

Report Title: Acute Dermal Toxicity of G0539.03 on SKH-HR1 Hairless Mice

Test Type: Acute Dermal Toxicity

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

Test Substance: G0539.03 is 5% Octopirox in propylene glycol/ethanol/water (1/2/1). The test material was diluted in neutral and alkaline pH vehicles.

Species: Mice

of Animals: 40 female (10 per group)

Test Conditions: Total of 40 female SKH-HR1 Hairless mice (10 per group) exposed dermally 1) neutral pH vehicle, 2) alkaline pH vehicle, 3) 5% G0539.03 (167 mg/kg BW) in a neutral pH vehicle and 4) 5% G0539.03 (167 mg/kg BW) in alkaline pH vehicle.

Results: No biologically significant treatment related effects seen in any animals with regard to body weights, hematology and macroscopic anatomy. One animal exhibited signs of neurotoxicity (myoclonus) which occurred in acute dermal treatment of the test material in the alkaline vehicle.

Study #: B88-0215

Report Date: 12-5-88

QA Statement/GLP compliance: Yes

Accession #: 34922



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES
P O BOX 1962, CINCINNATI, OHIO 45247

The following study was audited by the Quality Assurance Unit:

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

STUDY NUMBER: 888-0215

NOTEBOOK NUMBER: YS-986, VS-2398

DIVISIONAL REQUEST DOCUMENT: PHSE 132

TSIN: G0539.03

TYPE OF STUDY: Acute Dermal Toxicity

PORTION(S) OF STUDY AUDITED:	AUDITOR:	DATE AUDITED:	DATE REPORTED TO STUDY DIRECTOR:
Test Substance Handling, Test System, Environmental Conditions, Dosing, Observations	H. P. Bauer	8/30/88	8/30/88
Necropsy	H. P. Bauer	9/2/88	9/2/88
Study Data	L. K. Klahm	11/9/88	11/9/88

The protocol was audited for compliance to the GLP regulations.

The final study report was audited. The results presented in this report accurately reflect the raw data of the study.

M.P. Bauer 12/5/88
Quality Assurance Unit Date

FINAL REPORT

ACUTE DERMAL TOXICITY OF G0539.03 ON SKN-HR: HAIRLESS MICE

Issue Date: 12-5-88

SUMMARY

The acute dermal toxicity of G0539.03 in an alkaline (pH 9) vs. neutral (pH 7) pH vehicle was studied using hairless mice. Clinical signs were closely monitored for 3 days after dosing. Body weights were recorded daily. Gross necropsy of all animals on the fourth day after dosing was carried out to determine anatomical and clinical pathological effects (if any) related to treatment.

One mouse showed clinical signs of neurotoxicity which appeared to be related to acute dermal treatment with G0539.03 in the alkaline vehicle. No other treatment related effects were observed in all other animals with regard to body weights, hematologic cellular and hemoglobin variables, and macroscopic anatomical alterations.

Test substance identification number (TSIN) #: G0539.03

Study #: 588-0215

IRD #: FRSE 132

In-Life Start Date: August 30, 1988

In-Life Completion Date: September 2, 1988

Test Site: S-36, BTP, Miami Valley Laboratories, Ross, OH

Division: Beauty Care Technology

Study Director: G. G. Hillebrand, Ph.D.

Study Technician: D. A. Heitmayer

Divisional Toxicologist: W. B. Gibson, Ph.D., D.A.B.T.

Neurotoxicologist: J. P. Ross, D.V.M., Ph.D.

Veterinary Pathologists: R. H. Brunner, D.V.M., D.A.C.V.P., C. L. Alden, D.V.M., D.A.C.V.P.

Necropsy Technician: W. E. Wyder

Clinical Pathologists: K. W. Weingand, D.V.M., Ph.D., D.A.C.V.P. and M. J. Laytart, M.T.(A.S.C.P.)

Statisticians: R. D. Bruce, Ph.D. and P. J. Sprong

Purpose: To determine the acute dermal toxicity potential of G0539.03 in an alkaline (pH 9.3) or neutral pH vehicle.

Test Substance: 100% G0539.03 is a white powder and stable at room temperature. Test solutions will be prepared immediately before use.

Test System: Female albino Skh-HR1 hairless mice (R#28-0471, Charles River) 12 weeks and 6 days old and weighing between 21 and 26.3 g. Inclusion into this study of the one mouse that weighed greater than 26 g (26.3 g) is a protocol deviation and does not effect the results.

Experimental Design: There were 4 test groups (10 mice per group). The pH values of the treatment solutions were slightly different from the estimated values stated in the approved protocol and are defined below as well as in the Protocol Amendment.:

<u>Group #</u>	<u>Cage/Animal #s</u>	<u>Treatment</u>
1)	1-10	Treatment with neutral pH (6.99) vehicle.
2)	11-20	Treatment with alkaline pH (9.28) vehicle.
3)	21-30	Treatment with 5% G0539.03 (approximately 167 mg/kg body weight) in a neutral pH (6.95) vehicle.
4)	31-40	Treatment with 5% G0539.03 in an alkaline pH (9.12) vehicle.

The vehicle for these studies was propylene glycol:ethanol:water (1:2:1). Animals were dosed once, observed daily for four days and then gross necropsied.

Location of Data:

All raw data pertaining to Study B88-0215 can be found in lab notebook VS-986 and VS-2398.

METHODS

Route of Administration of Test Substance: 100 ul of the test material was applied once to the dorsal skin (about 7 cm²) of each mouse with a Gilson Pipetman and spread evenly with the pipette tip. Dosing was in order of animal number. After dosing, animals were randomized on the cage rack by the Study Technician. Therefore, monitoring of animals post-dosing was in random order.

Identification of Animals: Animals were individually housed and identified by cage number. In addition, each animal was color-coded (tail marked with a felt-tipped pen) according to group.

