 ml of supernatant will be carefully decanted from each tube. From each sample a 1.0 ml aliquot will be counted in 10 ml of Readysafe cocktail, and the remainder will be frozen in liquid nitrogen and stored at -80°C for DNA analysis by the Burton method (Appendix B).

- 11) ^3H dpm will be quantified in a Beckman LS5801 liquid scintillation counter set to the standard tritium window (channels 0 - 400). The counter will have been calibrated with Beckman quenched tritium standards, and the H# method will be used to determine counting efficiency.

APPENDIX E

DIPHENYLAMINE ASSAY FOR DNA

(A modification of the method of Burton [Methods in Enzymology 12: 163-166, 1968])

Solutions

- 1) 1.6% aqueous acetaldehyde
1 ml cooled acetaldehyde + 50 ml deionized water (use pipette chilled in freezer, -20° C)
- 2) Diphenylamine reagent (store at room temperature, stable for 3 - 4 months.)
15 g diphenylamine dissolved in 1000 ml glacial acetic acid, then add 15 ml concentrated sulfuric acid.
- 3) DNA standard (store at 4° C, stable for at least 6 months, check by measuring absorbance at 260 nm)
Calf thymus DNA brought to a final concentration of 200 µg/ml in 5 mM NaOH, based on absorbance at 260 nm (1.0 AU = 50 µg/ml) not weight.
- 4) Working DNA standard (stable for at least 3 weeks, store at 4°)
Mix 5 ml of 200 µg/ml DNA standard + 5 ml 1 N perchloric acid (PCA), then heat in a sealed tube at 90° C in a waterbath for 20 min, then cool on ice.
- 5) 1 N PCA
42.9 ml 70% PCA, QS to 500 ml.
- 6) 0.5 N PCA
Dilute 1 N PCA, 1:2.
- 7) Working diphenylamine reagent, make fresh daily
0.1 ml 1.6% acetaldehyde for every 20 ml diphenylamine reagent.

Assay Procedure

- 1) Standards and unknown samples are prepared in a final volume of 625 µl of 0.5 N PCA in 12 X 75 mm plastic tubes. All tubes should have the same amount of PCA, so 0.5 N PCA is used to adjust the volume.
- 2) The standard curve is constructed using 0, 50, 150, 200, 250 and 300 µg of the working standard DNA.
- 3) For epidermal extracts, 225 µl of extract is adjusted to 625 µl with 0.5 N PCA.
- 4) To each tube 1.25 ml of working diphenylamine reagent is added. Tubes are sealed, vortexed, and then incubated in a waterbath at 30°C overnight.
- 5) Absorbance is measured at 600 nm after -17 hr. The spectrophotometer flow cell is zeroed with a 2:1 mixture of glacial acetic acid and 0.5 N PCA. (Diphenylamine precipitates in water.)

APPENDIX C
Statistical Summary

Data summarized in Table 1 (pg. 9), 1st experiment, Octopirox groups compared to Control groups.

ECHO CHECK OF 88944 CONTROL CARDS

2/20/92

5 1 -1

1 -1

1 -1

1 -1

1 -1

1 -1

2HRC:2HRO

4HRC:4HRO

6HRC:6HRO

8HRC:8HRO

20HRC:200

110-111 MUTA MOUSE TIME COURSE

DNA dp=ug DNA

1

(5A4, #7.0)

99

\$\$\$GRPNAM

2HR CONTROL

2HR OCTOPIROX

4HR CONTROL

4HR OCTOPIROX

6HR CONTROL

6HR OCTOPIROX

8HR CONTROL

8HR OCTOPIROX

20HR CONTROL

20HR OCTOPIROX

\$\$HISTGM

99 1 4

93 1

94 1

99 1 SUMMARY OF STATISTICAL ANALYSES \$\$\$STATBL 0 0-2 1 2 0 2 0 1 0

MUTATION TIME COURSE

- DNA dpm/ug DNA

2/20/92

	2HR	CONT	2HR	OCTO	4HR	CONT	4HR	OCTO
MIDPOINT								
310.								
290.								
270.								
250.								
230.								
210.	X							
190.	XX				X			
170.					X			
150.								
130.					X			
110.					X		X	
90.								
70.								
50.							XX	
30.								
10.			XXX				X	
N		3		3		4		4
AVERAGE		200.00		15.83		147.25		54.53
STD.DEV		8.72		1.64		33.81		46.43
MINIMUM		194.0		14.6		114.0		3.5
MAXIMUM		210.0		17.7		187.0		116.0

	6HR	CONT	6HR	OCTO	8HR	CONT	8HR	OCTO
MIDPOINT								
310.								
290.								
270.								
250.								
230.								
210.								
190.								
170.	XX				XX			
150.					X			
130.	X							
110.							X	
90.			X				X	
70.			X					
50.			X					
30.			X					
10.	X							
N		4		4		3		3
AVERAGE		121.38		59.65		179.67		124.13
STD.DEV		74.89		26.08		15.04		36.89
MINIMUM		11.5		33.1		164.0		96.4
MAXIMUM		170.0		91.7		194.0		166.0

