3/30/98

ln S vs pci/cell

DMF Cysteamine 1.3 mM

Exp. Curve fit to ln S Data

Exp 5 $DMF = \frac{0.228}{0.130} = 1.75$

Exp 6 $DMF = \frac{0.164}{0.0814} = 2.01$

Exp 7 $DMF = \frac{0.132}{0.0776} = 1.70$

$\overline{DMF} = 1.82 \pm 0.17$

ln S vs mbq/cell

DMF

1.76

$C_0 = \frac{1}{0.187} = 8.431$ ~~5.349~~

$C_0 = \frac{1}{0.2086} = 4.79$

DMF

2.01

$C_0 = \frac{1}{0.165} = 6.06$

$C_0 = \frac{1}{0.332} = 3.01$

$C_0 = \frac{1}{0.204} = 4.902$

1.70

$C_0 = \frac{1}{0.348} = 2.88$

1.82 ± 0.17

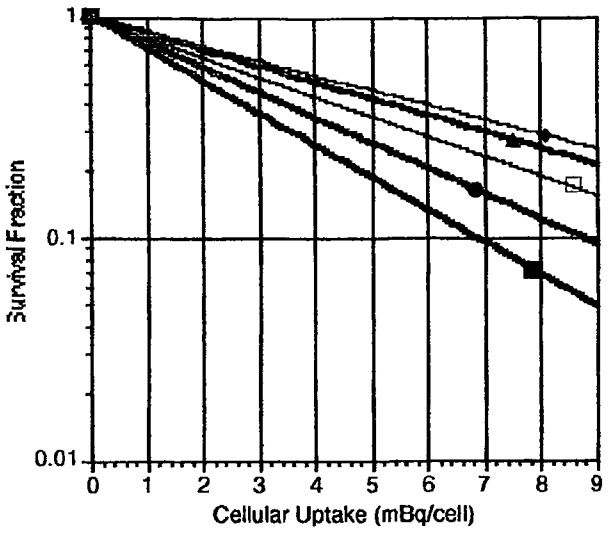
Expt	MEA	mbq/cell	DMF
1	~~(DMF)~~ 0	2986	~~1.82~~
9	0.65	3.80	1.27
	1.30	5.844	1.96
	1.95	6.587	2.19
	2.60	4.845	1.62

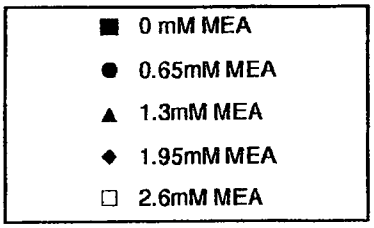
Single ~~hit~~ multitarget fit

(do is taken from Log SF vs. uptake data)

d	1.3 mM MEA		0 mM MEA		DMF
	Co	n	Co	n	
1	2.46	6.83	2.12	2.39	

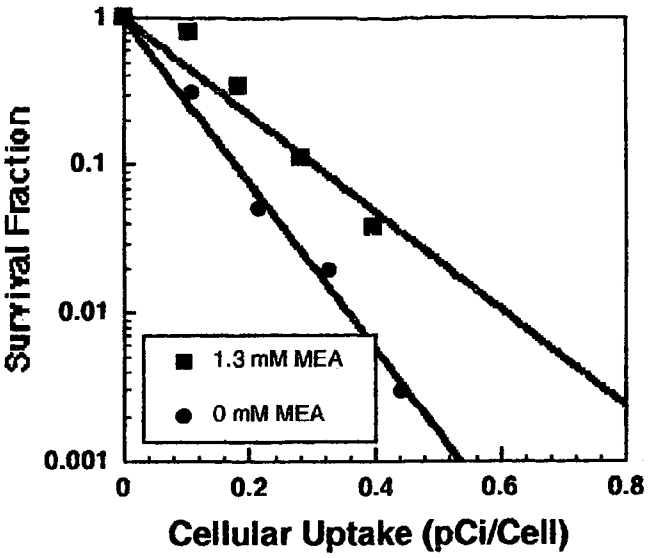
Expt #1


1
0.1
0.01
Survival Fraction
0
1
2
3
4
5
6
7
8
9
Cellular Uptake (mBq/cell)