Animal Monitoring: Each individual animal in the study was carefully monitored for pharmacological/clinical signs of toxicity by Dr. J. P. Ross, D.V.M., Ph.D.. These monitoring sessions were done by observing the animal's locomotor behavior and response to stimulation (e.g., air puffs and noise). The initial 3 monitoring sessions (the first just prior to dosing, the second 1 hour after dosing and the third 4 hours after dosing) were carried out with the mouse in a clear plastic observation cage (without a top) separate from its home cage. The remainder of the observation sessions during the study were done while the animal was in its home cage with the top removed. In addition, all animals in the study were watched closely while in their home cages on the animal rack throughout the day for changes in their locomotor activity by the Study Technician (this was done by viewing the animal through the clear plastic home cage while on the animal rack). If changes were noted, the animal (in its home cage) was pulled from the rack for more detailed monitoring by Dr. Ross. Animal monitoring records were kept for each animal.

Animal Weighing: Animals were weighed using a top loading balance (Sartorius Model 3802NP, NV#16303, lab notebook # VE125). Weighings were performed just prior to dosing and at the end of each day of the study.

Gross Necropsy:

Animals were randomized for gross necropsy according to the Standard Operating Procedures of the Facility. Necropsy was carried out by W. E. Wyder and supervised by C. L. Alden (animal # 33 only) and R. K. Brunner (all other animals). Body weights were determined just prior to euthanasia which was by ether asphyxiation. After euthanasia, blood was collected into EDTA containing tubes from the posterior vena cava using a syringe equipped with a needle.

Statistical Analysis:

Hematology data from the G0539.03 treatment groups (3 and 4) were compared with their respective vehicle control groups (1 and 2), the vehicle control groups were compared with each other, and the G0539.03 treatment groups were compared with each other. Group average body weight data from the G0539.03 treatment groups were compared with each other and G0539.03 treatment group 4 was compared to vehicle control group 2. The statistical evaluations were made by analysis of variance techniques and were conducted at a 5%, two-sided risk level.

RESULTS AND CONCLUSIONS

Clinical Signs:

One animal (#33) exhibited signs of neurotoxicity (myoclonus) which appeared to be treatment related. These signs began approximately 4 hours after dosing, continued for at least the next 4 hours and were not present the following morning of day 2 (see attached report by J. P. Ross). This animal was observed not to eat or drink after the start of the myoclonus signs and was moribund the morning of Day 3 at which point the animal was euthanized for gross necropsy. No other significant clinical signs were observed in the other animals.

Body Weights:

Comparisons were made for the change in group average body weight from the initial weight to the final weight at either necropsy or at the end of Day 3 (in-life phase of the study). These comparisons were made for group 2 vs. group 4 and for group 3 vs. group 4 (in both cases group 4 excluded animal #33 which became moribund and was sacrificed on Day #3). No significant changes in group average body weight which were deemed treatment related were observed. See attached Summary of Statistical Analysis of Body Weights.

Animal #33 showed a continuous decrease in body weight (from 23.2 g initial weight to 15.7 g necropsy weight) from the time it was dosed to the time of necropsy. This loss in body weight probably resulted from the animal refraining from eating and drinking.

Anatomic Pathology:

Macroscopic changes were not observed at gross necropsy in any animal which were considered related to treatment with G0539.03. See the Gross Necropsy Report which is included with the Pathology Report.

Clinical Pathology:

No biologically significant test substance treatment effects were evident on hematologic cellular and hemoglobin variables in mice treated topically with G0539.03 when compared to vehicle controls. A potential synergistic effect of test substance and alkaline vehicle resulted in mild increments of RBC, HGB, HCT, WBC, and Lymph. These increments were not thought to be of biological significance. See attached Pathology Report.

 12-5-84

G. G. Hillebrand
Study Director

PROTOCOL

ACUTE DERMAL TOXICITY OF GO 539.03 ON SKH-HR1 HAIRLESS MICE

Issue Date: 8-25-88

Test substance identification number (TSIN) # GO 539.03

DRD #: FMSE 132

Study # B88-0215

Division: Beauty Care Technology

Study Director: G. G. Killebrand

Test Site: S-36, BTF, Miami Valley Laboratories, Ross, OH

Purpose: To determine the acute dermal toxicity potential of GO 539.03 in an alkaline (pH 9.3) or neutral pH vehicle.

Proposed Start Date: August 30, 1988

In-Life Completion Date: September 2, 1988

Justification: Significant toxicity was seen in a previous investigational study when GO 539.03 (5% solution at pH 9.3) was administered to the dorsal skin of chronically UV-irradiated Skh-HR1 hairless mice. In those experiments, GO 539.03 treatment was followed 2 hours later by a full formula sunscreen and irradiation with simulated solar radiation (lab notebook ref. VS-2398, pp. 12 and 14). No toxicity has been observed in investigational studies when GO 539.03 was applied in a neutral pH vehicle to hairless mice. This study is being run for the purpose of determining the toxicity potential of GO 539.03 in a alkaline or neutral pH vehicle without sunscreen or irradiation treatment. This study is being conducted in accordance with GLP regulations.

Test System: Female albino Skh-HR1 hairless mice (Charles Rivers) about 10 weeks old and weighing between 21 and 26 g.

Test Substance: 100% GO 539.03 is a white powder and stable at room temperature. Test solutions will be prepared immediately before use.

Identification of Animals: Animals will be individually housed and identified by cage number. In addition, each animal will be color-coded (tail marked with a felt-tipped pen) according to group.

Experimental Design: There will be 4 test groups (10 mice per group):

<u>Group #</u>	<u>Cage/Animal #s</u>	<u>Treatment</u>
1)	1-10	Treatment with neutral pH (7.0) vehicle.
2)	11-20	Treatment with alkaline pH (9.3) vehicle.
3)	21-30	Treatment with 5% GO 539.03 (approximately 167 mg/kg body weight) in a neutral pH (7.0) vehicle.
4)	31-40	Treatment with 5% GO 539.03 in an alkaline pH (9.3) vehicle.

The vehicle for these studies is propylene glycol:ethanol:water (1:2:1). Animals will be dosed once, observed daily for four days and then gross necropsied.

Route of Administration of Test Substance: 100 ul of the test material will be applied once to the dorsal skin (about 7 cm²) of each mouse with a Gilson Pipetman and spread evenly with the pipette tip.

Animal Care and Diet: Mice will have free access to laboratory chow and water according to the Standard Operating Procedures of the Test Facility.

Environmental Conditions: Will follow the Standard Operating Procedures of the Test Facility.

