V79 COLONY FORMING ASSAY

100% labeled cells

Experiment Name : ^{131}IUdR toxicity (crossed dose); **Exp. #** : 1.1; **Investigator:** A. Bishayee **Date:** 01/29/98

1. Set the ~~rocker roller~~ shaker at 37°C incubator with 5% CO_2, set the Coulter Counter, wash cells (from two 150 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 10 ml MEMB for each flusk, pool, vortex, pass five times through 3 cc syringe with 21 gauge needle, perform cell count by transfering 100 ul in Coulter cup containing 20 ml isotone (Coulter balanced electrolyte solution)
2. Dilute to ~40,00,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 4518666·6 cells/ml cells/ml)
3. Transfer 1 ml of cell suspension into ten 12 ml tubes (Falcon plastic test tube, 17x100 mm) labeled 1-10 both on cap and wall
4. Roll the tubes for 3-4 h at 37°C, 5% CO_2 **Date/Time:** 01/29/98; 2-30 p.m.
5. Prepare MEMB containing radioactivity in hood

   3.5 µl ^{131}IUdR (prepared on 01/29/98) + 3 ml MEMB
6. After 3-4 h, remove test tubes from roller and add MEMB with or without radioactivity according to Table below. **Date/Time:** 01/29/98; 6-15 p.m.

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{131}IUdR (ml) [2.0 uCi/ml]
1	0	1.0	1.0	0
2	0	1.0	1.0	0
3	0.010	1.0	0.990	0.010
4	0.025	1.0	0.975	0.025
5	0.050	1.0	0.950	0.050
6	0.100	1.0	0.900	0.100
7	0.250	1.0	0.750	0.250
8	0.500	1.0	0.500	0.500
9	1.000	1.0	0.000	1.000

7. Return test tubes to roller for 12 h . **Date/Time:** 01/29/98; 6-30 p.m.

8. While test tubes are rolling label 40 (4x10) gamma-tubes (13 X 100 mm VWR glass test tube)
9. After ~12 h incubation period, remove tubes and centrifuge at 2000 rpm at 4°C for 10 min (***precooled centrifuge***). **Date/Time:** 01/30/98; 9-00 a.m.
10. **During centrifugation, obtain ice**
12. Remove buckets from centrifuge and carefully remove 100 µl of supernatant and place in prelabeled gamma-tube.
13. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
14. Centrifuge tubes for 10 min at 2000 rpm, 4°C
15. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
16. Centrifuge tubes for 10 min at 2000 rpm, 4°C
17. Decant supernatant, click tubes, vortex, resuspend in 10 ml MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, resuspend in 200 ul ice cold MEMA ,transfer the cell suspension in polypropylene microcentrifuge tubes with attached caps (Helena Plastics, 400 ul) using pipet tips
20. Again add 200 ul ice cold MEMA, resuspend and transfer the cell suspensions in the same polypropylene microcentrifuge tubes (Total volume ~400 ul)
21. Centrifuge tubes for 5 min at 1000 rpm, 4°C
22. Transfer tubes at 10°C for 72 h. **Date/Time:** 01/30/98; 11-30 a.m.
23. Transfer 10 ul supernatant in three sets of tubes containing small pieces of tissue paper from 100 ul supernatant removed earlier and count them for radioactivity
    Date/Time: 01/30/98; 11-45 a.m.
24. After 72 h, carefully remove the supernatant from the top, resuspend pellet in 200 ul wash MEMA and transfer the content to ten 12 ml tubes (Falcon plastic test tube, 17x100 mm, labeled 1-10 both on cap and wall) containing 10 ml wash MEMA by using pasteur pipet
    Date/Time: 02/02/98; 9-45 a.m.
25. Again add 200 ul wash MEMA in microcentrifuge tubes, resuspend and transfer the cell suspensions in 12 ml tubes
26. Centrifuge the tubes for 10 min at 2000 rpm, 4°C ***(precooled centrifuge)***
27. Labeling and preparation of dilution tubes and colony dishes
    - load 69 60 mm petri dishes with 4 ml MEMA
    - load 36 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 1.5; 2.2, 2.3, 2.4, 2.5; X.2, X.3, X.4, X.5 etc.
28. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
29. Centrifuge tubes for 10 min at 2000 rpm, 4°C
30. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
31. Centrifuge tubes for 10 min at 2000 rpm, 4°C

32. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
33. Determine cell concentration by transfering 100 µl to Coulter cup
34. Vortex tube, transfer 0.5 ml into dilution tube X.5, vortex tube X.5 and transfer 0.5 ml to tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3 and vortex tube X.3 and transfer 0.5 ml to tube X.2. Keep tubes on ice.
35. Transfer 1 ml from dilution tubes into dishes labeled X.2, X.3, X.4 (in triplicate). Only X.2 should be seeded for control T-tubes.
36. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
37. Incubate petridishes for 1 week
38. Count gamma tubes for radioactivity **Date/Time :** 02/02/98; 2-45 p.m.
39. After 1 week, wash colonies 3 times with normal (1X) saline,and 2 times with methanol. Stain colonies with 0.0 5% crystal violet
40. Count colonies. There must be between 25 and 250 colonies for the flask to be a valid data point.

Expt # 1-1

Take two 150 cm^2 flasks containing 80-90% confluent cells 01/29/98
Suspend in 10 ml HEMB for each flask.

Initial Cell count = 1111, 1127, 1151 (Counting time = 1-14 Sec)
Avg. cell count = 1129.6 (Manometer select = 50 µl)
Cell Conc. = 1129.6 × 4000 = 4518666.6 Cells/ml

For dilution,

Vol. of original cell suspension taken = $\frac{44000000}{}$

=

Take ml of cell suspension + ml MEMB = 11 ml.

After dilution,

Final count = 943
Avg. Count = 968
Cell conc. = 975
956

Expt # 1.1 01/29/98

Preparation of ^{131}IUdR in MEMβ (2 μCi/ml)

Stock ^{131}IUdR 1.71 μCi/ μl

Prepare 3 ml of 2 μCi/ml ⇒ 6 μCi required

From Stock 1.71 μCi ⇒ 1 μl

6 μCi ⇒ $\frac{1}{1.71} \times 6$

⇒ 3.50 μl.

i) Pipet 3.50 μl ^{131}IUdR from Stock in hood
ii) Keep it at RT for ~2-3 hr
iii) Add 3 ml of MEMβ

01/27/98

5·19567

wt of Chloramid T tube = 5·18300 g

wt of " + Chl. T = ~~5·23170~~

Chl T = 0·0126 = 12·67 mg

wt of Pot. meta bisulfite tube = 5·19136 g

" " " " + Pot. = 5·24000

Pot. meta = 0·04864

wt of 2L-Deoxyuridine tube = 5·25464

wt of 2L+Deo = 5·26438

2L-Deoxy = 0·00974

Ph. buffer = $\frac{12·67}{2}$ = 6·33 ml

dH_2O = $\frac{48·64}{10}$ = 4·86 ml

Ph. buffer = $\frac{9·77}{2}$ = 4·88 ml

0·02

2 — 1 ml

1 — $\frac{1}{2}$

0·02

TABLE- 1

Expt. # : 1·1 **Date/Time :** 01/30/98; 11-45 a.m.