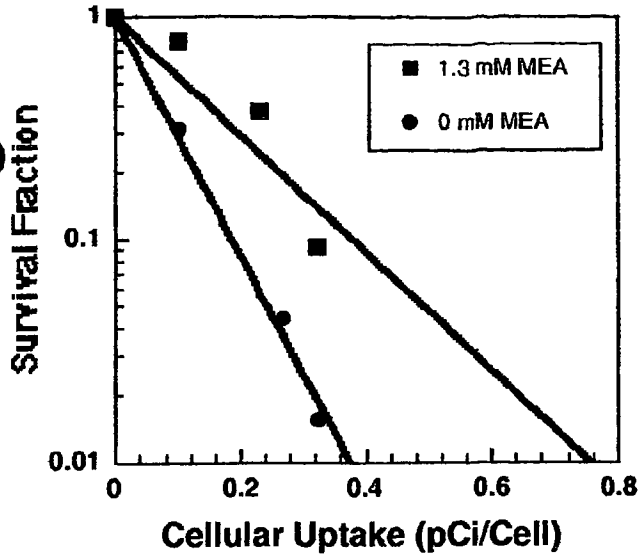

0 mM MEA
0.65mM MEA
1.3mM MEA
1.95mM MEA
2.6mM MEA

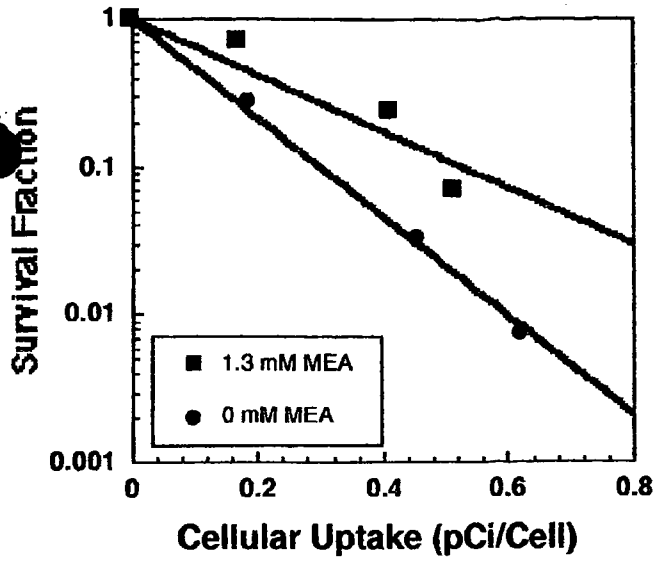
MEA Survival

Expt # 7



MEA Survival
Expt #6





MEA Survival

Expt #5

DATA EXP # 7

	Label	A	B	C	D	E
Label		1.3 mM MEA	SF	0 mM MEA	SF	
1		0	1	0	1	
2		0.1039	0.8089	0.1102	0.3151	
3		0.1842	0.3373	0.2164	0.05	
4		0.2831	0.1109	0.325	0.0192	
5		0.3981	0.0385	0.4428	0.0029	

DATA for Expt # 6

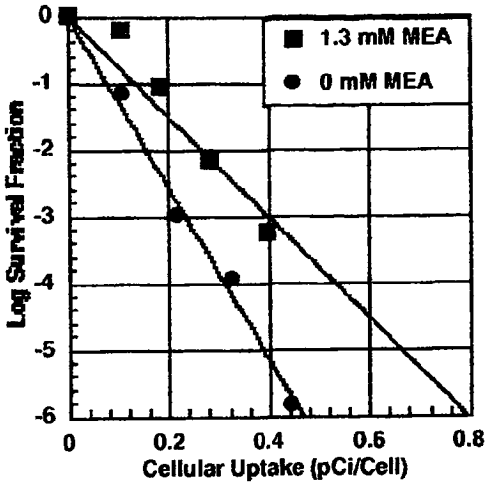
	Label	A	B	C	D	E
Label		1.3 mM MEA	SF	0 mM MEA	SF	
1		0	1	0	1	
2		0.103	0.7757	0.1034	0.3084	
3		0.2332	0.375	0.2679	0.0445	
4		0.3219	0.0908	0.3254	0.0154	

