

Report Title: Clonal Transformation Assay Using Syrian Golden Hamster Embryo (SHE) Cells

Test Type: Clonal Transformation

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

Test Substance(s): G0539.04 – Octopirox in DMSO

Species: Syrian Golden Hamster Embryo (SHE) Cells

Test Conditions: SHE cells were cultured in the presence of OP for 7 days then fixed/stained. 5 doses of OP ranging from 0.1 to 0.35 ug/ml.

Results: Octopirox induced statistically significant increase in the morphological transformation frequency at a concentration of 0.3 ug/ml. No significant response occurred below this concentration.

Study #: B89-0174

Report Date: 1/22/90

QA report/GLP compliance: Yes

Accession #: 35709



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THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 398707, CINCINNATI, OHIO 45239-8707

The following study was audited by the Quality Assurance Unit:

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

STUDY NUMBER: B89-0174

NOTEBOOK NUMBER: YE-1268

DIVISIONAL REQUEST DOCUMENT: BY0856

TSIN: G0539.04

TYPE OF STUDY: Clonal Transformation

PORTION(S) OF STUDY AUDITED:	AUDITOR:	DATE AUDITED:	DATE REPORTED TO STUDY DIRECTOR:
Prep of target and feeder cells	E. A. Bannan	3/14/89	3/14/89
Dosing solution prep	E. A. Bannan	3/15/89	3/15/89
Dosing of plates	E. A. Bannan	3/15/89	3/15/89

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
Quality Assurance Unit

2/9/90
Date

CELL TRANSFORMATION REPORT
Human & Environmental Safety Division

R.A. LeBoeuf
G.A. Kerckaert

Study #: B89-0174
Notebook #: YE-1268

Clonal Transformation Assay Using Syrian Golden
Hamster Embryo (SHE) Cells

Testing Facility: Biological Testing Facility
Miami Valley Laboratories
The Procter & Gamble Company
P. O. Box 39175
Cincinnati, Ohio 45247

Date Study Initiated: 2/28/89

Date Study Completed: 4/10/89

Test Substance Identification Number: G0539.04

Division Request Document Number: BY0856

Test Substance Description: Off- white Powder

Storage Conditions: Room Temperature

Positive Control Chemical: Benzo[a]pyrene Lot # KV01511KV

Sponsor's Divisional Toxicologist: G.S. Allgood
Procter & Gamble

Study Director: R.A. LeBoeuf
Procter & Gamble

Study Technician: G.A. Kerckaert
Procter & Gamble

Archived at: Biological Testing Facility
Miami Valley Laboratories
The Procter & Gamble Company
P. O. Box 39175
Cincinnati, Ohio 45247

I. SUMMARY

G0539.04 was tested in the in vitro Syrian Hamster Embryo (SHE) cell transformation assay to determine its potential to induce morphological transformation. G0539.04 was tested at concentrations ranging from 0.1 to 0.35 $\mu\text{g/ml}$. Treatment with 0.3 $\mu\text{g/ml}$ yielded a statistically significant increase in morphological transformation frequency compared to the solvent control. Concentrations tested below 0.3 $\mu\text{g/ml}$ did not cause a significant response. Concentrations of G0539.04 at or above 0.3 $\mu\text{g/ml}$ were extremely toxic to SHE cells as indicated by a reduction in plating efficiency compared to controls.

II. PURPOSE

The objective of this study was to determine the potential for G0539.04 to cause a significant increase in morphological transformation frequency compared to solvent controls in the in vitro Syrian hamster Embryo transformation assay. The transformation activity of the test substance was compared to that of a solvent control (DMSO) and to that of benzo[a]pyrene which is routinely used in this assay as a positive control.