Diet and Water Analysis: None needed (no known contaminants expected which would interfere with this study).

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

Skin Preparation: None needed.

Animal Monitoring after Dosing: Animals will be weighed before dosing. Animals will be dosed in the morning of 8-30-88. Monitoring will take place in a plastic observation cage. Animals will be monitored for pharmacological/clinical signs of toxicity by Dr. J.P. Ross, D.V.M., Ph.D. and neuro-toxicologist. Animal monitoring will be performed just prior to dosing and at least every two hours for the first eight hours and at least 3 times per day on the second and third days following dosing (8/31-9/1). Animals will be weighed at the end of the first day and the end of each subsequent day of the study. Gross necropsies will be performed on moribund or expired animals (as soon as possible after death or held under refrigeration) by trained necropsy personnel (W.E. Wyder) under the supervision of a veterinary pathologist. Also, gross necropsies will be performed on animals surviving to the end of the study (the morning of 9-2-88).

Gross Necropsy:

Animals will be randomized for gross necropsy according to the Standard Operating Procedures of the Facility. The gross necropsy will be done according to the Standard Operating Procedures of the Facility and supervised by a veterinary pathologist. Body weights will be determined just prior to euthanasia. Mice will be euthanized by ether asphyxiation. Blood will then be collected into EDTA containing tubes from the posterior vena cava using a syringe equipped with a needle. A differential white blood count and hemogram (includes WBC, RBC, Hgb, HCT, MCV, MCH, MCHC, and PLT) will be done on the blood sample. All gross observations for all organs will be recorded. Any tissues showing gross alterations that the attending pathologist considers potentially treatment related will be saved (in 10% neutral buffered formalin at a dilution of 1 part tissue to >10 parts fixative) for possible histopathological evaluation.

Statistical Analysis:

Hematology data from the GO 539.03 treatment groups will be compared with their respective vehicle control groups, the vehicle control groups will be compared with each other, and the GO 539.03 treatment groups will be compared with each other. The statistical evaluations will be made by analysis of variance techniques. Provided that Bartlett's test of homogeneity of variance is not

significant, groups should be compared using the least significant difference (LSD) criterion. If Bartlett's test is significant, comparisons should be made by a t-test technique which makes allowance for unequal variances. In this latter case, Wilcoxon's rank sum test should also be applied. All statistical tests should be conducted at a 5%, two-sided risk level.

A suitable reference for the statistical techniques mentioned above is Snedecor, G. W. and Cochran, W.G., Statistical Methods, 6th Edition, Iowa State University Press (1967). The appropriate text is: analysis of variance, pp. 277-279; least significant difference, pp. 271, 272, 278; Bartlett's test, pp. 296-298; Wilcoxon's rank sum test, pp. 130-132; t-test, unequal variances, pp. 114-116.

Report:

A final report will be prepared and will include the following data, summarized by group: the weights of animals during the study; summary of clinical observations; the number and identity of animals that died and the time of death after dosing; contributing scientist's reports pertaining to hematology, gross necropsy or histopathology (if any); a summary of all findings of toxicological significance. The report shall conform to all requirements outlined in Section 58.185, sub part J, Good Laboratory Practices Regulations.

G. G. Hillebrand
G. G. Hillebrand *8/24/68*
Study Director

W. B. Gibson
W. B. Gibson
Divisional Toxicologist

PROTOCOL AMENDMENT

ACUTE DERMAL TOXICITY OF GO 539.03 ON SKH-2P1 HAIRLESS MICE

Issue Date: 11-7-88

Study #: 888-0215

ORD #: FR88E 132

Test substance identification number (TSIN) #: GO 539.03

Study Director: G. G. Hillebrand

Divisional Toxicologist: W. B. Gibson

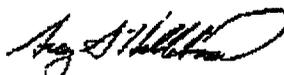
Original Protocol: The pH values of each treatment were defined as follows:

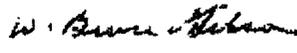
<u>Group #</u>	<u>Cage/Animal #s</u>	<u>Treatment</u>
1)	1-10	Treatment with neutral pH (7.0) vehicle.
2)	11-20	Treatment with alkaline pH (9.3) vehicle.
3)	21-30	Treatment with 5X GO 539.03 (approximately 167 mg/kg body weight) in a neutral pH (7.0) vehicle.
4)	31-40	Treatment with 5X GO 539.03 in an alkaline pH (9.3) vehicle.

Revised Protocol: The actual pH values of each treatment were as follows:

<u>Group #</u>	<u>Cage/Animal #s</u>	<u>Treatment</u>
1)	1-10	Treatment with neutral pH (6.99) vehicle.
2)	11-20	Treatment with alkaline pH (9.28) vehicle.
3)	21-30	Treatment with 5X GO 539.03 (approximately 167 mg/kg body weight) in a neutral pH (6.95) vehicle.
4)	31-40	Treatment with 5X GO 539.03 in an alkaline pH (9.12) vehicle.

Reason for Change: The original protocol stated estimates of the final pH values for each treatment. The actual pH values of the treatments determined the day of treatment preparation (8/30/88) were slightly different from these estimates and were deemed acceptable for the study by the Study Director.


G. G. Hillebrand
Study Director


W. B. Gibson
Divisional Toxicologist

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCB)

FORM 132

DATE: NOV 1987

TEST: 60587.02

Test Article
 (Product/Ingredient): Octopirox[®]
 Physical Form: Solid
 Color: Off-White
 Storage Conditions:

Brand Notebook Ref: HR-5479-15
 pH: 8-10 (1% solution in water)
 Expiration Date: 12/88
 Hazards: Corrosive

Room Temperature _____
 Other _____

Stability Testing is:

Completed
 In Progress

D.O.T. Hazard Classification: Non-hazardous/Not combustible

These chemicals/test articles have been checked Yes
 against the TSCA inventory list. No

Formulated Composition

<u>Component^a</u>	<u>(C.A.S.)^b</u> <u>(Number)</u>	<u>Target</u> <u>Nominal</u> <u>Component</u> <u>Level</u>	<u>Target</u> <u>Actual</u> <u>Component</u> <u>Level</u>	<u>Measured</u> <u>Component</u> <u>Level</u>	<u>Stock</u> <u>Code</u> <u>No.</u>	<u>Lot Number</u>
Octopirox [®]	-	100	100	99.5	EX288	2

- (a) Use chemical or CFA names which uniquely identifies each component. Common, non-chemical names such as (Targitol 15-5-0 or yellow dye #10) should be indicated in parentheses following the chemical name. Non-definitive identification (e.g., EC-Bass, Arquad) is not acceptable.
- (b) CAS numbers should be included whenever available. They are especially important for main components of the formulation.