Tube #	Medium count for 10 ul (cpm)	Avg. cpm	dpm [cpm/0.142]	µCi/ml (A_t) on counting [dpm/22200]	µCi/ml (A_o) on addition [$A_t/e^{-\lambda t}$]	
1	3, 2, 4	0	0	0	0	
2	4, 5, 3	0	0	0	0	
3	17, 23, 20	20	140·84	0·0063	~~0·005~~	0·0067
4	48, 36, 47	43·66	307·51	0·00138	~~0·012~~	0·0146
5	73, 89, 68	76·66	539·30	0·0243	~~0·022~~	0·0258
6	163, 172, 139	158	1112·67	0·0501	~~0·047~~	0·0532
7	378, 364, 406	389·33	2741·78	0·1235	~~0·1161~~	0·1313
8	763, 847, 818	809·33	5699·53	0·2567	~~0·2414~~	0·2730
9	1587, 1607, 1625	1606·33	11312·20	0·5095	~~0·4793~~	0·5420
~~10~~						

0·01
0·02
0·05
0·1
0·25
0·5
1

$e^{-\lambda t}$

$= e^{-\frac{\ln 2}{t_{1/2}} \times 17.25\ h}$

$= e^{-\frac{0.693}{193.2} \times 17.25}$

$= e^{-0.0618}$

$= ~~1·063~~\ 0·940$

$A_o = \frac{A_t}{~~1·063~~\ 0·940}$

TABLE-2

Expt. # : 1·1 **Date/Time :** 02/02/98; 2-45 p.m.

Tube #	Radioactivity for 300 ul cell suspension (cpm)	Avg. cpm	dpm [cpm/0.142]	µCi/ml (A_t) on counting [dpm/666000]	µCi/ml (A_o) after 12 h incubation [$A_t/e^{-\lambda t}$]
1	0, 2, 1	0	0	0	0
2	2, 2, 0	0	0	0	0
3	105, 106, 105	105·33	741·78	0·00113	0·00149
4	250, 261, 278	263	1852·11	0·00278	0·00367
5	433, 475, 433	447	3147·88	0·00472	0·00624
6	1064, 1108, 1091	1087·66	7659·6	0·01150	0·01521
7	2562, 2552, 2472	2528·66	17807·51	0·02673	0·03535
8	4694, 4883, 4924	4833·66	34039·85	0·05111	0·06760
9	9588, 9790, 9829	9735·66	68561·03	0·10294	0·13616
10					

$A_o = A_t / e^{-\lambda t}$

$= A_t / e^{-\frac{\ln 2}{193.2} \times 77.75}$

$= A_t / e^{-\frac{0.693 \times 77.75}{193.2}}$

$= A_t / e^{-0.278}$

$= A_t / 0.756$

72h + 5.75 = 77.75h

02/02/98 : 2-45 p.m.

01/30/98 : 9-00 a.m

TABLE-3

Expt. # : 1.1 **Date/Time :** 02/02/98; 11-00 a.m.

Tube #	Coulter count for 100 ul cell suspension (Manometer Select : 50 µl)	Avg. count	Cells/ml [Avg. count x 400D]	pCi/cell [uCi/cell x 10^6 / Cells/ml]	
1	290, 310, 324	308	1232000	0	
2	514, 536, 510	520	2080000	0	
3	539, 535, 487	520·33	2081333·3	0·00087	0·00071
4	464, 464, 469	472·33	1889333·3	0·00194	
5	438, 436, 422	432	1728000	0·00361	
6	499, 486, 503	496	1984000	0·00766	
7	517, 537, 493	515·66	20626666	0·01713	
8	439, 464, 467	456·66	1826666·6	0·03700	0·5 -
9	442, 429, 440	437	1748000	0·07789	1·0
~~10~~					