III. MATERIALS AND METHODS

The procedures described in Protocol C45 (Clonal Transformation Assay Using SHE Cells) were used, without deviation from the protocol. A test substance solubility test showed DMSO was the solvent of choice. The test substance Cytotoxicity Assay was done to determine a concentration that yielded at least 50% reduction in SHE cell plating efficiency. 0.35 $\mu\text{g/ml}$ test substance yielded a plating efficiency of approximately 50% of that of the solvent control. Plating efficiency inhibition is considered to be an indicator of toxicity in this assay. Five test substance dose groups (0.1, 0.2, 0.25, 0.3, and 0.35 $\mu\text{g/ml}$), a solvent (DMSO) control group, two positive control groups (2.5 and 10 $\mu\text{g/ml}$ benzo[a]pyrene), and a nontreated control group were tested in the transformation assay. SHE cells were cultured in the presence of test solutions for seven days at which time the cultures were fixed and stained. Clone counts were done to determine plating efficiency, and transformed clones were scored to assess transformation frequency. The transformation assay was run in duplicate, and the resultant data were pooled.

IV. RESULTS

The raw data from the Cytotoxicity Assay and the Transformation Assay (both tests) are included in the Appendix. Table 1 and Figure 1 summarize the cytotoxicity data. Plating efficiency was reduced by 50% at a concentration of G0539.04 of between 0.3 and 0.35 $\mu\text{g/ml}$ G0539.04 compared to control. Table 2 and Figure 2 summarize the transformation assay data. G0539.04 at 0.3 $\mu\text{g/ml}$ caused a statistically significant increase in transformation frequency ($p < 0.047$) compared to control. Concentrations below 0.3 $\mu\text{g/ml}$ had no effect on morphological transformation frequency. Transformation frequency was also not increased at concentrations of G0539.04 greater than 0.3 $\mu\text{g/ml}$, most likely due to G0539.04 cytotoxicity.

V. CONCLUSION

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Since G0539.04 yielded a significant increase in morphological transformation frequency at only one dose level (0.3 ug/ml), further study of this compound is justified. One question that is raised is the role of G0539.04 cytotoxicity in G0539.04 transformation. Most of the known rodent or human carcinogens examined with this assay to date induce a significant increase in transformation frequency at multiple doses at least one dose of which is not cytotoxic. Examples of this are the benzo[a]pyrene dose groups (Table 2). Benzo[a]pyrene at 2.5 and 10 ug/ml resulted in significantly positive transformation frequencies, while plating efficiencies at these doses were only slightly less than that of the solvent control.

 1-19-90
R.A. LeBoeuf
Study Director

 1/22/90
G.A. Kerckaert
Study Technician

Table 1

G0539.04 EFFECTS ON PLATING EFFICIENCY-CYTOTOXICITY ASSAY

Treatment ($\mu\text{g/ml}$)	Plating Efficiency ^a (%)	Plating Efficiency ^b (% of Control)
Control	49.9 +/- 1.95	100
0.1	52.3 +/- 3.70	104.8
0.2	46.9 +/- 4.65	94
0.3	32.8 +/- 3.43	65.7
0.4	4.27 +/- 2.12	8.6
0.5	0	0

^a Colony number/plate/75 target cells plated X 100. Values shown represent (mean +/- S.E.M.) with five replicates.

^b Plating efficiency expressed here as % of DMSO control.

Table 2

G0539.04 TRANSFORMATION ASSAY-SUMMARY TABLE

Treatment ($\mu\text{g/ml}$)	Plating Efficiency (mean +/- SEM)	Total Colonies Scored	# AC ^a	# TC ^b	% AC ^c	% TC ^c
Control	46.93 +/- 0.88	1408	0	6	0	0.43
0.1	45.40 +/- 0.91	1362	3	1	0.22	0.07
0.2	39.04 +/- 0.87	1171	4	2	0.34	0.17
0.25	31.07 +/- 1.25	932	1	3	0.11	0.32
0.3	15.04 +/- 1.18	451	1	6	0.22	1.33*
0.35	3.47 +/- 0.58	104	0	1	0	0.96
NTC ^d	48.54 +/- 1.02	1456	1	3	0.07	0.21
2.5 BaP	42.31 +/- 0.97	1269	1	18	0.08	1.42*
10 BaP	41.42 +/- 1.20	1243	3	25	0.24	2.01*

^a AC = Altered Colonies.

^b TC = Transformed Colonies.

^c % AC and %TC = # AC/Total colonies scored X 100 and # TC/Total colonies scored X 100 respectively.

^d NTC = Nontreated Control.

* Transformation frequency significantly different (P<0.05) from solvent control.