ANALYSIS

<u>Date Submitted</u>	<u>Submitter Code Or Lab Notebook</u>	<u>Analysis</u>	<u>(X) *</u>	<u>Measured Value</u>	<u>Limits</u>	<u>Laboratory Testing Or Data Source</u>
5/8/87	85149005	NCT	X	Pass	Pass	SWTC Micro
	88025018	NCT		Pass	Pass	" "
	85149005	Active Assay		99.5X	98.0-101.5X	SWTC Analy
	88025017	Active Assay		99.5X	" "	" "
	85149005 and 88025017	IR		Pass	Must match standard spectrum	SWTC Analy
	85149005 and 87127003	Appearance		Pass	Pass	CR

* Analysis required by toxicologist or microbiologist

Approvals: The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product are being/have been obtained and evaluated by Products Research.

Process Development: Caroline R. Ladd 11/1/88
 (Signature) (Date)

Products Research: N.A.
 (Signature) (Date)

Acceptable Microbial Susceptibility: James J. Kurbela 1/26/88
 (Signature) (Date)

GMP Quality Assurance: Thomas R. Johnston 2/2/77
 (Signature) (Date)

(Attach batch records for GMP review)

finished products will be retained by Quality Assurance (# samples)

This substance has been appropriately characterized and is acceptable for testing outlined in the attached DRD.

Division Toxicologist: Attila 2/2/88
 (Signature) (Date)

VA1/005
 8/87 Division Toxicologist, Bruce Gibson W. Bruce Gibson 8/25/88

INTERDEPARTMENTAL CORRESPONDENCE

FROM: J. F. Ross

Date: 4/1/68

TO: G. G. Hillebrand

R/L: NON-DISSE-
TIONARY

SUBJECT: Observations in Study B68-0215

The following summarizes observations made during the conduct of B68-0215, acute dermal dosing of octopirox-containing solutions in mice.

One animal (#35) exhibited signs of neurotoxicity. These began approximately four hours after dosing and were characterized by tight circling and asynchronous myoclonus involving all four limbs. Muscles of the head were not involved at this time, and reaction to external stimuli (auditory, tactile, were normal or perhaps even greater than normal. No signs of autonomic stimulation (urination, salivation, tearing) were exhibited.

During the next hour the myoclonus progressed rostrally to include the muscles of the eyelids, ears and perhaps the mouth. At this time the myoclonic contractions were synchronous, although the forelimbs appeared to be less involved than the hindlimbs. This condition changed little during the remainder of the day.

On the following morning (Day 2) myoclonus was no longer present. However, the mouse had no spontaneous locomotor activity. There were depressed spinal reflexes indicated by failure to extend the limbs when lifted by the tail. The response to tail pinch was poorly directed, although the response to a puff of air was relatively normal.

During the day this animal remained depressed and was without locomotor activity. There was apparent aphagia and adipsia.

On the third morning, the animal was hypothermic and moribund and was therefore sacrificed for necropsy.

Additional observations of minor increases or decreases in locomotor activity or response to air puff seen in other mice are deemed incidental. Ocular lesions consisting of blepharospasm and/or corneal opacities were observed incidentally in other mice during the observations for neurotoxicity.

Joseph F. Ross
Joseph F. Ross

cc:File

STUDY 688-0215
SUMMARY OF STATISTICAL ANALYSES

10/12/48

		GROUP 1	GROUP 2	GROUP 3	GROUP 4	OTHER TESTS	PROB
		F	F	F	F		
FINAL	AVG	24.49	24.10	24.13	24.69	GRPS:264	0.365
WT.-BEC GMS	SE	0.45	0.54	0.44	0.25	GRPS:364	0.423
	N	10	10	10	10		
	PROB		0.511	0.630	0.748		
INITIAL	AVG	24.36	23.42	23.79	24.74	GRPS:264	0.065
WT. GMS	SE	0.44	0.49	0.50	0.15	GRPS:364	0.022*
	N	10	10	10	9		
	PROB		0.218	0.353	0.604		
FINAL	AVG	23.82	23.25	23.59	24.39	GRPS:264	0.051
WT.-LIFE GMS	SE	0.34	0.50	0.41	0.27	GRPS:364	0.169
	N	10	10	10	9		
	PROB		0.310	0.000	0.324		
BODY WT.	AVG	0.13	0.60	0.60	0.10	GRPS:264	0.060
CHANGE WGT.	SE	0.13	0.27	0.23	0.17	GRPS:364	0.372
	N	10	10	10	9		
	PROB		0.067	0.159	0.121		
BODY WT.	AVG	-0.54	-0.17	-0.20	-0.37	GRPS:264	0.541
CHANGE LIFE	SE	0.16	0.23	0.26	0.20	GRPS:364	0.604
	N	10	10	10	9		
	PROB		0.241	0.280	0.590		

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

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: K. W. Weingand, M. J. Laytart,
D. L. Rothacker

DATE: November 10, 1968

TO: G. G. Hillebrand

R/L: NON-DISCRETIONARY

SUBJECT: Pathology Report B88-0215,
PHBSE-132, TSIN G0539.03

C O N C L U S I O N S

C L I N I C A L P A T H O L O G Y

No biologically significant test substance treatment effects were evident on hematologic cellular and hemoglobin variables in mice treated topically with G0539.03 when compared to vehicle controls. A potential synergistic effect of test substance and alkaline vehicle resulted in mild increments of of RBC, HGB, HCT, WBC, and LYMPH. These increments were not thought to be of biological significance.

A N A T O M I C P A T H O L O G Y

Macroscopically, no alterations were noted which could be attributed to test substance administration.