MUTATION TIME COURSE

- DNA dpm/ug DNA

20/92

	20HR	CONT	20HR	OCTO
MIDPOINT				
310.				
290.				
270.				
250.				
230.				
210.				
190.			X	
170.			X	
150. XX			X	
130.				
110. X				
90.				
70.				
50.				
30.				
10. X				
N	4		3	
AVERAGE	101.18		173.00	
STD. DEV	43.96		22.00	
MINIMUM	9.7		151.0	
MAXIMUM	150.0		195.0	

MUTA HOUSE TIME COURSE
 RESPONSES 1- 1: 10 GROUPS WITH SIZES

2/20/92

3 3 4 4 4 4 3 3 4 3

			DNA dpm/ ug DNA
GROUP1	4C1	CONTROL	194.0
GROUP1	4C3	CONTROL	196.0
GROUP1	4C4	CONTROL	210.0
GROUP AVERAGES			200.00
STANDARD ERRORS			5.03
GROUP2	4O2	OCTOPX	14.6
GROUP2	4O3	OCTOPX	15.2
GROUP2	4O4	OCTOPX	17.7
GROUP AVERAGES			15.83
STANDARD ERRORS			0.95
GROUP3	3C1	CONTROL	187.0
GROUP3	3C2	CONTROL	125.0
GROUP3	3C3	CONTROL	114.0
GROUP3	3C4	CONTROL	163.0
GROUP AVERAGES			147.25
STANDARD ERRORS			16.90
GROUP4	3O1	OCTOPX	116.0
GROUP4	3O2	OCTOPX	45.4
GROUP4	3O3	OCTOPX	3.5
GROUP4	3O4	OCTOPX	53.2
GROUP AVERAGES			54.53
STANDARD ERRORS			23.22
GROUP5	2C1	CONTROL	170.0
GROUP5	2C2	CONTROL	11.5
GROUP5	2C3	CONTROL	136.0
GROUP5	2C4	CONTROL	168.0
GROUP AVERAGES			121.38
STANDARD ERRORS			37.44
GROUP6	2O1	OCTOPX	44.8
GROUP6	2O2	OCTOPX	69.0
GROUP6	2O3	OCTOPX	91.7
GROUP6	2O4	OCTOPX	33.1
GROUP AVERAGES			59.65
STANDARD ERRORS			13.04
GROUP7	1C1	CONTROL	164.0
GROUP7	1C3	CONTROL	194.0
GROUP7	1C4	CONTROL	181.0
GROUP AVERAGES			179.67
STANDARD ERRORS			8.69
GROUP8	1O2	OCTOPX	110.0
GROUP8	1O3	OCTOPX	166.0
GROUP8	1O4	OCTOPX	96.4
GROUP AVERAGES			124.13
STANDARD ERRORS			21.30
GROUP9	5C1	CONTROL	140.0
GROUP9	5C2	CONTROL	105.0
GROUP9	5C3	CONTROL	9.7
GROUP9	5C4	CONTROL	150.0
GROUP AVERAGES			101.18
STANDARD ERRORS			31.98
GROUP0	5O1	OCTOPX	173.0
GROUP0	5O2	OCTOPX	151.0
GROUP0	5O3	OCTOPX	195.0

MUTA USE TIME COURSE

2/20/92

DNA dpm/
ug DNA

GROUP AVERAGES	173.00
STANDARD ERRORS	12.70

AVERAGE= 114.68 POOLED
 STD.DEV= 42.58 C.V.= 37.13%
 HARM.MEAN LSD= 67.02

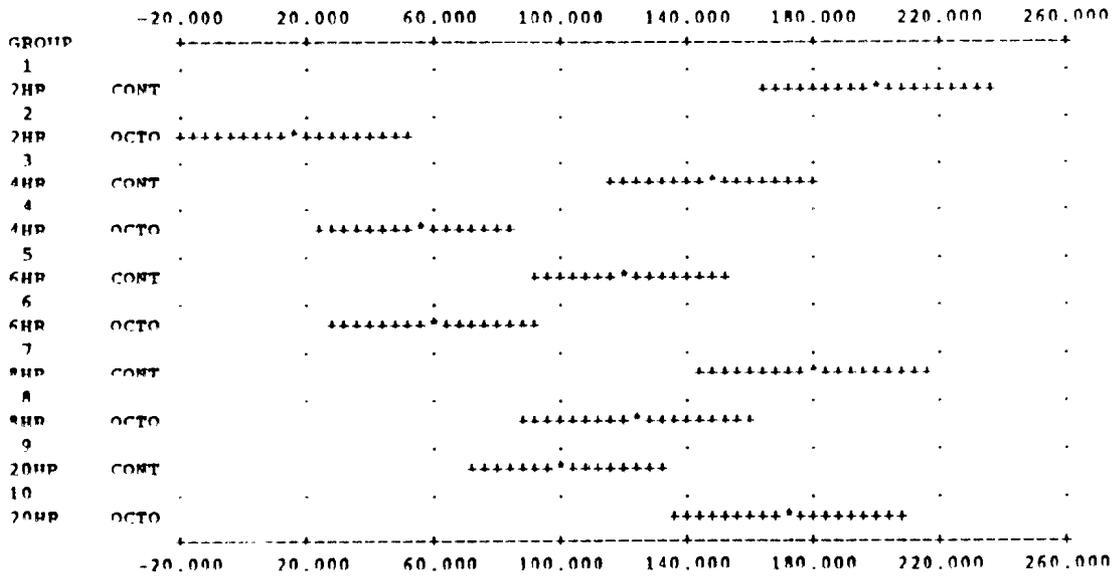
GROUP	NO	AVERAGE	STD.ERROR	GRP.STD.DEV	SIGN.DIFF.	T	PROB
2HR CONT	3	200.00	24.59	8.72			
2HR OCTO	3	15.83+	24.59	1.64	71.65	-35.96*	0.001
4HR CONT	4	147.25	21.29	33.81	67.02	-2.99*	0.048
4HR OCTO	4	54.53+	21.29	46.43	67.02	-6.12*	0.007
6HR CONT	4	121.38+	21.29	74.89	67.02	-2.08	0.126
6HR OCTO	4	59.65+	21.29	26.08	67.02	-10.04*	0.001
8HR CONT	3	179.67	24.59	15.04	71.65	-2.03	0.130
8HR OCTO	3	124.13+	24.59	36.89	71.65	-3.47	0.064
20HR CONT	4	101.18+	21.29	63.96	67.02	-3.05	0.052
20HR OCTO	3	173.00	24.59	22.00	71.65	-1.98	0.156