Fig. 1. G0539.04 toxicity on SHE cells. 75 SHE target cells were plated/60 mm plate. ▲, plating efficiency expressed as % of targeted cells. Data points represent mean \pm SEM of the plating efficiency from 5 plates/G0539.04 concentration.

Figure 1 G0539.04 TOXICITY

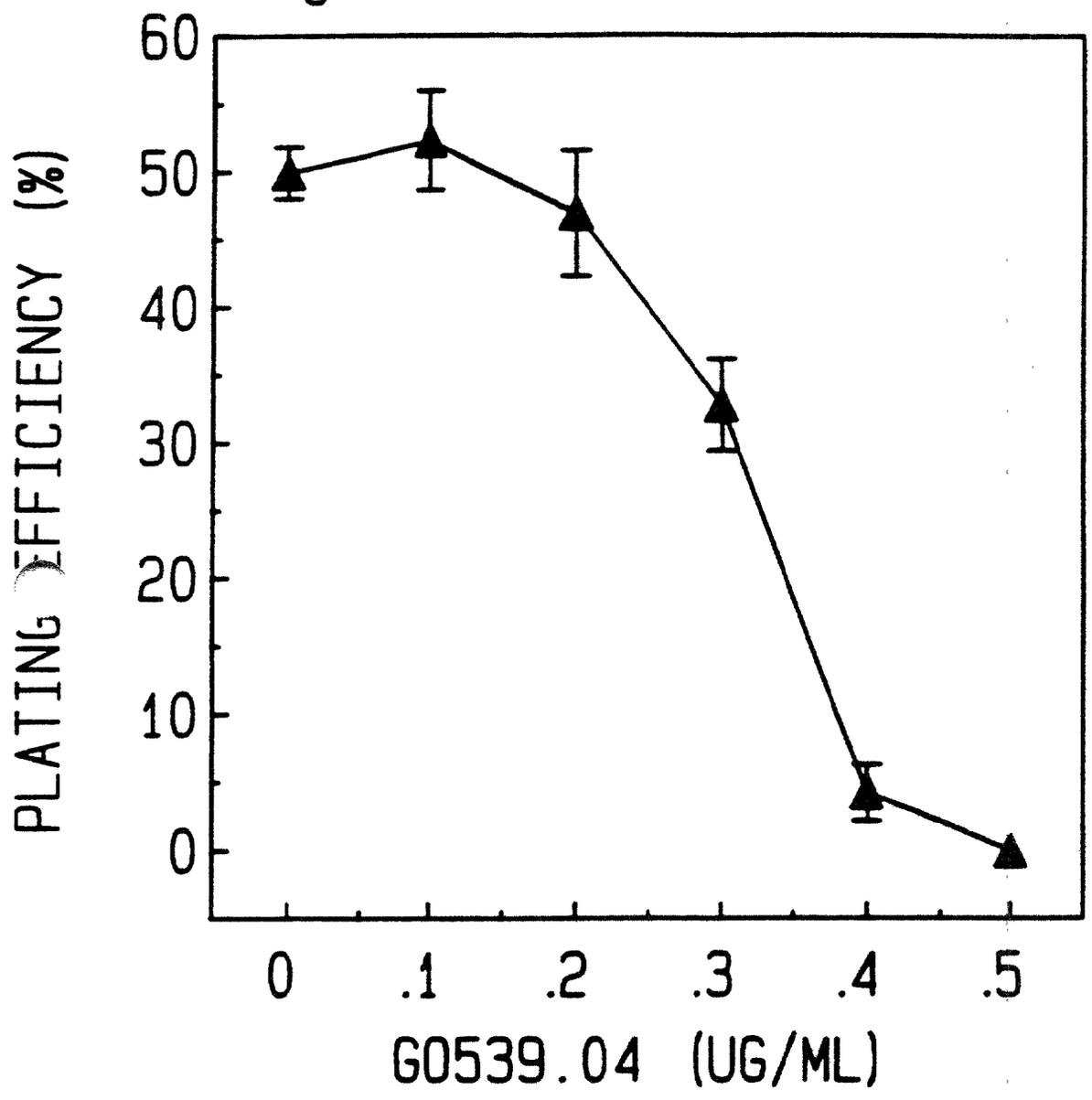
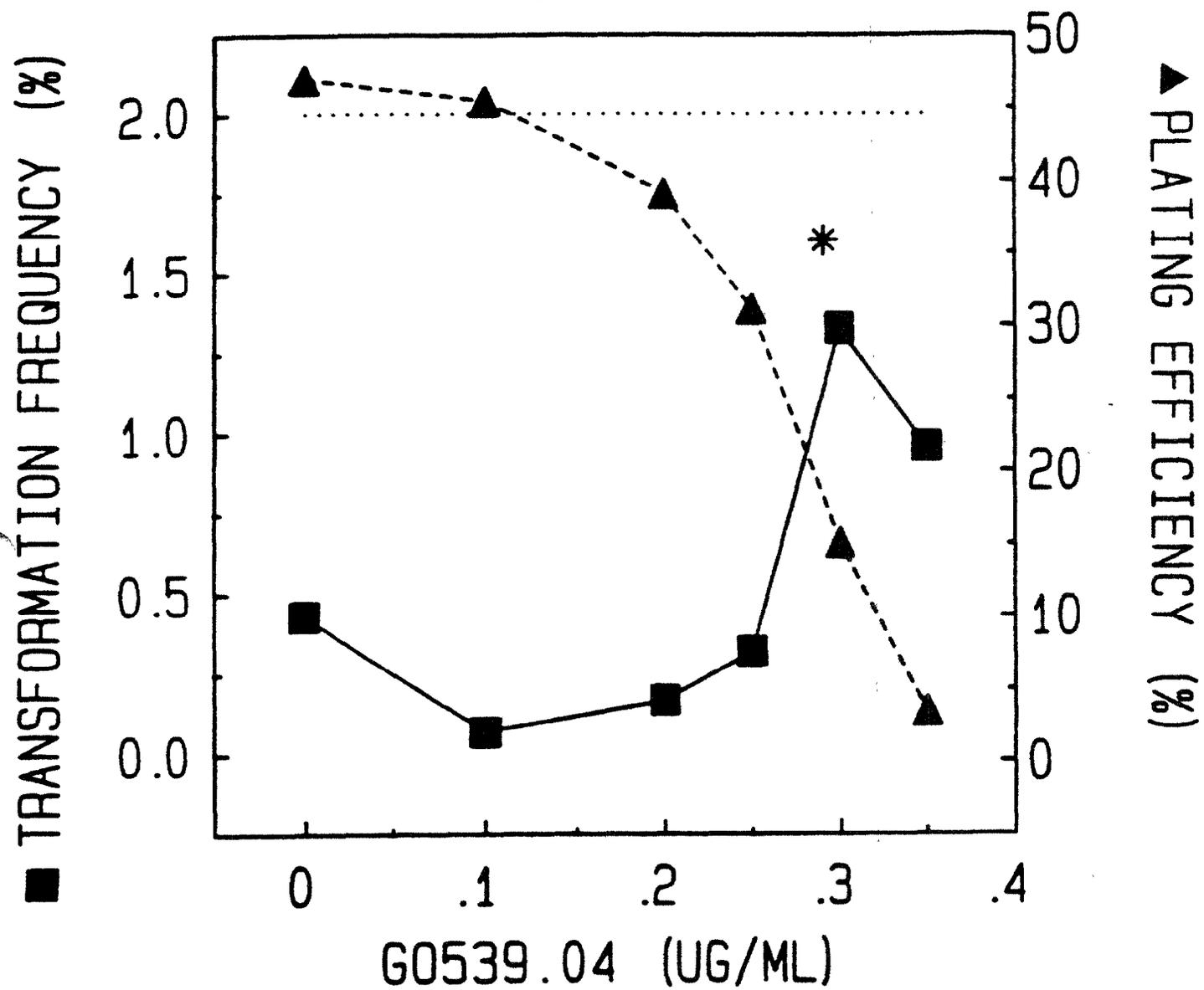


Fig 2. G0539.04 transformation of SHE cells. ▲ , plates efficiency expressed as % of targeted cells; ■ transformation frequency expressed # of transformed colonies/total colonies scored X 100. * denotes that the indicated data point is statistically different ($P < 0.047$) from unmarked points on the curve. Horizontal dotted line indicates the transformation frequency of the 10 $\mu\text{g/ml}$ benzo[a]pyrene control.

