

OBSERVATION TABLES AND FIGURES

In Vitro Assays

REVERSE MUTATION ASSAYS (AMES RESULTS)

TABLE: 1 (CYTOTOXICITY)

Table: 1.1 Lawn and Mean Revertants

Strain TA 98					
Without S9 mix				With S9 mix	
Sr. No.	Dose levels (µl/plate)	Mean No. of revertants per plate	Lawn	Mean No. of revertants per plate	Lawn
1	NC	18.5	Normal	20.0	Normal
2	VC	18.5	Normal	22.0	Normal
3	0.00005	16.5	Normal	31.0	Normal
4	0.0005	15	Normal	24.5	Normal
5	0.005	8	Normal	24.5	Normal
6	0.05	10	Normal	25.0	Normal
7	0.5	4.5, toxicity	MLI	16.0	Normal
8	5.0	-, Toxic	LA	20.0	Normal

Table: 1.2 Lawn and Mean Revertants

Strain TA 100					
Without S9 mix				With S9 mix	
Sr. No.	Dose levels µl/plate	Mean No. of revertants per plate	Lawn	Mean No. of revertants per plate	Lawn
1	NC	142	Normal	154.0	Normal
2	VC	147	Normal	146.5	Normal
3	0.00005	82	Normal	144.0	Normal
4	0.0005	88	Normal	135.0	Normal
5	0.005	82	Normal	197.5	Normal
6	0.05	58.5	Normal	153.5	Normal
7	0.5	44	MLI	0.000	LA
8	5.0	-	LA	-	LA

Note: NC = Negative Control, VC = Vehicle control, MLI = Moderate lawn inhibition, LA = Lawn absent, n = 2.

PLATE INCORPORATION METHOD

TABLE: 2 (REVERTANT FREQUENCY – MAIN STUDY-I)

Table: 2.1 - Strain TA 1537(-S9)								
Group	G1	G2	G3	G4	G5	G6	G7	PC (SA)
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	0.5
Replicate1	4	7	13	11	14	16	9	688
Replicate2	4	11	15	9	27	33	14	692
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLI	Normal
Fold Value	NA	NA	1.56	1.11	2.28	2.72	1.28	76.67
Mean	4	9	14	10	20.5	24.5	11.5	690
S.D.	0	2.8284	1.414	1.414	9.192	12.02	3.536	2.8284
S.E.	0	2.0003	1	1	6.501	8.501	2.5	2.0003
Regression Equation: $y = 2.1429x + 4.7857$, $r = 0.13$, $n = 2$								

REVERTANT FREQUENCY – MAIN STUDY-I

Table: 2.2 - Strain TA 98 (-S9)								
Group	G1	G2	G3	G4	G5	G6	G7	2-NF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	50
Replicate1	18	14	14	17	9	24	25	1336
Replicate2	14	26	12	17	15	20	28	1364
Fold Value	NA	NA	0.65	0.85	0.60	1.10	1.33	67.50
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mean	16	20	13	17	12	22	26.5	1350
S.D.	2.828	8.4853	1.414	0	4.243	2.828	2.121	19.799
S.E.	2	6.0009	1	0	3	2	1.5	14.002
Regression Equation: $y = 1.2321x + 13.143$, $r = 0.79$, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation, S.E. = Standard Error, MLI = Mild Lawn Inhibition, SA = Sodium Azide and NF = Nitrofluorene.

REVERTANT FREQUENCY – MAIN STUDY-I

Table: 2.3 - Strain TA 1535 (-S9)								
Group	G1	G2	G3	G4	G5	G6	G7	9-Aa
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	50
Replicate1	15	10	7	11	12	21	22	140
Replicate2	10	19	6	12	10	18	23	152
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLI	Normal
Fold Value	NA	NA	0.45	0.79	0.76	1.34	1.55	10.07
Mean	12.5	14.5	6.5	11.5	11	19.5	22.5	146
S.D.	3.536	6.364	0.707	0.707	1.414	2.121	0.707	8.4853
S.E.	2.5	4.5007	0.5	0.5	1	1.5	0.5	6.0009
Regression Equation: $y = 1.5893x + 7.6429$, $r = 0.82$, $n = 2$								