SOURCE	DF	SS	MS	F	PROB
TOTAL	35	611668.938			
MEAN	1	460302.531			
GROUPS	9	106031.813	11781.313	6.497*	0.000
RESIDUAL	25	45314.561	1813.182		

CONTRAST NO.	NO	CONTRAST	MS	F	PROB	T	PROB	F(LOF)	PROB
1	1	184.167	50876.043	28.056*	0.000	35.96*	0.001		
2HR:2HR		1.00 -1.00	-R- -R-	-R- -R-	-R- -R-	-R- -R-	-R- -R-		
2	2	92.725	17195.852	9.483*	0.005	3.23*	0.020		
4HR:4HR		-R- -R-	1.00 -1.00	-R- -R-	-R- -R-	-R- -R-	-R- -R-		
3	3	61.725	7619.951	4.202	0.051	1.56	0.200		
6HR:6HR		-R- -R-	-R- -R-	1.00 -1.00	-R- -R-	-R- -R-	-R- -R-		
4	4	55.533	4625.928	2.551	0.123	2.41	0.106		
8HR:8HR		-R- -R-	-R- -R-	-R- -R-	1.00 -1.00	-R- -R-	-R- -R-		
5	5	-71.825	8843.708	4.877*	0.037	-2.09	0.107		
20HR:20H		-R- -R-	-R- -R-	-R- -R-	-R- -R-	-R- -R-	1.00 -1.00		

* SIGNIFICANT F-TEST OR T-TEST(5%RISK). T-TESTS USE WELCH'S PROCEDURE
 + SIGNIF.MULT.COMPARISON(5%RISK, POOLED VARIANCE), METHOD=LEAST SIGNIF. DIFF.
 BARTLETT'S CHI-SQ (9 DF)= 21.44* PROB=0.011(DIST.FREE OPTION IS INDICATED)

BAR GRAPH PLOT. APPROXIMATE LSD'S (5 PERCENT RISK LEVEL)



MUTATION USE TIME COURSE
SUMMARY OF STATISTICAL ANALYSES

2/20/92

	2HR		2HR		4HR		4HR		6HR		6HR		PROB
	N	CONT	OCTO	CONT	OCTO	CONT	OCTO	CONT	OCTO	CONT	OCTO		
DNA dpm/ ug DNA	AVG	200.0	15.8***	147.3*	54.5**	121.4	59.7***						
	SE	5.0	0.9	16.9	23.2	37.4	13.0						
	PROB		0.001	0.048	0.007	0.126	0.001						

	4HR		4HR		20HR		20HR		OTHER TESTS	PROB
	N	CONT	OCTO	CONT	OCTO	CONT	OCTO			
DNA dpm/ ug DNA	AVG	179.7	124.1	101.2	173.0	12.7	2HRC:2HRO	0.001***	✓	
	SE	8.7	21.3	32.0	12.7	4HRC:4HRO	0.020*			
	PROB	0.130	0.064	0.052	0.156	6HRC:6HRO	0.200			
						8HRC:8HRO	0.106			
						20HRC:20O	0.107			

SYMBOLS APPEARING BY GROUP AVERAGES/MEDIANS INDICATE DIFFERENCES FROM 2HR CONT
 *, **, *** DENOTE SIGNIFICANCE BY NORMAL DISTN. METHODS WITH P<.05, .01, .001 RESPECTIVELY

Data summarized in Table 1 (pg 9), 2nd experiment, Octopirox groups compared to Control groups

ECHO BOOK OF RA944 CONTROL CARDS

2/20/

4 1 -1
1 -1
1 1
1 -1

1HRC:1HRO 2HRC:2HRO 3HRC:3HRO 4HRC:4HRO

1 A-111 MUTA MOUSE TIME COURSE

DNA dpμ/ug DMA

1
(5AA,F7 0)

99				\$\$GRPRAM			
1HR	CONTROL	1HR	OCTOPIROX	2HR	CONTROL	2HR	OCTOPIROX
3HR	CONTROL	3HR	OCTOPIROX	4HR	CONTROL	4HR	OCTOPIROX
99 1	4			\$\$HISTGM			