Figure 2 G0539.04
TRANSFORMATION



WORKSHEET FOR TRANSFORMATION ASSAY

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FIXING-STAINING OF PLATES AND COLONY COUNTS

DATE 3/30/89

PERFORMED BY H. Schubert

On seven days post dosing, plates were NaOH fixed and Giemsa stained, using the procedures described in Protocol #C45, section III B6, pp 12-13.

Colonies in each plate were counted and scored manually, using a

stereo-microscope. () - ALTERED COLONIES. * () - TRANSFORMED COLONIES.

	Feeder Only	Non-Treated Controls	2.5 ug/ml B(a)P	10 ug/ml B(a)P	Solvent Control	0.1 ug/ml	0.2 ug/ml G0539.04	0.25 ug/ml	0.3 ug/ml	C
		41 ()	35 () ()	39 () ()	40	36 ()	36	25 ()	12 () ()	
	NO GROWTH	32	36 ()	30 ()	36	35	27	26	11	
		37	39	40	37	31	28	25	17	
		44	27	36	30	35	34	17	17	
		41	32	30	31	30	35	32	13	4
		26	27 ()	25 () ()	35	39 ()	33	30 ()	20 () ()	
		41	35	40 ()	36	36	26	28	8	
		32	34	31	34	30	35	23	11	
		28	38	32	35	40	31	37	11	6
		29	31	34	34	30	26	34	13	
		36	35 ()	34 () ()	39 ()	39 ()	32 ()	25	20 ()	6
		41	35 ()	30 ()	42	41	28	32	9	0
		29	31	45 ()	36	36	28	30	10	8
		35	31	34	36	30	35	25	18	1
		29	33	36	38	42	30	27	15	2
		40	29 ()	32 ()	38 ()	38	33 ()	29	13	3
		32	34 () ()	36 ()	31	35	30	22	17	7
		38	34	38 ()	42	42	27	24	15	2
		42	42	34 ()	33	36	37	21	21	8
		36	30	37	32	36	25	26	23	0
Cloning Eff. \bar{x}	-	47.29	44.60	46.19	47.67	47.81	41.07	35.86	19.60	5.
SD. Dev.	-	7.33	5.25	6.06	4.60	5.36	5.06	6.31	5.71	4.
SD. ERROR	-	1.64	1.18	1.36	1.03	1.20	1.13	1.41	1.28	0.
.. FREQ.	-	0.14	0.15	0.29	0	0.28	0.16	0.19	0.34	0.
COL. FREQ.	-	0	1.20	1.88	0.28	0.14	0.16	0.19	1.36	1..
TOTAL COL.	-	709	669	693	715	717	616	538	294	8
# ALT. COL.	-	1	1	2	0	?	1	1	1	0
# TRANS. COL.	-	0	8	13	2	1	1	1	4	

WORKSHEET FOR TRANSFORMATION ASSAY

FIXING-STAINING OF PLATES AND COLONY COUNTS

000109

DATE 3/23/89 PERFORMED BY H. Kerkovitch

On seven days post dosing, plates were MeOH fixed and Giemsa stained, using the procedures described in Protocol #C45, section III B6, pp 12-13.

Colonies in each plate were counted and scored manually, using a

stereo-microscope. () = ALTERED COLONIES. (*) = TRANSFORMED COLONIES.