DATA for Expt # 5

↘	Label	A	B	C	D	E
Label		1.3 mM MEA	SF	0 mM MEA	SF	
1		0	1	0	1	
2		0.167	0.7036	0.1844	0.2778	
3		0.4082	0.2419	0.4526	0.0339	
4		0.5113	0.0694	0.6181	0.0075	

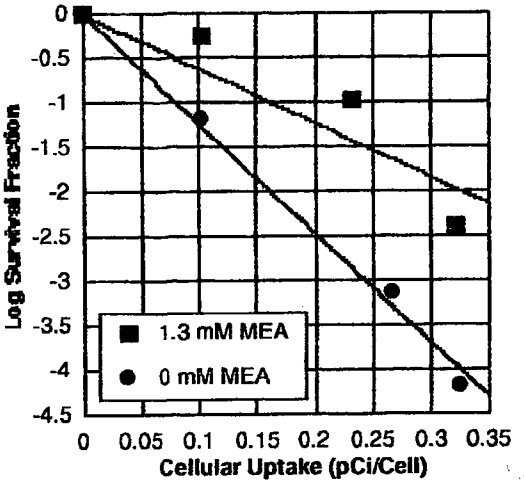
MEA Survival – Log

Expt #7



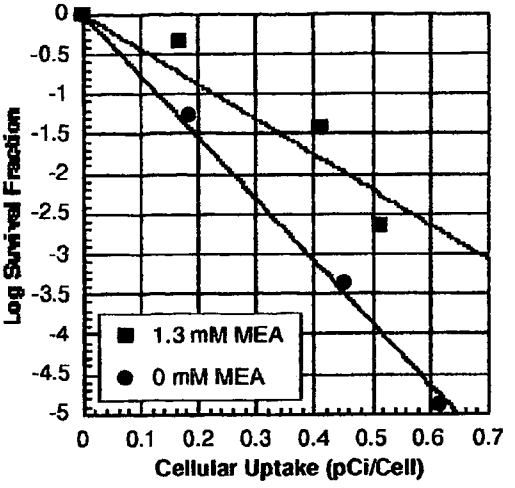
MEA survival - log

Expt #6



MEA Survival - Log

Expt 5



DATA EXPT 7 ('log)

	F	G	H	I
Label	1.3 mM MEA	SF	0 mM MEA	SF
1	0	0	0	0
2	0.1039	-0.212079978	0.1102	-1.154865230
3	0.1842	-1.086782536	0.2164	-2.995732273
4	0.2831	-2.199126384	0.325	-3.952844999
5	0.3981	-3.257097037	0.4428	-5.843044541

DATA for Expt # 6 (Cag)

	F	G	H	I
Label	1.3 mM MEA	SF	0 mM MEA	SF
1	0	0	0	0
2	0.103	-0.253989431	0.1034	-1.176357637
3	0.2332	-0.980829253	0.2679	-3.112266089
4	0.3219	-2.399095993	0.3254	-4.173387769

DATA for Expt #5 (Log)

	F	G	H	I
Label	1.3 mM MEA	SF	0 mM MEA	SF
1	0	0	0	0
2	0.167	-0.351545266	0.1844	-1.280853848
3	0.4082	-1.419230861	0.4526	-3.384340264
4	0.5113	-2.667868411	0.6181	-4.892852258

V79 COLONY FORMING ASSAY

Experiment Name : ^{131}IUdR + 50-200 ug/ml MEA; **Exp. # :** 1;
Investigator: A. Bishayee **Date:** 08/24/98