93 1

94 1

99 1 SUMMARY OF STATISTICAL ANALYSES \$\$STATRL 0 0-2 1 2 0 2 0 1 0

	1HR	CONT	1HR	OCTO	2HR	CONT	2HR	OCTO
MIDPOINT								
310.					X			
290.								
270.								
250.								
230.								
210.					X			
190.	XXX				X			
170.					X			
150.					X			
130.								
110.								
90.	X							
70.			X					
50.			X					
30.			X				XX	
10			X				XX	
N	4		4		4		4	
AVERAGE	162.10		35.63		206.75		18.18	
STD. DEV	54.47		28.17		72.89		6.61	
MINIMUM	80.4		1.3		147.0		12.1	
MAXIMUM	190.0		69.0		300.0		26.5	

	3HR	CONT	3HR	OCTO	4HR	CONT	4HR	OCTO
MIDPOINT								
310.								
290.								
270.								
250.								
230.								
210.								
190.								
170.					XX			
150.	X							
130.								
110.								
90.	Y							
70.								
50.								
30			XXXX				XXXX	
10	X				XX			
N	3		4		4		4	
AVERAGE	84.60		27.78		93.88		55.70	
STD. DEV	75.62		4.43		94.12		2.77	
MINIMUM	3.4		21.9		9.9		52.6	
MAXIMUM	153.0		31.8		179.0		59.3	

MUTATION TIME COURSE

RESPONSES 1-1: 8 GROUPS WITH SIZES

/20/92

			DNA dpm/ µg DNA
GROUP1	1C1	CONTROL	190.0
GROUP1	1C2	CONTROL	188.0
GROUP1	1C3	CONTROL	190.0
GROUP1	1C4	CONTROL	80.4
GROUP AVERAGES			162.10
STANDARD ERRORS			27.24
GROUP2	101	OCTOPX	1.3
GROUP2	102	OCTOPX	43.9
GROUP2	103	OCTOPX	69.0
GROUP2	104	OCTOPX	28.3
GROUP AVERAGES			35.63
STANDARD ERRORS			14.18
GROUP3	2C1	CONTROL	163.0
GROUP3	2C2	CONTROL	147.0
GROUP3	2C3	CONTROL	208.0
GROUP3	2C4	CONTROL	309.0
GROUP AVERAGES			206.75
STANDARD ERRORS			36.45
GROUP4	201	OCTOPX	13.7
GROUP4	202	OCTOPX	26.5
GROUP4	203	OCTOPX	12.1
GROUP4	204	OCTOPX	20.4
GROUP AVERAGES			18.18
STANDARD ERRORS			3.31
GROUP5	3C1	CONTROL	97.4
GROUP5	3C2	CONTROL	3.4
GROUP5	3C4	CONTROL	153.0
GROUP AVERAGES			84.60
STANDARD ERRORS			41.66
GROUP6	301	OCTOPX	26.0
GROUP6	302	OCTOPX	30.5
GROUP6	303	OCTOPX	21.9
GROUP6	304	OCTOPX	31.8
GROUP AVERAGES			27.78
STANDARD ERRORS			2.22
GROUP7	4C1	CONTROL	179.0
GROUP7	4C2	CONTROL	9.9
GROUP7	4C3	CONTROL	14.6
GROUP7	4C4	CONTROL	172.0
GROUP AVERAGES			93.88
STANDARD ERRORS			47.16
GROUP8	401	OCTOPX	55.0
GROUP8	402	OCTOPX	59.3
GROUP8	403	OCTOPX	55.9
GROUP8	404	OCTOPX	52.6
GROUP AVERAGES			55.70
STANDARD ERRORS			1.39

POOLED
 AVERAGE= 85.61 STD.DEV= 53.40 C.V.= 62.38%
 HARM.MEAN LSD= 79.77

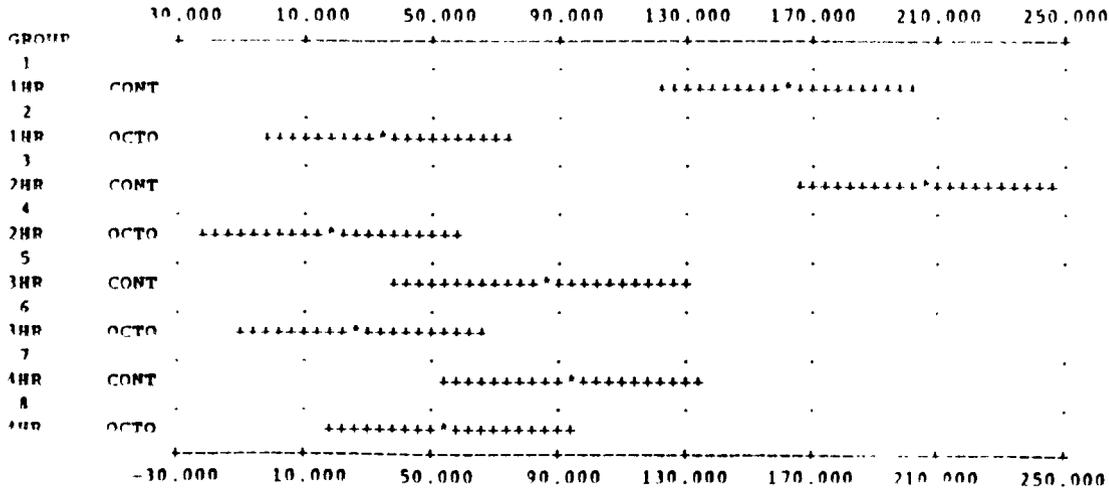
GROUP	NO	AVERAGE	STD.ERROR	GRP.STD.DEV	SIGN.DIFF.	T	PROB
1HR CONT	4	162.10	26.70	54.47			
1HR OCTO	4	35.63+	26.70	28.37	78.16	-4.12*	0.011
2HR CONT	4	206.75	26.70	72.89	78.16	0.98	0.367
2HR OCTO	4	18.18+	26.70	6.61	78.16	-5.25*	0.013
3HR CONT	3	84.60	30.83	75.62	84.42	-1.51	0.216
3HR OCTO	4	27.78+	26.70	4.43	78.16	-4.92*	0.016
4HR CONT	4	93.88	26.70	94.32	78.16	-1.25	0.268
4HR OCTO	4	55.70+	26.70	2.77	78.16	-3.90*	0.030