	Feeder Only	Non-Treated Controls	2.5 ug/ml B[a]P	10 ug/ml B[a]P	Solvent Control	0.1 ug/ml	0.2 ug/ml	0.25 ug/ml	0.3 ug/ml	0.5 ug/ml
		34(*)	29(*)	32(*)	42(*)	28(*)	34(*)	21(*)	0	5
	NO SPOWTH	40	38(*)	25(*)	34	26	27	21	6	0
		35	31(*)	25(*)	37	27	21	18	6	3
	SPOWTH	34	22	22	30	31	26	19	0	0
		30	36	29	32	36	23	22	7	0
		36(*)	32(*)	24(*)	35	28	24(*)	17(*)	9	1
		44	33(*)	26(*)	43	32	31	17	0	0
		40	24	24	32	36	27	31	6	2
		38	29	32	31	31	33	21	3	1
		43	33	36	39	33	26	12	12	0
		33(*)	26(*)	24(*)	34(*)	39	29(*)	21	11(*)	2
		34	23(*)	31(*)	31	31	26	24	11	0
		33	28(*)	31	42	38	32	20	14	0
		39	32	28	42	34	27	23	11	2
		35	35	23	31	33	24	15	16	4
		39	34(*)	28(*)	33	32	37(*)	26	9(*)	2
		42	24(*)	33(*)	36	27	27	12	11	0
		37	26	27	34	33	24	17	12	1
		44	33	19	29	39	26	13	6	1
		37	32	31	26	31	26	24	7	0
MEAN EFF. \bar{x}	—	49.80	40.01	36.47	46.20	43.00	37.01	26.27	10.47	1.6
Dev.	—	5.31	6.15	5.78	6.44	5.25	5.27	6.40	6.15	1.9
ERROR	—	1.19	1.38	1.29	1.44	1.17	1.18	1.43	1.38	0.1
COL. FREQ.	—	0	0	0.18	0	0.16	0.54	0	0	0
ALT. FREQ.	—	0.10	1.67	2.18	0.58	0	0.18	0.51	1.27	0
TOTAL COL.	—	747	600	550	693	645	555	394	157	2
# ALT. COL.	—	0	0	1	0	1	3	0	0	0
# TRANS. COL.	—	3	10	12	4	0	1	2	2	0

WORKSHEET FOR CYTOTOXICITY ASSAY

FIXING-STAINING OF PLATES AND COLONY COUNTS

000193

DATE 3/13/89

PERFORMED BY J. Schubert

On seven days post dosing, plates were MeOH fixed and Giemsa stained, using the procedures described in Protocol #C45, section III B6, pp 12-13. Colonies in each plate were counted and scored manually, using a stereo-microscope.

COLONY COUNTS	Feeder Only	Non treated Control	Solvent Control DMSO	0.1 ug/ml	0.2 ug/ml	0.3 ug/ml	0.4 ug/ml	0.5 ug/ml
				← G0539.04 →				
	UB	44	35	40	42	24	0	0
	UB	36	35	44	35	15	7	0
	UB	32	43	31	38	25	0	0
	UB	42	37	46	39	30	2	0
	UB	45	37	35	22	28	7	0
PLATING EFF. %	—	53.1	49.9	52.3	46.9	32.8	4.27	0
STD. DEV.	—	7.44	4.36	8.30	10.41	7.69	4.75	0
STD. ERROR	—	3.32	1.95	3.70	4.65	3.43	2.12	0

Doses of TEST SUBSTANCE to be used in TRANSFORMATION ASSAY 0, 0.2, 0.25, 0.3, 0.35 ug/ml.

STUDY DIRECTOR R. A. Leung DATE 3/13/89

Routing Approval 000* 94

BCD SAFETY TEST REQUEST DOCUMENT

A. Material/Product: Octopirox
Dept. Charge No.: 4185-0527-7506
Est. Total Cost: \$14.0M* (MVL)
Indexing Terms: Octopirox
Head & Shoulders transformation assay

Orig. SH *
Orig. AD v
Toxicologist
HSS SH
P&RS AD
Tox. Office
H&ESD Liais
Oper. Sect.

Initials Data
KBF 2/23/89
RSH 1/27/89
HSS 2/6/89
P&RS 2/6/89
Tox. Office 2/6/89

Return to BCD HSS

Sample Identification

B. TSIN Description
60639.04 Octopirox

C.

Safety Test

-vitro syrian hamster embryo (SHE) cell transformation assay

Table with 5 columns: Estimated Cost (\$14.0M*), Amount of Test Substance Needed (15g), Comments (Test follow-up validation of assay for chelators), Test Facility (MVL)

* MVL cross-charge

D.

Originating Brand Staff: [Signature] 1/27/89 4492 241
Signature Date Phone # Mail Box #

E.

Division Toxicologist: [Signature] 2/6/89 4098
Signature Date Phone #

Risk Level: N/A
(Human Studies Only)