REVERTANT FREQUENCY – MAIN STUDY-I

Table: 2.4 - Strain TA 100 (-S9)								
Group	G1	G2	G3	G4	G5	G6	G7	SA
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	5.0
Replicate1	154	144	98	124	86	73	37 T*	1064
Replicate2	136	158	120	130	79	88	45 T*	860
Lawn	Normal	Normal	Normal	Normal	Normal	MLi	I	Normal
Fold Value	NA	NA	0.72	0.84	0.55	0.53	0.27	6.37
Mean	145	151	109	127	82.5	80.5	41	962
S.D.	12.73	9.8995	15.56	4.243	4.95	10.61	5.657	144.25
S.E.	9.001	7.0011	11	3	3.501	7.501	4.001	102.02
Regression Equation: $y = -17.125x + 173.64$, $r = 0.84$, $T^* =$ moderately toxic, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation,
 S.E. = Standard Error, 9 - Aa = 9- Aminoacridine and
 SA = Sodium Azide, MLI = Moderate Lawn Inhibition, I = Lawn Inhibition.

Chapter 3

REVERTANT FREQUENCY – MAIN STUDY-I

Table: 2.5 Strain TA 102 (-S9)								
Group	G1	G2	G3	G4	G5	G6	G7	MMC
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	0.5
Replicate1	314	328	335	359	434	422	1097	1692
Replicate2	349	296	290	445	383	434	1042	2184
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	1.00	1.29	1.31	1.37	3.43	6.21
Mean	331.5	312	312.5	402	408.5	428	1070	1938
S.D.	24.75	22.627	31.82	60.81	36.06	8.485	38.89	347.9
S.E.	17.5	16.002	22.5	43.01	25.5	6.001	27.5	246.04
Regression Equation: $y = 90.839x + 103$, $r = 0.98$, $n = 2$								

Table: 2.6 - Strain TA 1537 (+S9)								
Group	G1	G2	G3	G4	G5	G6	G7	2An
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	50
Replicate1	3	13	14	8	13	8	16	44
Replicate2	3	7	8	15	7	11	19	49
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLi	Normal
Fold Value	NA	NA	1.10	1.15	1.00	0.95	1.75	4.65
Mean	3	10	11	11.5	10	9.5	17.5	46.5
S.D.	0	4.2426	4.243	4.95	4.243	2.121	2.121	3.5355
S.E.	0	3.0005	3	3.501	3	1.5	1.5	2.5004
Regression Equation: $y = 1.4821x + 4.4286$, $r = 0.74$, $n = 2$								

Table: 2.7 - Strain TA 1535(+S9)								
Group	G1	G2	G3	G4	G5	G6	G7	SA
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	0.5
Replicate1	27	27	31	40	40	28	37	2040
Replicate2	27	27	52	36	42	42	17	1886
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLi	Normal
Fold Value	NA	NA	1.54	1.41	1.52	1.30	1.00	72.70
Mean	27	27	41.5	38	41	35	27	1963
S.D.	0	0	14.85	2.828	1.414	9.899	14.14	108.89
S.E.	0	0	10.5	2	1	7.001	10	77.012
Regression Equation: $y = 0.5536x + 31.571$, $r = 0.38$, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation, S.E. = Standard Error, MLi = Mild Lawn Inhibition, SA = Sodium Azide, 2-An = 2-Aminoanthracene and MMC = Mitomycin-C.

Chapter 3

REVERTANT FREQUENCY – MAIN STUDY-I

Table: 2.8 - Strain TA 100 (+S9)								
Group	G1	G2	G3	G4	G5	G6	G7	2-AF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	20
Replicate1	131	187	70	179	35	182	167	2100
Replicate2	172	102	94	179	44	206	195	2026
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	0.57	1.24	0.27	1.34	1.25	14.28
Mean	151.5	144.5	82	179	39.5	194	181	2063
S.D.	28.99	60.104	16.97	0	6.364	16.97	19.8	52.326
S.E.	20.5	42.506	12	0	4.501	12	14	37.006
Regression Equation: $y = 5.1786x + 118.07$, $r = 0.40$, $n = 2$								

Table: 2.9 - Strain TA 98(+S9)								
Group	G1	G2	G3	G4	G5	G6	G7	2-AF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	20
Replicate1	14	57	36	54	42	43	46	844
Replicate2	50	44	45	46	23	62	23	1880
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	0.80	0.99	0.64	1.04	0.68	26.97
Mean	32	50.5	40.5	50	32.5	52.5	34.5	1362
S.D.	25.46	9.1924	6.364	5.657	13.44	13.44	16.26	732.56
S.E.	18	6.501	4.501	4.001	9.501	9.501	11.5	518.08
Regression Equation: $y = 0.125x + 41.286$, $r = 0.23$, $n = 2$								