1. Set the rocker-roller at 37°C incubator, set the Coulter Counter, wash cells (from 75 cm^2 flusk, subcultured 1:2, 24h before) with PBS, trypsinize cells, resuspend in 7 ml MEMB, 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 ~4,00,000 cells/ml in MEMB (final volume 11 ml) [Actual count : 414,400 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:** 08/24/98; 5-00 p.m
5. Prepare MEMB containing radioactivity in hood

   26.5 µl ^{131}IUdR (prepared on 8/13/98) + 4 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:** 08/24/98; 7-50 p.m

Tube #	^{131}IUdR uCi/ml	Cells in MEMB (ml)	MEMB (ml)	MEMB+ ^{131}IUdR (ml) [1.2 uCi/ml]	MEMA+ MEA 200 ug/ml (ml)	MEMA (ml)	MEA Conc. (ug/ml)
1	0	1.0	1.0	0	0	2.0	0
2	0	1.0	1.0	0	0.5	1.5	50
3	0	1.0	1.0	0	1.0	1.0	100
4	0	1.0	1.0	0	1.5	0.5	150
5	0	1.0	1.0	0	2.0	0	200
6	0.4	1.0	0.33	0.67	0	2.0	0
7	0.4	1.0	0.33	0.67	0.5	1.5	50
8	0.4	1.0	0.33	0.67	1.0	1.0	100
9	0.4	1.0	0.33	0.67	1.5	0.5	150
10	0.4	1.0	0.33	0.67	2.0	0	200

7. Return test tubes to roller for 12 h, increase the elevation angle of the roller.

   Date/Time: 08/24/98; 8-00 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:** 08/25/98; 9-15 a.m.
10. **During centrifugation, move roller to 10°C and obtain ice**
11. Prepare 11 ml of sterile MEA (200 ug/ml) in MEMA , **put on 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 wash MEMA
18. Centrifuge tubes for 10 min at 2000 rpm, 4°C
19. Decant supernatant, click tubes, vortex, resuspend in 2 ml ice cold MEMA containing 0 or 100 ug/ml of MEA as per Table. Keep on ice!
20. Transfer tubes to roller at 10°C for 72 h. **Date/Time:** 08/25/98; 11-00 a.m.
21. 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: 08/25/98; 11-00 a.m.

21. After 72 h, remove tubes and **place on ice**, add 8 ml ***ice cold*** wash MEMA.

    Date/Time: 08/28/98; 3-00 p.m
22. Centrifuge tubes for 10 min at 2000 rpm, 4°C (***precooled centrifuge***)
23. Labeling and preparation of dilution tubes and colony dishes
    - load 57 60 mm petri dishes with 4 ml MEMA
    - load 30 T-tubes with 4.5 ml MEMA and label them 1.2, 1.3, 1.4, 2.2, 2.3, 2.4, X.2, X.3, X.4, etc.
24. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
25. Centrifuge tubes for 10 min at 2000 rpm, 4°C
26. Decant supernatant, click tubes, vortex, resuspend in 10 ml wash MEMA
27. Centrifuge tubes for 10 min at 2000 rpm, 4°C
28. Decant supernatant, click tubes, vortex, resuspend in 2 ml wash MEMA, pass five times through 3 cc syringe with 21 gauge needle
29. Determine cell concentration by transfering 100 µl to Coulter cup
30. Vortex tube, transfer 0.5 ml into dilution tube X.4, vortex tube X.4 and transfer 0.5 ml to tube X.3, vortex tube X.3 and transfer 0.5 ml to tube X.2 and vortex. Keep tubes on ice.
31. 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.

2200 µg
= 2.2 mg.

Stock : 200 mg/ml
Stock required = 11 ml.

900 ~ 1 ½

32. Transfer 300 µl of cell suspension (in triplicate) to gamma tubes for each tube
33. Incubate petridishes for 1 week
34. Count gamma tubes for radioactivity **Date/Time :** 08/28/98 ; 7-00 p.m
35. After 1 week, wash colonies 3 times with normal (1X) saline, and 2 times with methanol. Stain colonies with crystal violet
36. Count colonies (50 or more cells). There must be between 25 and 250 colonies for the dish to be a valid data point.