SOURCE	DF	SS	MS	F	PROB
TOTAL	31	420295.750			
MEAN	1	227182.406			
GROUPS	7	127520.680	18217.240	6.388*	0.000
RESIDUAL	23	65592.664	2851.855		

CONTRAST NO.	DF	SS	MS	F	PROB	T	PROB	F(LOF)	PROB
1	1	126.475	31991.854	11.218*	0.003	4.12*	0.011		
1HRC:1HRO		1.00 -1.00	-R- -R-	-R- -R-	-R- -R-	-R-	-R-		
2	1	188.575	71121.063	24.939*	0.000	5.15*	0.014		
2HRC:2HRO		-R- -R-	1.00 -1.00	-R- -R-	-R- -R-	-R-	-R-		
3	1	56.825	5535.566	1.941	0.177	1.30	0.323		
3HRC:3HRO		-R- -R-	-R- -R-	1.00 -1.00	-R- -R-	-R-	-R-		
4	1	38.175	2914.661	1.022	0.323	0.81	0.478		
4HRC:4HRO		-R- -R-	-R- -R-	-R- -R-	-R- -R-	1.00 -1.00			

* SIGNIFICANT F-TEST OR T-TEST (5% RISK). T-TESTS USE WELCH'S PROCEDURE
 + SIGNIF. MULT. COMPARISON (5% RISK, POOLED VARIANCE), METHOD=LEAST SIGNIF. DIFF.
 BARTLETT'S CHI-SQ. (7 DF)= 17.27* PROB=0.000 (DIST.FREE OPTION IS INDICATED)

RAP GRAPH PLOT. APPROXIMATE LSD'S (5 PERCENT RISK LEVEL)



MUTATION USE TIME COURSE
SUMMARY OF STATISTICAL ANALYSES

2/20/92

	1HR		1HR		2HR		2HR		3HR	
	CONT		OCTO		CONT		OCTO		CONT	
	N	4	4	4	4	4	4	4	3	
DNA dpm/	AVG	162.1	35.6*	206.8	18.2*	84.6				
ug DNA	SE	27.2	14.2	36.4	3.3	43.7				
	PROB		0.011	0.367	0.013	0.216				

	3HR		4HR		4HR		OTHER TESTS	PROB
	OCTO		CONT		OCTO			
	N	4	4	4	4	4		
DNA dpm/	AVG	27.8*	93.9	55.7*	1HRC:1HRO	0.011*		
ug DNA	SE	2.2	47.2	1.4	2HRC:2HRO	0.014*		
	PROB	0.016	0.268	0.030	3HRC:3HRO	0.323		
					4HRC:4HRO	0.478		

SYMBOLS APPEARING BY GROUP AVERAGES/MEDIANS INDICATE DIFFERENCES FROM 1HR CONT
*, **, *** DENOTE SIGNIFICANCE BY NORMAL DISTN. METHODS WITH P<.05, .01, .001 RESPECTIVELY

Data summarized in Table 2 (pg 10), Octopirox-dosed groups compared to Control groups

ECHO CHECK OF B8944 CONTROL CARDS

1 1
0 0.75 2.5 7.5 25 75

-1

3/27/92

2

1 6-111 STUDY032792

dpm/uq

1

(5A4, P6.0)

99

CONTROL

0.75MG

\$\$GRPAM

2.5MG

7.5MG

25MG

75.0MG

90 1 6

\$\$HISTGM

93 1

94 1

99 1

SUMMARY OF STATISTICAL ANALYSES \$\$STATBL 0 0-2 1 2 2 2 0 0 0

STUDY 0

- dpm/ug

/92

	CONTROL	0.75MG	2.5MG	7.5MG	25MG	75.0MG
MIDPOINT						
310.						
290.						
270.						
250.						
230.						XX
210.						
190.						X
170.						X
150.						X
130.						XX
110.						XX
90.		XX				
70.		XXXX				
50.					X	
30.		XX	X			XX
10.	X	X	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
N	10	9	10	10	10	10
AVERAGE	143.69	56.87	13.21	8.41	11.97	14.22
STD. DEV	62.21	25.84	5.38	3.44	15.37	10.58
MINIMUM	17.3	19.8	5.5	1.8	0.2	4.2
MAXIMUM	226.6	88.0	23.0	14.2	54.3	31.9