Table: 2.10 - Strain TA 102(+S9)								
Group	G1	G2	G3	G4	G5	G6	G7	2-AF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	20
Replicate1	374	440	406	135 T*	38 T**	—	—	904
Replicate2	309	414	442	120 T*	20 T**	—	—	1112
Lawn	Normal	Normal	Normal	T*	I	I	I	Normal
Fold Value	NA	NA	0.99	0.30	0.07	—	—	2.36
Mean	341.5	427	424	127.5	29	—	—	1008
S.D.	45.96	18.385	25.46	10.61	12.73	—	—	147.08
S.E.	32.5	13.002	18	7.501	9.001	—	—	104.02
Regression Equation: $y = -91.371x + 544.63$, $r = 0.75$,								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation, S.E. = Standard Error SA = Sodium Azide and 2-AF = 2-Aminofluorene. T*= moderately toxic, T**= Toxic, I = Inhibition, '—' = Highly toxic (No growth) , $n = 2$



AMES ASSAY

Revertant Frequency: Plate Incorporation Method
(In the absence of metabolic activation)

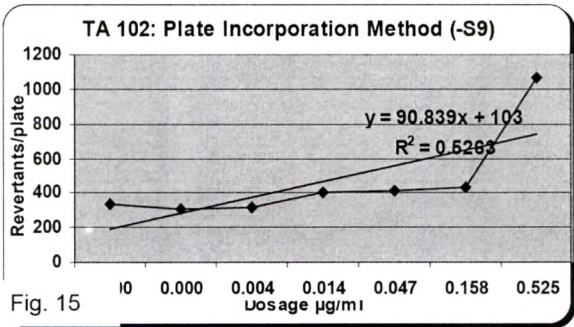
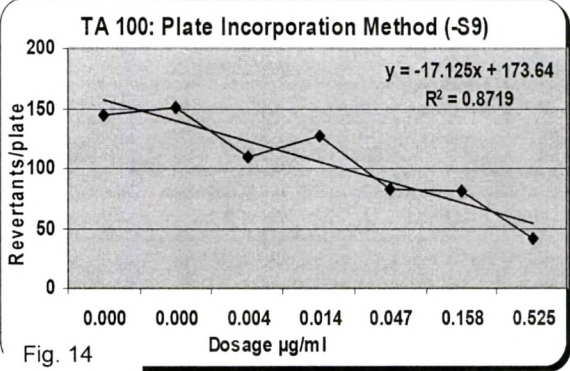
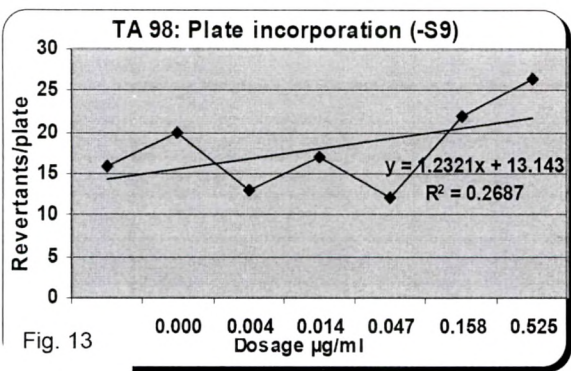
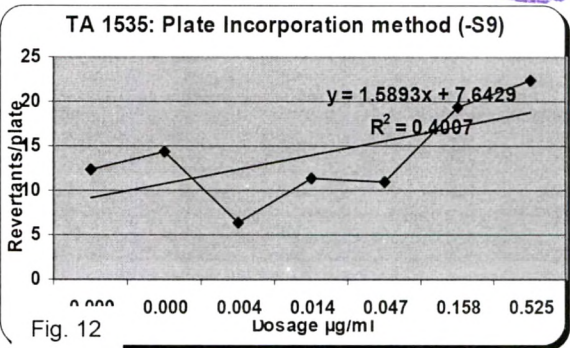
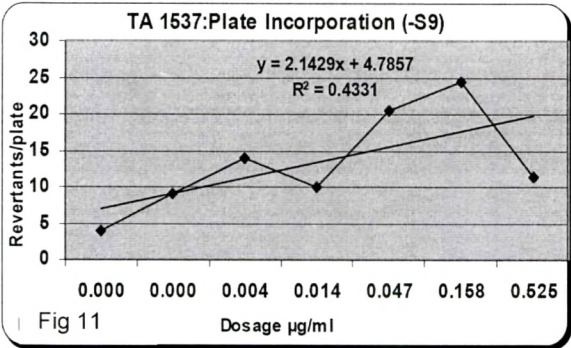