Expt. #1 08/24/98 ;

Initial Cell count = 6721, 6831, 6847
Avg. cell count = 6799.6
Cell conc. = 6799.6 × 400
= 2719866 cells/ml

For dilution,
Vol. of Cell suspension taken = $\frac{4400000}{2719866}$ ml
= 1.61 ml

Take 1.6 ml cells + 9.4 ml MEMS = 11 ml.

After dilution,
Final cell count = 1077, 1012, 1019
Avg. cell count = 1036
Cell conc. = 1036 × 400
= 414,400 cells/ml

Expt. # 1

08/24/98

Preparation of 4 ml of 1.2 μCi/ml ^{131}I UdR
= 4.8 μCi required

Stock on 08/13/98 at 2-00 pm = 0.398 μCi/ml
on 08/24/98 at 8-00 pm = 0.151 μCi/ml.

---

11 days + 6 hr
= 270 hr

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

$= 0.398 \times e^{-\frac{0.693 \times 270}{193.2}}$

$= 0.398 \times 0.379$

$= 0.151$ μCi/ml

Stock required $= \frac{4}{0.151} = 26.47$ ml.

① Take 26.5 ml Stock
② Keep at RT for 3 & hr
③ Add 4 ml of MEMB

TABLE-1

Expt. # : 1 **Date/Time :** 08/25/98 ; 11-00 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, 1				
2	1, 1, 0				
3	2, 2, 1				
4	1, 1, 2				
5	3, 2, 2				
6	811, 781, 785	793·6	5589·2	0·2517	0·2656
7	778, 786, 826	796·6	5610·3	0·2527	0·2666
8	786, 854, 769	803	5654·9	0·2547	0·2688
9	721, 798, 759	759·3	5347·4	0·2408	0·2541
10	826, 894, 854	858	6042·2	0·2721	0·2872

08/24/98; 8-00 p.m

12h + 3h = 15h

$e^{-\lambda t}$

$e^{-\frac{0.693 \times 15}{193.2}}$

$= 0.9476$

TABLE-2

Expt. # : 1 **Date/Time :** 08/28/98; 7:00 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					
2					
3					
4					
5	3017, 3098, 3122				
6	3119, 3221, 3293	3079	21683·0	0·0325	0·0436
7	3209, 3119, 3214	3211	22612·6	0·0339	0·0454
8	3314, 3213, 3209	3180	22399	0·0336	0·0451
9	3197, 3213, 3301	3245	22854	0·0343	0·0460
10		3237	22795	0·0342	0·0459

08/25/98; 9-15 a.m.

72h + 10h = 82h

$e^{-\lambda t}$

$= e^{-\frac{0.693 \times 82}{193.2}}$

$= 0.7451$

TABLE-3

Expt. # : 1 **Date/Time :**

Tube #	Coulter count for 100 ul cell suspension	Avg. count	Cells/ml [Avg. count x 400]	pCi/cell [uCi/cell x 10^6 Cells/ml]
1	456, 432, 441	443	177200	
2	520, 556, 531	535	214266	
3	475, 485, 461	473	189466	
4	572, 536, 559	555	222266	
5	595, 606, 585	595	238133	
6	499, 512, 526	512	204933	0·2127
7	602, 617, 622	613	245466	0·1849
8	566, 555, 545	555	222133	0·2030
9	523, 535, 517	525	210000	0·2190
10	495, 505, 485	495	198000	0·2318