STUDY01
 RESPONSES 1 1: 6 GROUPS WITH SIZES 10 10 10 10 10 10

/92

dp=11d

GROUP 1 1	CONTROL	222.0
GROUP 1 2	CONTROL	17.3
GROUP 1 3	CONTROL	104.1
GROUP 1 4	CONTROL	114.5
GROUP 1 5	CONTROL	140.0
GROUP 1 6	CONTROL	175.8
GROUP 1 7	CONTROL	123.0
GROUP 1 8	CONTROL	128.4
GROUP 1 9	CONTROL	226.6
GROUP 1 10	CONTROL	185.2
GROUP AVERAGES		143.69
STANDARD ERRORS		19.67
GROUP 2 1	0.75	80.0
GROUP 2 2	0.75	25.1
GROUP 2 3	0.75	61.7
GROUP 2 4	0.75	27.8
GROUP 2 5	0.75	88.0
GROUP 2 6	0.75	75.9
GROUP 2 7	0.75	19.8
GROUP 2 8	0.75	62.9
GROUP 2 9	0.75	70.6
GROUP 2 10	0.75	-
GROUP AVERAGES		56.87
STANDARD ERRORS		8.61
GROUP 3 1	2.5	23.0
GROUP 3 2	2.5	9.6
GROUP 3 3	2.5	15.3
GROUP 3 4	2.5	5.5
GROUP 3 5	2.5	13.4
GROUP 3 6	2.5	18.1
GROUP 3 7	2.5	8.5
GROUP 3 8	2.5	8.4
GROUP 3 9	2.5	17.5
GROUP 3 10	2.5	12.8
GROUP AVERAGES		13.21
STANDARD ERRORS		1.70
GROUP 4 1	7.5	11.7
GROUP 4 2	7.5	7.5
GROUP 4 3	7.5	9.1
GROUP 4 4	7.5	10.0
GROUP 4 5	7.5	5.5
GROUP 4 6	7.5	9.9
GROUP 4 7	7.5	1.8
GROUP 4 8	7.5	14.2
GROUP 4 9	7.5	7.8
GROUP 4 10	7.5	6.6
GROUP AVERAGES		8.41
STANDARD ERRORS		1.09
GROUP 5 1	25	10.0
GROUP 5 2	25	13.5
GROUP 5 3	25	7.3
GROUP 5 4	25	54.3
GROUP 5 5	25	6.6
GROUP 5 6	25	0.2

STUDY 0

1/92

dpm/ug

GROUP 5 7 25	7.7
GROUP 5 8 25	11.3
GROUP 5 9 25	2.6
GROUP 5 10 25	6.2
GROUP AVERAGES	11.97
STANDARD ERRORS	4.86
GROUP 6 1 75	33.9
GROUP 6 2 75	4.2
GROUP 6 3 75	8.7
GROUP 6 4 75	9.2
GROUP 6 5 75	18.4
GROUP 6 6 75	8.9
GROUP 6 7 75	32.4
GROUP 6 8 75	9.6
GROUP 6 9 75	8.5
GROUP 6 10 75	8.4
GROUP AVERAGES	14.22
STANDARD ERRORS	3.35

POOLED
 AVERAGE= 41.13 STD. DEV= 28.71 C.V.= 69.79%
 HARM. MEAN LSD= 26.01

GROUP	NO	AVERAGE	STD. ERROR	GRP. STD. DEV	SIGN. DIFF.	T	PROB
CONTROL	10	143.69	9.08	62.21			
0.75MG	9	56.87+	9.57	25.84	26.48	-4.04*	0.002
2.5MG	10	13.21+	9.08	5.38	25.77	-6.61*	0.000
7.5MG	10	8.41+	9.08	3.44	25.77	-6.87*	0.000
25MG	10	11.97+	9.08	15.37	25.77	-6.50*	0.000
75.0MG	10	14.22+	9.08	10.58	25.77	-6.49*	0.000

SOURCE	DF	SS	MS	F	PROB
TOTAL	59	285154.094			
MEAN	1	99819.648			
GROUPS	5	141660.094	28332.020	34.382*	0.000
RESIDUAL	53	43674.344	824.044		

REGRESSION COMPONENTS OF GROUPS SUM OF SQS.

X-VALUES USED BELOW: 0.00 0.75 2.50 7.50 25.00 75.00

LIN. REGR.	1	21985.684	21985.684	26.680*	0.000
LACK OF FIT	4	119674.414	29918.604	36.307*	0.000
QUADRATIC	1	28949.848	28949.848	35.131*	0.000
LACK OF FIT	3	90724.563	30241.521	36.699*	0.000

* SIGNIFICANT F-TEST OR T-TEST (5% RISK). T-TESTS USE WELCH'S PROCEDURE
 + SIGNIF. MULT. COMPARISON (5% RISK, POOLED VARIANCE), METHOD=LEAST SIGNIF. DIFF.
 PARTIETT'S CRT SO (5 DF)= 81.71+ PROB=0.000 (DIST. FREE OPTION IS INDICATED)