uci/ml

0·01

0·025

0·05

0·1

0·25

0·5

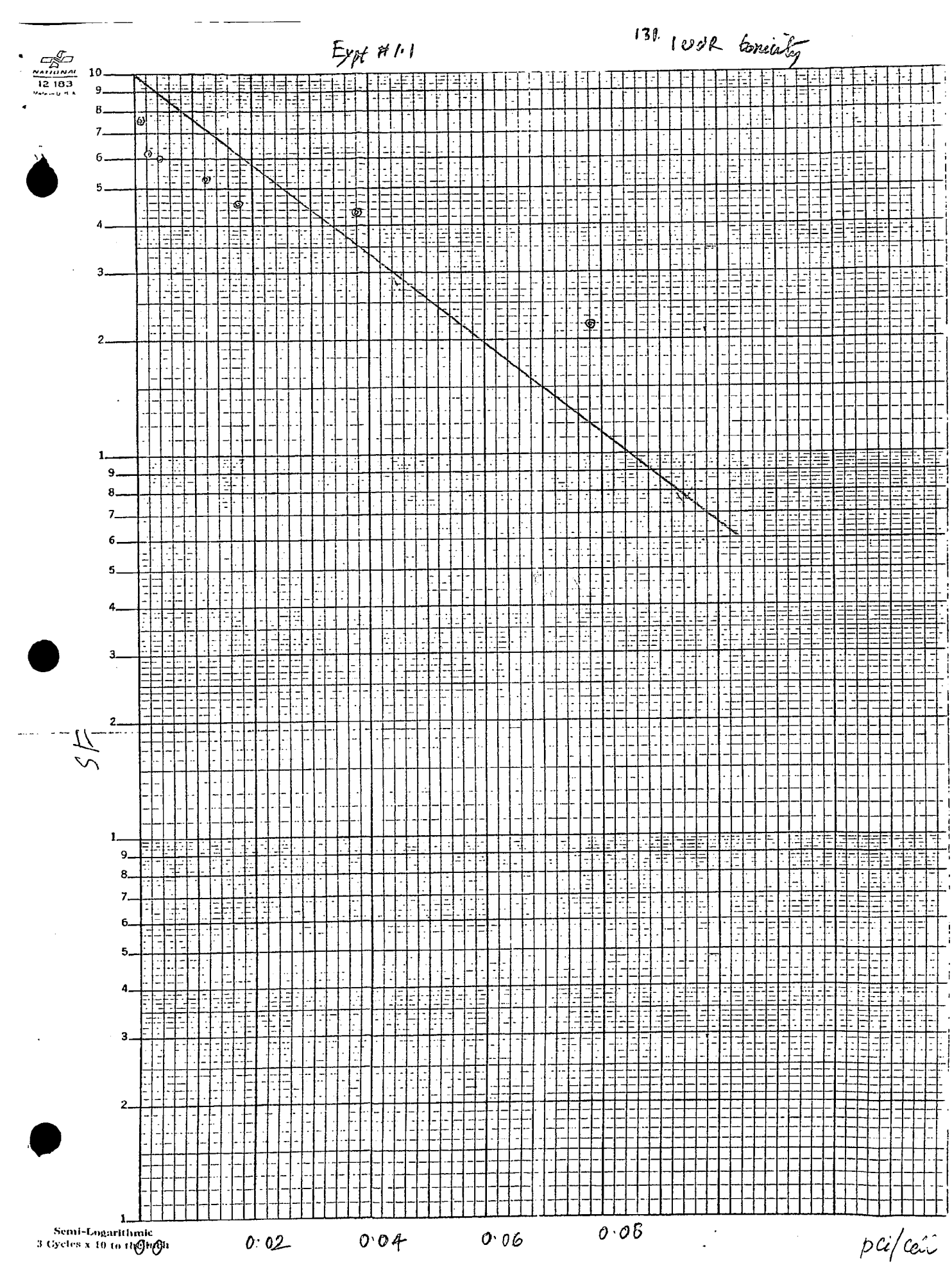
1·0

Expt # : 1·1 **Date :** 02/09/98

Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	44	37	44	41·66*	—
2·2	118	107	113	112·66	—
3·2	73	91	93	85·66	0·7603
4·2	71	78	66	71·66	0·6360
5·2	69	71	68	69·33	0·6153
6·2	60	61	62	61	0·5414
7·2	55	45	60	53·33	0·4733
8·2	44	54	53	50·33	0·4467
9·2	28	25	31	28	0·2485