TABLE-4

Expt. #: 1

Date: 09/04/98

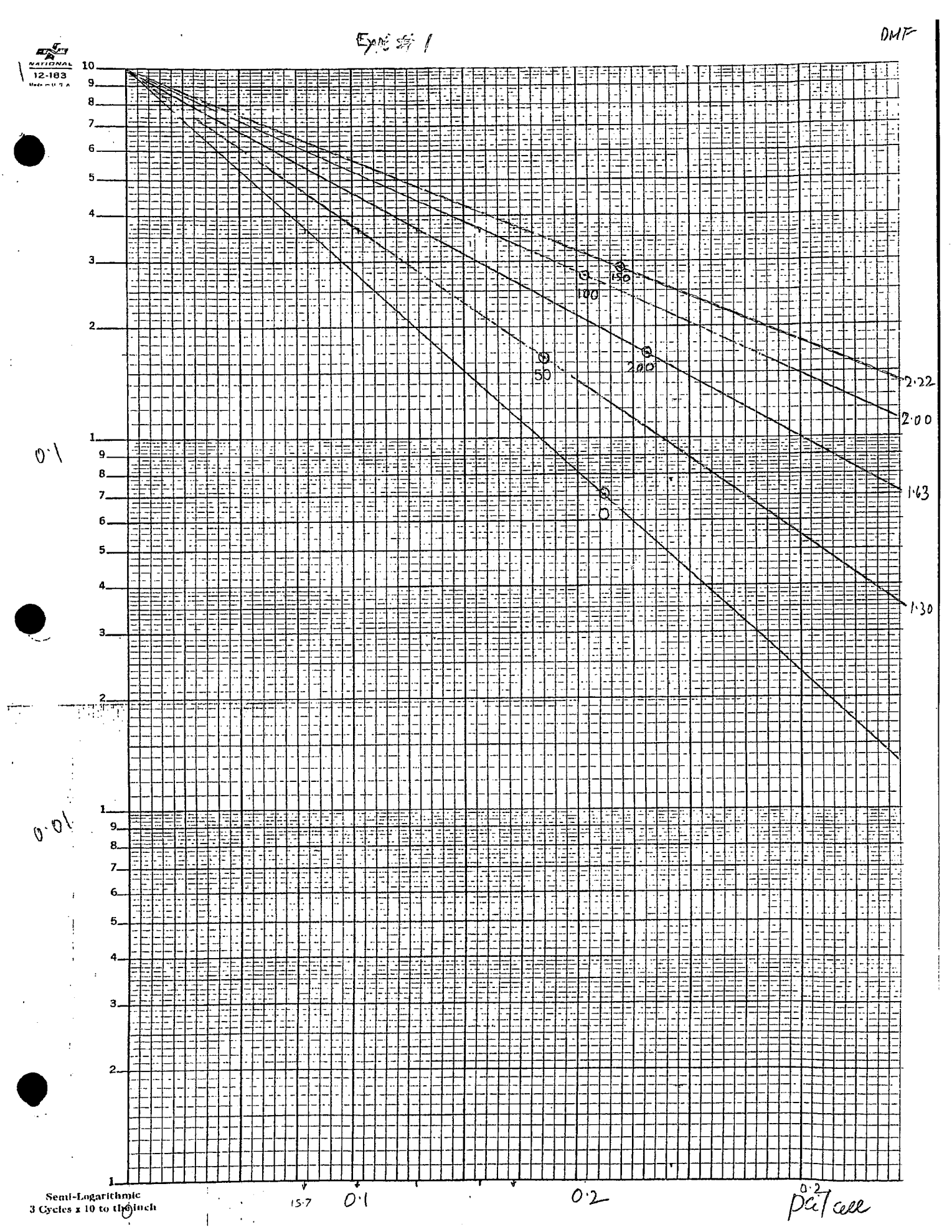
Colony Counts and Survival Fraction

Tube.dilution	Colony 1	Colony 2	Colony 3	Avg Colony	SF
1·2	123	113	136	124	
2·2	116	128	108	117·33	0·9462
3·2	109	103	106	106	0·8548
4·2	92	76	86	84·66	0·6827
5·2	75	85	66	75·33	0·6075
6·3	99	80	88	8·9	0·0717
7·3	194	201	187	19·4	0·1653
8·2	29	35	25	29·33	0·2766
9·2	26	25	23	24·66	0·2912
10·3	120	129	136	12·83	0·1703

MEA Conc. (µg/ml)	DMF
0	1
50	1·3
100	2
150	2·2
200	1·63

Expt 1

DMF



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