I N T R O D U C T I O N

Four groups of 10 female albino Skh-HR1 hairless mice (Charles River) were treated once dermally with either vehicle control substance at pH 6.99, vehicle control substance at pH 9.29, 5% G0539.03 at pH 6.95, or 5% G0539.03 at pH 9.12 and observed for 4 days. The purpose of this study was to determine the acute dermal toxicity potential of G0539.03 in an alkaline or neutral pH.

M A T E R I A L S A N D M E T H O D S

E X P E R I M E N T A L M O D E L

TYPE OF STUDY:	Acute Dermal Toxicity
SPECIES:	Mouse
STRAIN (SOURCE):	Skh-HR1 albino, hairless
SEX:	Female
INITIAL WEIGHT RANGE:	21 - 26 g
TEST SUBSTANCE DOSE:	5% G0539.03
VEHICLE CONTROL SUBSTANCE DOSE:	Propylene glycol:ethanol:water (1:2:1)
ROUTE OF EXPOSURE:	Dermal application
DOSING REGIMEN:	Once
TEST DOSE VOLUME:	100 μ l
OBSERVATION:	4 days

ANIMAL GROUP ASSIGNMENT

<u>GROUP</u>	<u>ANIMAL #/SEX</u>	<u>TREATMENT and DOSAGE (100 µl)</u>
1	# 1 - 10/female	Vehicle substance at pH 6.99
2	# 11 - 20/female	Vehicle substance at pH 9.28
3	# 21 - 30/female	5% G0539.03 at pH 6.95
4	# 31 - 40/female	5% G0539.03 at pH 9.12

CLINICAL PATHOLOGY

Blood for hematologic analyses was collected at necropsy on 9/1/88 for animal #33 (moribund) and on 9/2/88 for all other mice. Whole blood was collected from the vena cava into tubes containing an appropriate amount of EDTA anticoagulant. Hematologic analyses were performed at the Clinical Pathology Laboratory (MVL).

Hematology

- White blood cell count (WBC)
- Red blood cell count (RBC)
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLT)
- Differential leukocyte count
 - Segmented neutrophils (SEGS)
 - Band neutrophils (BANDS)
 - Lymphocytes (LYMPH)
 - Monocytes (MONO)
 - Eosinophils (EOS)
 - Basophils (BASO)

Hematologic results were analyzed statistically by R. D. Bruce and P. J. Sprong using Procter and Gamble Computer Program #88944. This program's output has listed statistical comparisons of each group (2-4) to Vehicle Control Group 1. Group 2 is the Vehicle Control Group for Treatment Group 4. The statistical comparison of Group 4 to Group 1 is not experimentally meaningful and will not be discussed further.

All statistical tests were conducted at a 5%, two-sided risk level. Data from the Clinical Pathology testing and statistical analyses were further evaluated by K. W. Weingand, D.V.M., Ph.D., Diplomate, A.C.V.P. and M. J. Laytart, M.T.(A.B.C.P.).

ANATOMIC PATHOLOGY

Refer to the report submitted by Pathology Associates, Inc. (Anatomic Pathology Appendix I).

RESULTS

CLINICAL PATHOLOGY

Summaries of the hematologic statistical analyses are given in Clinical Pathology Table 1. Individual animal hematologic data are given in Clinical Pathology Appendix I. Data for animal #33 (moribund) are given in Appendix II. Data from the study are located in notebook Y2-1291 in the Clinical Pathology Laboratory.

Some data values are missing (see table below) and are noted as -M- in the data tables. The amount of missing data was small and did not affect the overall evaluation of the data.

TABLE OF MISSING DATA

<u>Group</u>	<u>Animal #</u>	<u>Test</u>	<u>Reason</u>
1	1	All	No sample received due to technical difficulties in obtaining blood
1	3	Differential	Quantity Not Sufficient (QNS)
1	4	Differential	QNS
2	15	Differential	QNS
2	16	Differential	QNS
2	20	Differential	QNS
3	24	Differential	QNS
3	25	Differential	QNS
3	30	Differential	QNS
4	33	Differential	QNS
4	35	All	Sample clotted

No statistically significant group differences were seen in the following variables: MCH, PLT, BANDS, BASO, EOS, MONO, AND SEGS. No statistically significant differences were noted when Vehicle Control Groups 1 and 2 were compared.

ANATOMIC PATHOLOGY

Refer to the report submitted by Pathology Associates, Inc. (Anatomic Pathology Appendix I).

RESULTS

CLINICAL PATHOLOGY

Summaries of the hemtologic statistical analyses are given in Clinical Pathology Table 1. Individual animal hematologic data are given in Clinical Pathology Appendix I. Data for animal #33 (moribund) are given in Appendix II. Data from the study are located in notebook YB-1201 in the Clinical Pathology Laboratory.

Some data values are missing (see table below) and are noted as -M- in the data tables. The amount of missing data was small and did not affect the overall evaluation of the data.

TABLE OF MISSING DATA

<u>Group</u>	<u>Animal #</u>	<u>Test</u>	<u>Reason</u>
1	1	All	No sample received due to technical difficulties in obtaining blood
1	3	Differential	Quantity Not Sufficient (QNS)
1	4	Differential	QNS
2	15	Differential	QNS
2	16	Differential	QNS
2	20	Differential	QNS
3	24	Differential	QNS
3	25	Differential	QNS
3	30	Differential	QNS
4	33	Differential	QNS
4	35	All	Sample clotted

No statistically significant group differences were seen in the following variables: MCH, PLT, BANDS, BASO, EOS, MONO, AND SEGS. No statistically significant differences were noted when Vehicle Control Groups 1 and 2 were compared.

Statistically significant differences were seen when Treatment Group 3 was compared to Vehicle Control Group 1. The average MCV and MCHC were statistically significantly increased and decreased, respectively, in Treatment Group 3 when compared to Vehicle Control Group 1. A statistically significant difference was also seen when Treatment Group 4 was compared to Vehicle Control Group 2. The average MCHC was statistically significantly decreased in Treatment Group 4 when compared to Vehicle Control Group 2. Although statistically significant, the magnitude of these mean group differences was minimal. These changes are not thought to be biologically significant or treatment-related and will not be discussed further.

Statistically significant changes were seen when Treatment Groups 3 and 4 were compared. The average RBC, HGB, HCT, WBC, and LYMPH were statistically significantly increased in Treatment Group 4 when compared to Treatment Group 3. These changes may be treatment-related.