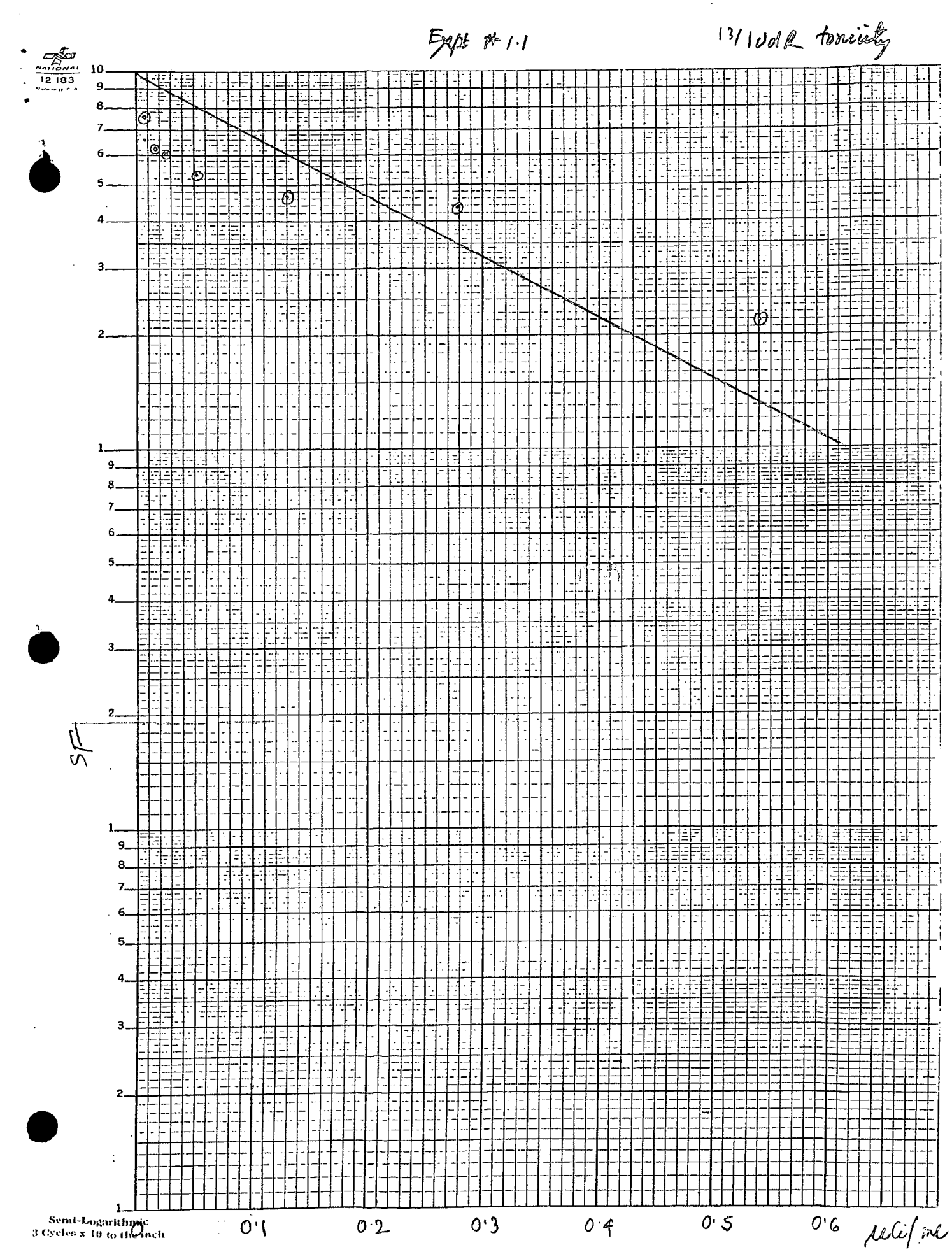
* not considered.



Semi-Logarithmic
3 Cycles x 10 to the Inch

Expt # 1.1

131 IUdR toxicity



B002116