TABLE: 3 - GROWTH
Revertant Frequency: Plate Incorporation Method
(In the presence of metabolic activation)

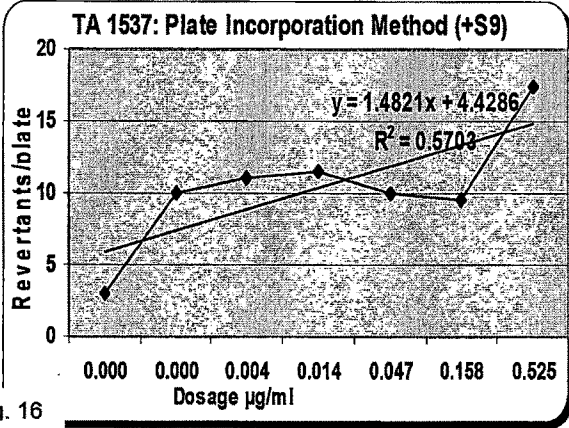


Fig. 16

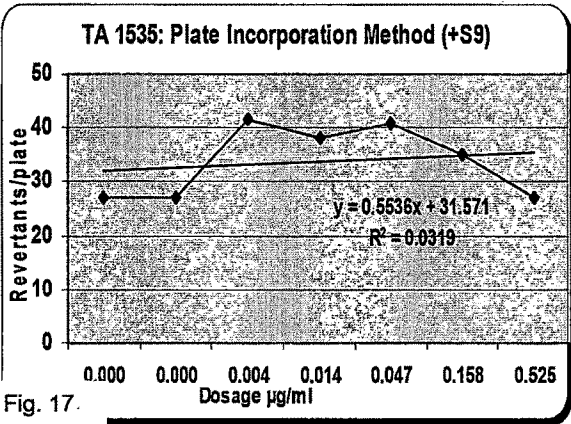


Fig. 17

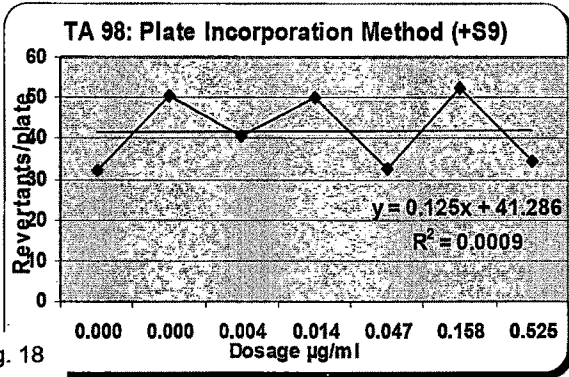


Fig. 18

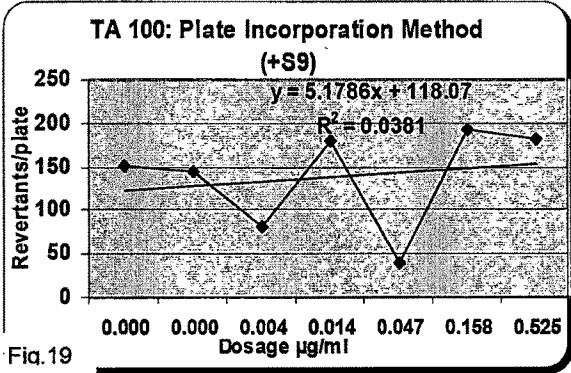


Fig. 19

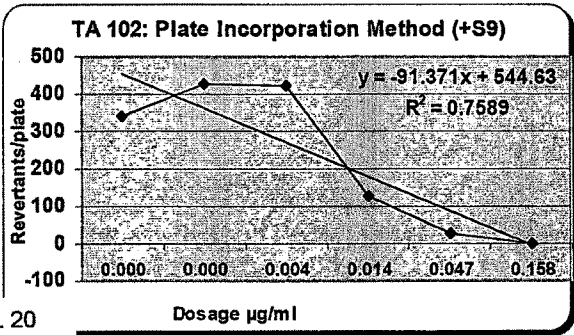


Fig. 20

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.2 - Strain: TA 1535 (-S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	PC (9-Aa)
Dose ($\mu\text{g}/\text{plate}$)	0	0	0.004	0.014	0.047	0.158	0.525	50
Replicate1	22	24	60	55	57	49	32	187
Replicate2	25	31	104	52	82	45	23	189
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLI	Normal
Fold Value	NA	NA	2.98	1.95	2.53	1.71	1.00	6.84
Mean	23.50	27.50	82.00	53.50	69.50	47.00	27.50	188.00
S.D.	13.65	16.26	52.20	30.92	42.00	27.12	16.21	1.41
S.E.	9.65	11.50	36.92	21.86	29.70	19.18	11.46	1.00
Regression Equation: $y = 10.799\ln(x) + 34.063$, $r = 0.33$, MLI = Mild Lawn Inhibition, $n = 2$								

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.3 - Strain: TA 98 (-S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	2-NF
Dose ($\mu\text{g}/\text{plate}$)	0	0	0.004	0.014	0.047	0.158	0.525	5.0
Replicate1	32	39	26	36	23	24	36	1784
Replicate2	44	25	22	101	37	30	28	1128
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	0.75	2.14	0.94	0.84	1.00	45.50
Mean	38.00	32.00	24.00	68.50	30.00	27.00	32.00	1456.00
S.D.	22.74	19.76	14.00	51.18	18.66	15.79	18.61	463.86
S.E.	16.09	13.97	9.90	36.20	13.19	11.16	13.16	328.05
Regression Equation: $y = -1.3713\ln(x) + 37.599$, $r = 0.06$, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation,
S.E. = Standard Error, 9- Aa = 9- Aminoacridine, 2 -NF = 2-Nitrofluorene.

Chapter 3

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.4 - Strain: TA 100 (-S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	SA
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	5.0
Replicate1	119	119	108	99	126	95	89	1064
Replicate2	127	121	117	160	127	106	120	860
Lawn	Normal	Normal	Normal	Normal	Normal	MLI	MLI	Normal
Fold Value	NA	NA	0.94	1.08	1.05	0.84	0.87	8.02
Mean	123.00	120.00	112.50	129.50	126.50	100.50	104.50	962.00
S.D.	71.13	69.29	65.11	80.74	73.01	58.19	62.00	144.25