ANATOMIC PATHOLOGY

Refer to the report submitted by Pathology Associates, Inc. (Anatomic Pathology Appendix I).

DISCUSSION

CLINICAL PATHOLOGY

There were no biologically significant test-substance treatment effects evident on hematologic analytes when comparing Treatment Groups 3 and 4 with Vehicle Control Groups 1 and 2, respectively. The comparison of Treatment Groups 3 and 4 showed statistically significant increments in RBC, HGB, HCT, WBC, and LYMPH in Treatment Group 4. The increased HGB and HCT are due primarily to the increment in RBC. The increased WBC is due primarily to the increment in LYMPH. The magnitude of these changes is minimal and probably not biologically significant or specifically test substance related. These mild changes may be the result of alkaline vehicle test substance synergistic effects. Mild increments in a multitude of hematologic variables, such as observed in Treatment Group 4, are sometimes related to systemic dehydration and hemoconcentration.

ANATOMIC PATHOLOGY

Refer to the report submitted by Pathology Associates, Inc. (Anatomic Pathology Appendix I).

Gary R. Johnson
REVIEWING ANATOMIC PATHOLOGIST

Kurt W. Weingard
CLINICAL PATHOLOGIST

CLINICAL PATHOLOGY TABLE 1a

STATISTICAL ANALYSIS SUMMARY

RESPONSE:		PERIOD 1 VALUES											
		RBC MILLION	HGB (GM/DL)	HCT (%)	MCV (FL.)	MCH (PG.)	MCHC (G)	WBC THOUSAND	PLATELET THOUSAND	HAMA THOUSAND	ESR THOUSAND	EOS THOUSAND	LYMPH THOUSAND
GROUP 1	AVG.	9.056	14.73	41.16	47.7	16.34	34.33	2.41	962.6	0.000	0.000	0.019	2.010
	S.E.	0.110	0.31	0.30	0.2	0.10	0.35	0.14	82.2	0.000	0.000	0.010	0.110
GROUP 2	AVG.	9.173	15.14	44.37	49.3	16.51	34.33	2.54	940.7	0.000	0.000	0.013	1.307
	S.E.	0.362	0.78	0.93	0.4	0.17	0.17	0.22	52.4	0.000	0.000	0.006	0.244
	P(C)	0.620	0.369	0.226	0.133	0.256	0.661	0.506	0.600	1.000	-	0.001	0.346
GROUP 3	AVG.	9.887	14.48	45.16	46.5	16.29	33.99	2.54	1024.7	0.005	0.000	0.016	2.157
	S.E.	0.272	0.28	0.77	0.3	0.09	0.20	0.22	30.2	0.005	0.000	0.007	0.185
	P(C)	0.501	0.400	0.037	0.043*	1.000	0.022*	0.751	0.960	0.710	-	0.902	0.640
GROUP 4	AVG.	9.418	13.33	46.30	49.4	16.31	32.13	3.23	1026.0	0.000	0.000	0.000	2.770
	S.E.	0.200	0.21	0.37	0.6	0.35	0.47	0.29	12.8	0.000	0.000	0.000	0.262
	P(C)	0.176	0.131	0.005**	0.046*	0.370	0.021*	0.620*	0.743	1.000	-	0.109	0.013*
GRPS:244	P(0)	0.346	0.523	0.066	0.122	0.945	0.016*	0.074	0.460	1.000	-	0.109	0.110
GRPS:244	P(0)	0.046*	0.030*	0.007**	0.217	0.573	0.623	0.020*	0.686	0.690	-	0.100	0.012*

P(C) DENOTES P-VALUE FOR COMPARISON OF OTHER GROUPS WITH CONTROL, GROUP 1 F
P(D) DENOTES P-VALUE FOR LINEAR REGRESSION OR FOR A CONTRAST.
*, **, *** DENOTE SIGNIFICANCE BY NORMAL DISTRIBUTION METHODS WITH P<.05, .01 OR .001 RESPECTIVELY.
+, **, *** DENOTE SIGNIFICANCE BY DISTRIBUTION-FREE METHODS WITH P<.05, .01 OR .001 RESPECTIVELY.
- INDICATES A STATISTICAL TEST NOT PERFORMED BECAUSE OF NO DATA OR WITHIN-GROUP VARIANCE = 0.

STATISTICAL ANALYSIS SUMMARY

0000215 HEMATOLOGY		PERIOD 1 VALUES	
RESPONSE		NUMO THOUSAND	SEGS THOUSAND
GROUP 1	AVG	0.106	0.400
F	S.E.	0.027	0.077
GROUP 2	AVG	0.092	0.331
F	S.E.	0.020	0.058
	P(C)	0.640	0.440
GROUP 3	AVG	0.102	0.420
F	S.E.	0.020	0.077
	P(C)	0.901	0.619
GROUP 4	AVG	0.106	0.341
F	S.E.	0.009	0.027
	P(C)	0.993	0.499
GRPS:264	P(0)	0.635	0.902
GRPS:364	P(0)	0.904	0.364

P(C) DENOTES P-VALUE FOR COMPARISON OF OTHER GROUPS WITH CONTROL, GROUP 1 F
P(0) DENOTES P-VALUE FOR LINEAR REGRESSION OR FOR A CONTRAST.