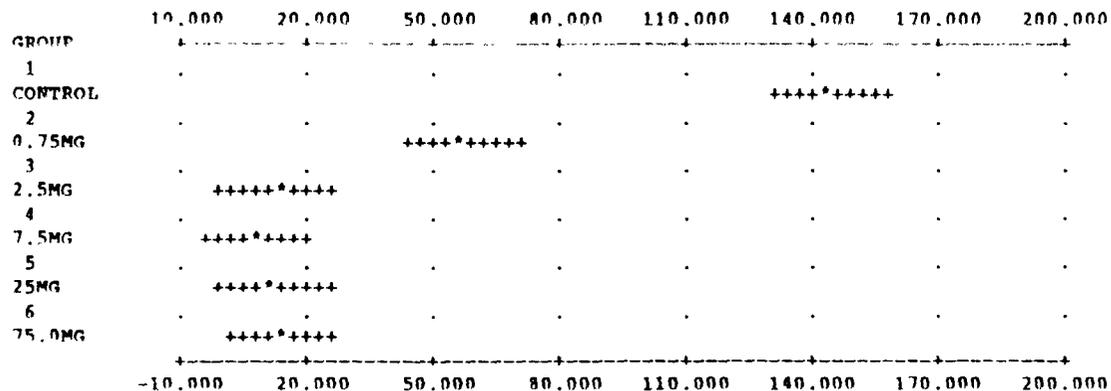
EQN FOR LINEAR REGRESSION

Y= 54.64402 + -0.72035*X

EQN FOR QUADRATIC REGRESSION

Y= 71.68475 + -4.44914*X + 0.04945*X*X
 YSTAR= 44.9905 YSTAR= -28.3998

BAR GRAPH PLOT, APPROXIMATE LSD'S (5 PERCENT RISK LEVEL)



DISTRIBUTION-FREE MULTIPLE COMPARISONS

GROUP	NO	MEDIAN	RANK SUM	RANK AVE	GRP-CONT	SIGN	DIFF
CONTROL.	10	134.20	529.0	52.90	0.00		15.20
0.75MG	9	62.90	404.0	44.89	-8.01		15.47
2.5MG	10	13.10	261.0	26.10*	-26.80		15.05
7.5MG	10	8.45	169.5	16.95*	-35.95		15.05
25MG	10	7.50	169.0	16.90*	-36.00		15.05
75.0MG	10	9.05	237.5	23.75*	-29.15		15.05

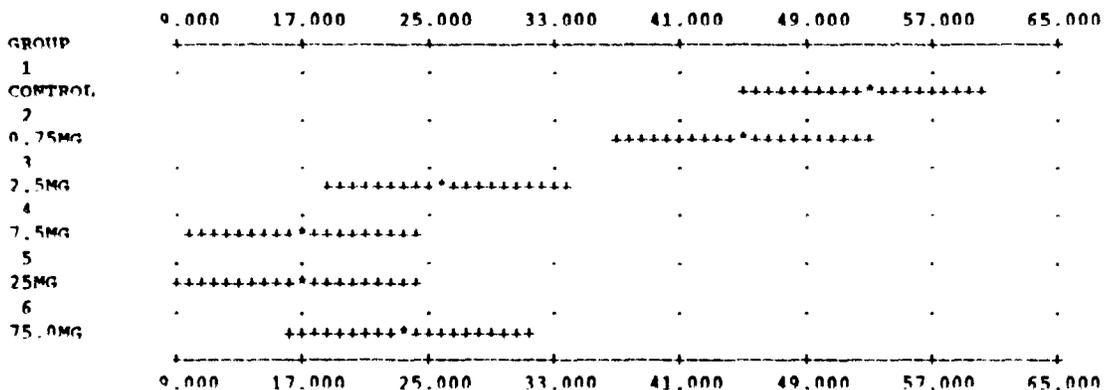
KRUSKAL-WALLIS STATISTIC= 37.98* (DF= 5) PROB=0.000
 F-RATIO BASED ON RANKS= 20.10* (DF= 4.90, 51.91) PROB=0.000
 JONCKHEERE'S STATISTIC= 299.0* (CMPLMNT= 1151.0) PROB=0.000 DOWN

*DENOTES SIGNIFICANCE AT 5% RISK. IF GROUPS ARE OF EQUAL SIZE THEN DIFFERENCES INDICATED BY + MAY ALSO BE CONSIDERED SIGNIFICANT. COMPARISONS ARE BASED ON AN ANALOG OF THE LSD.

DISTRIBUTION-FREE PAIR-WISE COMPARISONS WITH CONTROL GROUP

GROUP	RANK SUM	U	Z(U)	PROB	P(G>C)D+	D-	D	PCTILE	PROB
0.75MG	54.0	9.0*	-2.92	0.002	0.100	0.100	0.900	0.900*	100.0 0.001
2.5MG	58.0	3.0*	-3.53	0.000	0.030	0.000	0.900	0.900*	100.0 0.001
7.5MG	55.0	0.0*	-3.76	0.000	0.000	0.000	1.000	1.000*	100.0 0.000
25MG	56.0	1.0*	-3.69	0.000	0.010	0.000	0.900	0.900*	90.0 0.001
75.0MG	58.0	3.0*	-3.53	0.000	0.030	0.000	0.900	0.900*	100.0 0.001

BAR GRAPH PLOT. APPROXIMATE LSD'S (5 PERCENT RISK LEVEL)