S.E.	50.30	49.00	46.04	57.10	51.63	41.16	43.85	102.02
Regression Equation: $y = -7.4948\ln(x) + 125.77$, $r = 0.46$, MLI = Mild Lawn Inhibition, $n = 2$								

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.5 - Strain: TA 102 (-S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	MMC
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	0.5
Replicate1	381	466	513	438	446	319	333	904
Replicate2	502	418	380	454	395	456	336	1112
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	1.01	1.01	0.95	0.88	0.76	2.28
Mean	441.50	442.00	446.50	446.00	420.50	387.50	334.50	1008.00
S.D.	261.98	256.31	266.22	257.61	244.08	233.89	192.83	147.08
S.E.	185.28	181.27	188.28	182.19	172.62	165.41	136.37	104.02
Regression Equation: $y = -41.656\ln(x) + 467.66$, $r = 0.68$, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation, S.E. = Standard Error, SA = Sodium Azide, MMC = Mitomycin-C.

Chapter 3

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.6 - Strain: TA 1537 (+S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	2-An
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	5.0
Replicate1	6	10	21	19	14	7	5	189
Replicate2	9	12	18	20	12	12	3	254
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLi	Normal
Fold Value	NA	NA	1.77	1.77	1.18	0.86	0.36	20.14
Mean	7.50	11.00	19.50	19.50	13.00	9.50	4.00	221.50
S.D.	4.58	6.43	11.36	11.26	7.54	5.95	2.24	45.96
S.E.	3.24	4.55	8.03	7.96	5.34	4.20	1.59	32.50
Regression Equation: $y = -0.1754\ln(x) + 12.214$, $r = 0.02$, MLI = Mild Lawn Inhibition, $n = 2$								

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.7 - Strain: TA 1535(+S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	2-An
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	5.0
Replicate1	22	23	20	17	27	26	0	2680
Replicate2	27	18	15	23	30	25	0	1760
Lawn	Normal	Normal	Normal	Normal	Normal	MLi	I	Normal
Fold Value	NA	NA	0.85	0.98	1.39	1.24	0.00	108.29
Mean	24.50	20.50	17.50	20.00	28.50	25.50	0.00	2220.00
S.D.	14.36	12.10	10.41	11.92	16.50	14.64	0.30	650.54
S.E.	10.16	8.56	7.36	8.43	11.67	10.35	0.21	460.07
Regression Equation: $y = -4.8051\ln(x) + 25.352$, $r = 0.35$, MLI= Mild Lawn Inhibition, I = Inhibition, $n = 2$								

Note: G1- G7 are treatment groups, S. D. = Standard Deviation,
S.E. = Standard Error, 2-An = 2-Aminoanthracene.