DATA LISTING

8880215--HEMATOLOGY

PERIOD 1 VALUES

RESPONSE:	RBC	HGB	HCT	MCV	MCH	MCNC	WBC	PLATELET	BANDS	BASO	EOS	LYMPH	
	MILLION	(GM/DL)	(%)	(FL.)	(PG.)	(%)	THOUSAND	THOUSAND	THOUSAND	THOUSAND	THOUSAND	THOUSAND	
GROUP 1	ANIMAL												
GROUP 1	1	--	--	--	--	--	--	--	--	--	--	--	
F	2	9.47	15.5	45.1	48.	16.4	34.4	3.2	1093.	0.00	0.00	0.00	2.46
	3	9.14	14.9	43.8	48.	16.3	34.0	1.9	1066.	--	--	--	--
	4	9.25	15.2	44.1	48.	16.4	34.5	2.2	324.	--	--	--	--
	5	9.35	15.1	44.1	47.	16.1	34.2	2.1	975.	0.00	0.00	0.04	1.66
	6	8.25	13.3	39.7	48.	16.1	33.5	2.8	1056.	0.00	0.00	0.00	2.30
	7	9.08	14.3	42.9	47.	15.7	33.3	2.3	958.	0.00	0.00	0.07	1.86
	8	9.13	14.7	43.0	47.	16.1	34.2	2.8	979.	0.00	0.00	0.00	2.02
	9	8.93	14.7	42.7	48.	16.5	34.4	2.4	1123.	0.00	0.00	0.02	2.09
	10	8.90	14.9	43.0	48.	16.7	34.7	2.2	1089.	0.00	0.00	0.00	1.74
	AVERAGE	9.056	14.73	43.16	47.7	16.26	34.13	2.43	962.6	0.000	0.000	0.019	2.018
	STD. ERR.	0.118	0.21	0.50	0.2	0.10	0.15	0.14	82.2	0.000	0.000	0.010	0.110
GROUP 2	ANIMAL												
F	11	9.16	14.7	43.8	48.	16.0	33.6	3.2	1190.	0.00	0.00	0.00	2.62
	12	10.42	17.1	49.8	48.	16.4	34.3	2.7	982.	0.00	0.00	0.00	2.48
	13	8.43	13.6	39.9	47.	16.1	34.1	2.2	1015.	0.00	0.00	0.02	2.00
	14	8.94	14.9	43.1	48.	16.7	34.6	1.5	892.	0.00	0.00	0.00	1.08
	15	8.92	14.8	43.6	49.	16.6	33.9	3.2	933.	--	--	--	--
	16	8.79	15.2	44.3	50.	17.3	34.3	1.8	688.	--	--	--	--
	17	8.75	15.2	43.1	49.	17.4	35.3	3.0	1151.	0.00	0.00	0.00	2.40
	18	9.11	15.2	44.7	49.	16.7	34.0	3.7	1037.	0.00	0.00	0.04	3.18
	19	9.00	14.7	44.2	49.	16.3	33.3	2.9	553.	0.00	0.00	0.03	2.38
	20	10.21	16.0	47.2	46.	15.7	33.9	2.2	1046.	--	--	--	--
	AVERAGE	9.173	15.14	44.37	48.3	16.52	34.13	2.64	948.7	0.000	0.000	0.013	2.307
	STD. ERR.	0.202	0.29	0.83	0.4	0.17	0.17	0.22	62.4	0.000	0.000	0.006	0.244
GROUP 3	ANIMAL												
F	21	8.70	14.1	43.0	49.	16.2	32.8	2.1	1096.	0.00	0.00	0.00	1.85
	22	8.69	14.1	42.1	48.	16.2	33.5	2.0	1079.	0.00	0.00	0.04	1.72
	23	9.36	15.1	45.3	48.	16.1	33.3	2.7	983.	0.00	0.00	0.00	2.00
	24	9.38	15.3	46.1	49.	16.3	33.2	3.1	1182.	--	--	--	--
	25	8.63	14.1	40.8	47.	16.3	34.6	1.8	912.	--	--	--	--
	26	9.50	16.0	46.6	49.	16.8	34.3	3.3	965.	0.00	0.00	0.03	2.67
	27	9.02	14.3	43.7	48.	15.9	32.7	3.6	1062.	0.04	0.00	0.04	2.66
	28	7.92	13.3	40.4	51.	16.8	32.9	3.1	1026.	0.00	0.00	0.00	2.39
	29	9.44	15.2	45.4	48.	16.1	33.5	2.1	1081.	0.00	0.00	0.00	1.81
	30	8.23	13.3	40.2	49.	16.2	33.1	1.6	861.	--	--	--	--
	AVERAGE	8.887	14.48	43.36	48.6	16.29	33.39	2.54	1024.7	0.005	0.000	0.016	2.157
	STD. ERR.	0.172	0.28	0.77	0.3	0.09	0.20	0.22	30.2	0.005	0.000	0.007	0.155

DATA LISTING

0000215 HEMATOLOGY

PERIOD 1 VALUES

RESPONSE: MONO SEGS
 THOUSAND THOUSAND

GROUP	ANIMAL		
GROUP 1	1	-M-	-M-
F	2	0.13	0.61
	3	-M-	-M-
	4	-M-	-M-
	5	0.06	0.34
	6	0.14	0.36
	7	0.09	0.28
	8	0.03	0.76
	9	0.05	0.24
	10	0.24	0.22

AVERAGE	0.106	0.400
STD. ERR.	0.027	0.077

GROUP 2	11	0.19	0.38
F	12	0.11	0.11
	13	0.04	0.13
	14	0.06	0.36
	15	-M-	-M-
	16	-M-	-M-
	17	0.09	0.51
	18	0.04	0.44
	19	0.12	0.38
	20	-M-	-M-

AVERAGE	0.092	0.331
STD. ERR.	0.020	0.058

GROUP 3	21	0.06	0.19
F	22	0.04	0.20
	23	0.14	0.57
	24	-M-	-M-
	25	-M-	-M-
	26	0.13	0.46
	27	0.18	0.68
	28	0.12	0.59
	29	0.04	0.25
	30	-M-	-M-

AVERAGE	0.102	0.420
STD. ERR.	0.020	0.077

DATA LISTING

8880215 HEMATOLOGY

PERIOD 1 VALUES

RESPONSE:	RBC MILLION	HGB (GM/DL)	HCT (%)	MCV (FL.)	MCH (PG.)	MCHC (%)	WBC THOUSAND	PLATELET THOUSAND	BANDS THOUSAND	BASO THOUSAND	EOS THOUSAND	LYMPH THOUSAND
GROUP 4	31	9.22	15.5	45.9	50.	16.8	3.4	960.	0.00	0.00	0.00	2.92
F	32	10.35	14.7	49.0	47.	14.2	2.8	1078.	0.00	0.00	0.00	2.35
	34	9.60	16.4	47.7	50.	17.1	4.0	1042.	0.00	0.00	0.00	3.52
	35	-M-	-M-	-M-	-M-	-M-	-M-	-M-	-M-	-M-	-M-	-M-
	36	8.66	15.0	45.2	52.	17.3	2.9	1042.	0.00	0.00	0.00	2.52
	37	9.01	15.0	44.7	50.	16.6	3.5	1033.	0.00	0.00	0.00	2.90
	38	10.09	15.9	47.4	47.	15.8	1.9	1025.	0.00	0.00	0.00	1.62
	39	9.30	15.4	46.2	50.	16.6	4.5	987.	0.00	0.00	0.00	4.01
	40	9.11	14.7	44.3	49.	16.1	2.8	1025.	0.00	0.00	0.00	2.38
AVERAGE	9.418	15.33	46.30	49.4	16.31	33.13	3.23	1024.0	0.000	0.000	0.000	2.778
STD. ERR.	0.200	0.21	0.57	0.6	0.35	0.47	0.29	12.8	0.000	0.000	0.000	0.262