STUDY 2
 SUMMARY STATISTICAL ANALYSES

7/92

	CONTROL	0.75MG	2.5MG	7.5MG	25MG	75.0MG	OTHER TESTS	PROB
	N	10	9	10	10	10		
dpm/ug	AVG	143.7	56.9++	13.2+++	8.4+++	12.0+++	14.2+++ LINEAR REGRESSION	0.000***?
	SE	19.7	8.6	1.7	1.1	4.9	3.3	
	PROB		0.002	0.000	0.000	0.000	0.000	

SYMBOLS APPEARING BY GROUP AVERAGES/MEDIANS INDICATE DIFFERENCES FROM CONTROL
 *, **, *** DENOTE SIGNIFICANCE BY NORMAL DISTN. METHODS WITH P<.05, .01, .001 RESPECTIVELY
 +, ++, +++ DENOTE SIGNIFICANCE BY DISTN.-FREE METHODS WITH P<.05, .01, .001 RESPECTIVELY
 ? DENOTES SIGNIFICANT LACK OF-FIT TEST AT P<.05

APPENDIX I

INTERDEPARTMENTAL CORRESPONDENCE

From: G. M. Ridder

Date: 10/29/91

To: A. Erickson, R. L. Binder

Retention Limit:

Subject: Determination of Skin Treatment Areas by Image Analysis

Below are measurements of the skin treatment areas that you traced onto overhead transparencies. The image analysis system used was the Pathology workstation consisting of TCL-Image (Version 4.6 Biological Detection Systems) software running on a Macintosh IIx computer (Apple). The areas in square centimeters were determined by placing the transparencies onto a lighted macrostand and acquiring the images from television camera (Sony DXC327H). The results (in cm^2) were calibrated by measuring the total area of a 4 cm^2 region of graph paper and ratioing the pixel areas of the samples to the pixel area of the scale (average of five measurements of the scale). (Notebook Reference: VE 1386 pg133). This work was performed October 28, 1991)

Sample	Area(Pixels)	Area(sq cm)
Scale	57857	4.00
Scale	57411	4.00
Scale	57573	4.00
Scale	57901	4.00
Scale	57428	4.00
Skin 1-1	88573	6.15
Skin 1-2	97455	6.76
Skin 1-3	107303	7.45
Skin 2-1	85460	5.93
Skin 2-2	91081	6.32
Skin 2-3	106018	7.36
Skin 2-3 Back	111067	7.71

G. M. Ridder
G. M. Ridder

2/10/92

WTDS STUDY SUMMARY

TSIN	Test Material Name
G0539.05	OCTOPIROX
G0539.06	
<p>WTDS Accession Number: 36907 Archive Location: 1563 and WAC0020n</p> <p>DRD: BYCR 856S Sector: Unavailable Category: Unavailable Site: Unavailable Division: HUMAN AND ENVIRONMENTAL SAFETY DIVISION Toxicologist: BINDER, R.L. Test Type: LACZ MUTANT FREQUENCY Test System: MUTAMOUSE TISSUE TSCA 8(e) Status: A -- REVIEWED - NO REPORTABLE INFORMATION</p>	

Study Title: LACZ MUTANT FREQUENCY	
Lab Study Number: YB-1402 B91-0153	Report Date: 04/27/1992
Study Dates:	
Performing Laboratory: MIAMI VALLEY LABORATORIES BIOLOGICAL TEST FACILITY	
<p>Text Summary: DOSE GROUP, NUMBER, CONCENTRATION</p> <p>1, 10, 0 2, 10, 0.075 MG IN 0.1 ML ETHANOL 3, 10, 0.25 MG IN 0.1 ML ETHANOL 4, 10, 0.75 MG IN 0.1 ML ETHANOL 5, 10, 2.50 MG IN 0.1 ML ETHANOL 6, 10, 7.50 MG IN 0.1 ML ETHANOL</p> <p>EPIDERMAL DNA SYNTHESIS IN MUTA(TM)MOUSE WAS DETERMINED BY MEASURING THE INCORPORATION OF 3H-THYMIDINE INTO DNA. A DOSE OF 7.5 MG OF OCTOPIROX APPLIED IN 0.1 ML OF ETHANOL, CAUSED A RAPID INHIBITION OF EPIDERMAL DNA SYNTHESIS, WITH A MAXIMAL EFFECT 2 HOURS AFTER DOSING. THIS WAS FOLLOWED BY AN APPARENT REBOUND TO HIGHER THAN CONTROL LEVELS OF DNA SYNTHESIS WITHIN 20 HOURS. ALL DOSES OF OCTOPIROX TESTED CAUSED A STATISTICALLY SIGNIFICANT INHIBITION OF EPIDERMAL DNA SYNTHESIS. THE LOWEST DOSE (0.075 MG OR 11.3 UG/CM(2)) CAUSED A STATISTICALLY SIGNIFICANT INHIBITION OF EPIDERMAL DNA SYNTHESIS. THE LOWEST DOSE (0.075 MG OR 11.3 UG/CM(2)) CAUSED ABOUT A 60% INHIBITION OF THE INCORPORATION OF 3H-THYMIDINE INTO DNA. DOSES FROM 0.25 TO 7.5 MG (37.5 TO 1130 UG/CM(2)) ALL CAUSED A MAXIMAL INHIBITION OF EPIDERMAL DNA SYNTHESIS OF >90%.</p>	

TSIN:	G0539.05
TSIN:	G0539.06

<i>Test Material:</i>	OCTOPIROX

Original summary was prepared as a text document and subsequently reformatted to the tabular summary format.