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.8 - Strain: TA 98(+S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	2-AF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	20
Replicate1	25	24	34	39	42	40	16	3880
Replicate2	32	52	38	41	29	44	13	4376
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	0.95	1.05	0.93	1.11	0.38	108.63
Mean	28.50	38.00	36.00	40.00	35.50	42.00	14.50	4128.00
S.D.	16.82	26.03	20.88	23.11	21.48	24.24	8.21	350.72
S.E.	11.90	18.41	14.77	16.34	15.19	17.14	5.80	248.04
Regression Equation: $y = -1.296\ln(x) + 35.078$, $r = 0.09$, $n = 2$								

REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.9 - Strain: TA 100(+S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	2-AF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	20
Replicate1	107	109	133	144	181	175	167	608
Replicate2	118	122	174	140	164	179	195	702
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	MLI	Normal
Fold Value	NA	NA	1.33	1.23	1.49	1.53	1.57	5.67
Mean	112.50	115.50	153.50	142.00	172.50	177.00	181.00	655.00
S.D.	65.18	67.00	90.96	82.00	99.93	102.12	105.13	66.47
S.E.	46.10	47.38	64.33	57.99	70.67	72.22	74.35	47.01
Regression Equation: $y = 38.695\ln(x) + 103.45$, $r = 0.93$, MLI = Mild Lawn Inhibition, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation,
S.E. = Standard Error, 2-AF = 2-Aminofluorene

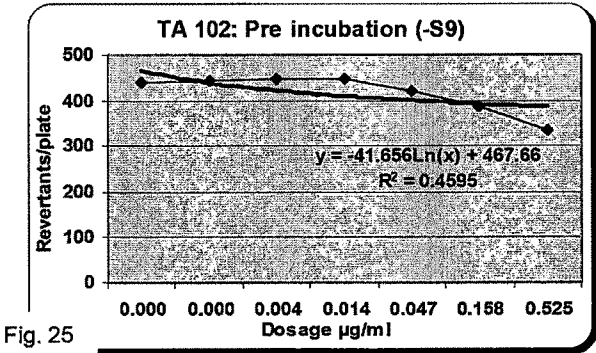
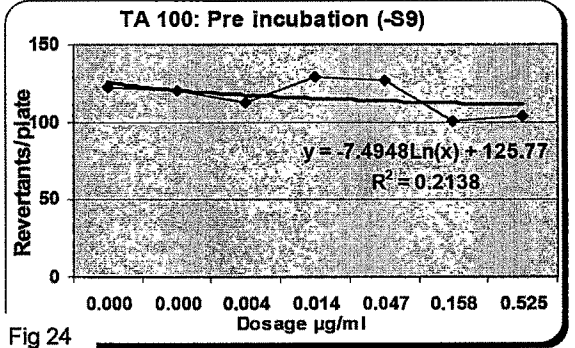
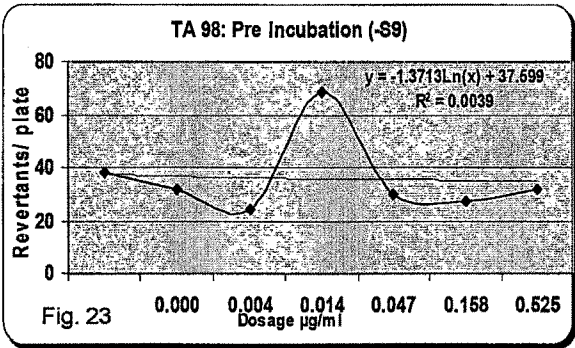
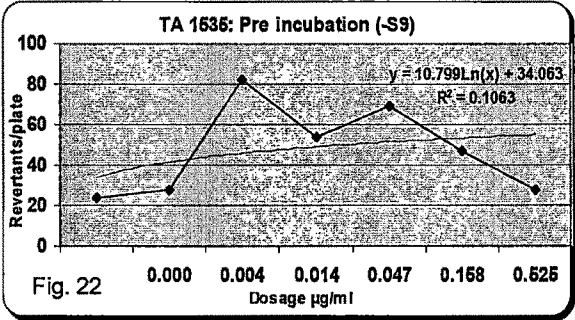
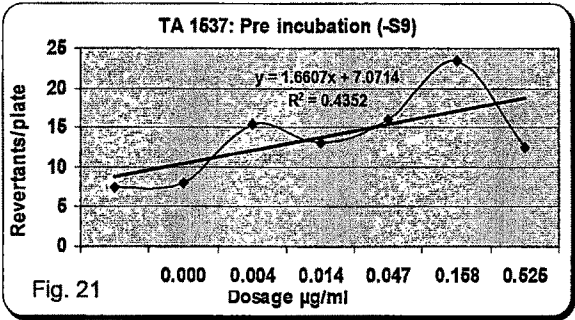
REVERTANT FREQUENCY – MAIN STUDY-II