DATA LISTING

0000215 HEMATOLOGY

PERIOD 1 VALUES

RESPONSE:		MONO THOUSAND	SEGS THOUSAND
GROUP 4	31	0.10	0.37
	32	0.11	0.34
	34	0.08	0.40
	35	--	--
	36	0.12	0.26
	37	0.14	0.46
	38	0.08	0.21
	39	0.14	0.36
	40	0.08	0.34
AVERAGE		0.106	0.341
STD. ERR.		0.009	0.027

Report: CLPXPR55 CLINICAL PATHOLOGY - HEMATOLOGY ALPHA RESULTS Run date: 10/28/88
 Study (Ref) no.: B88-0215 Run time: 08:59
 Period: 1 File no.: 88000617

Grp Id.	Animal Id.	Sex	Platelets	Polychromasia	Anisocytosis	Poikilocytosis	Rbc crenated	Smudge cells
0001	10	F	PLTS NORM	NOTED	1+		OCC	
	2			NOTED	1+		FEW	
	5			NOTED	1-		RARE	
	6		PLTS NORM	NOTED	1+		MOD	
	7		PLTS NORM	NOTED	1+		MOD	
	8		PLTS NORM	NOTED	NOTE1		RARE	
	9			NOTED	1+		OCC	
0002	11	F		NOTED	NOTE1		FEW	
	12			NOTED	NOTE1		MANY	
	13			NOTED	1+		MOD	
	14		PLTS NORM	NOTED	NOTE1		MANY	
	17		PLTS NORM	1+	1+		FEW	
	18			NOTED	NOTE1		FEW	
	19			NOTED	1+		FEW	
0003	21	F	PLTS NORM	1+	1+		MOD	
	22			NOTED	1+		OCC	
	23		PLTS NORM	1+	NOTE1		MOD	
	26			NOTED	1+		FEW	
	27			NOTED	NOTE1		FEW	
	28			NOTED	NOTE1		FEW	
	29		PLTS NORM	NOTED	1+		MOD	
0004	31	F	PLTS NORM	NOTED	1+		OCC	
	32			NOTED	NOTE1		MANY	
	34			NOTED	1+		MANY	
	36			NOTED	NOTE1		MOD	
	37			NOTED	1+		OCC	
	38			NOTED	1+		MANY	
	39			NOTED	NOTE1		MANY	
	40			NOTED	1+		FEW	

Report: CLPXPR55 CLINICAL PATHOLOGY - HEMATOLOGY ALPHA RESULTS Run date: 10/28/88
 Study (Ref) no.: B88-0215 Run time: 09:00
 Period: 1 File no.: 88000617

Grp Id.	Animal Id.	Sex	Plt Decrease	Plt Increase	Plt mkd Decrease	Plt mkd Increase	Plt clumped	Plt giant
0001	2	F					MOD	
	5						MANY	
	7						OCC	
	9						MANY	
0002	11	F					MOD	
	12						MANY	
	13						MANY	
	18						MANY	
	19						MANY	
0003	22	F					MANY	
	26						MANY	
	27						MANY	
	28						MANY	
0004	32	F					MANY	
	34						MANY	
	36						MOD	
	37						MANY	
	38						MOD	
	39						MANY	
	40						MOD	

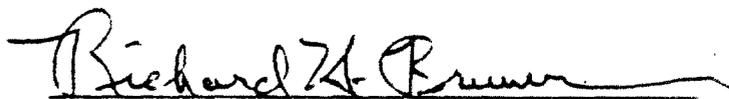
GROSS NECROPSY REPORT
FOR
PROCTER & GAMBLE
STUDY #B88-0215
THE ACUTE DERMAL TOXICITY OF G0539.03
TO SKH-HR1 HAIRLESS MICE

Gross necropsy examinations of 39 Skh-HR1 hairless mice used to evaluate the acute dermal toxicity of G0539.03 were conducted on September 2, 1988. All procedures were supervised by R. H. Bruner, D.V.M., Diplomate, A.C.V.P. An additional mouse assigned to this study (animal #33) became moribund during the exposure period and was sacrificed on September 1, 1988. The necropsy for this mouse was performed by C. L. Alden, D.V.M., Diplomate, A.C.V.P.

Macroscopic changes were not observed in any animal which were considered to be distinctly related to dermal applications of G0539.03. Liver tissues in animals #16, 19 & 40 exhibited very small (\pm 1mm), red, linear streaks over the ventral surface of the median lobe. These small streaks were thought to represent artifactual disruption of the liver capsule during postmortem manipulations. Animals #17, 31 & 39 displayed several indistinct, whitish, subcapsular streaks along the margins of the median liver lobe which measured approximately 1mm in length. Causative factors for these very faint streaks were not determined, but they were thought to represent a spontaneous change of little pathologic significance, and there was no distinct treatment relationship. Cut surfaces did not reveal that any capsular or subcapsular changes extended into the subjacent liver parenchyma. There was no evidence of gross lesions in the skin or subcutaneous tissue of any test animal. Inanition was thought to contribute to the moribund condition of animal #33.

Summary

Gross necropsy examination of 40 Skh-HR1 hairless mice used to evaluate the acute dermal toxicity of G0539.03 failed to identify gross lesions which were considered to be treatment related.



Richard H. Bruner, D.V.M.
Diplomate, A.C.V.P.

September 12, 1988

Date