Table: 4.10 - Strain: TA 102(+S9), Pre incubation method								
Group	G1	G2	G3	G4	G5	G6	G7	2-AF
Dose (µg/plate)	0	0	0.004	0.014	0.047	0.158	0.525	20
Replicate1	366	466	506	471	390	337	300	896
Replicate2	448	408	445	447	394	249	203	912
Lawn	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fold Value	NA	NA	1.09	1.05	0.90	0.67	0.58	2.07
Mean	407.00	437.00	475.50	459.00	392.00	293.00	251.50	904.00
S.D.	238.53	253.96	276.22	265.27	226.30	174.70	152.80	11.31
S.E.	168.69	179.61	195.34	187.60	160.04	123.55	108.06	8.00
Regression Equation: $y = -70.956\ln(x) + 474.27$, $r = 0.57$, $n = 2$								

Note: G1- G7 are treatment groups, S.D. = Standard Deviation,
S.E. = Standard Error, 2-AF = 2-Aminofluorene.

AMES TEST

Revertant Frequency: Pre Incubation Method
(In the absence of metabolic activation)



AMES TEST

Revertant Frequency: Pre Incubation Method
(In the presence of metabolic activation)

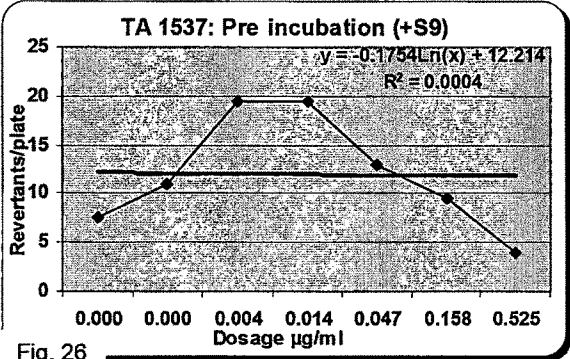


Fig. 26

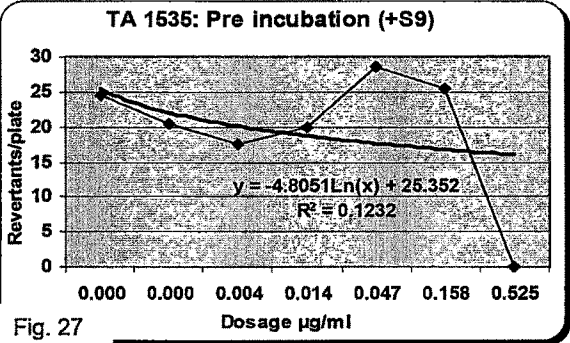


Fig. 27

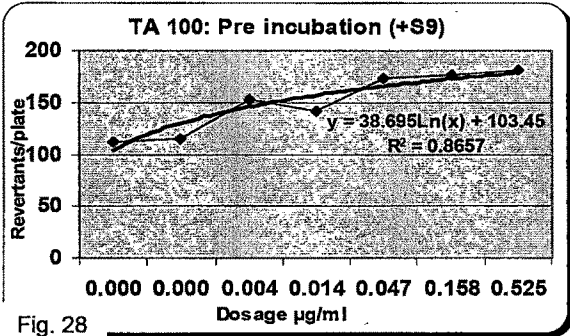


Fig. 28

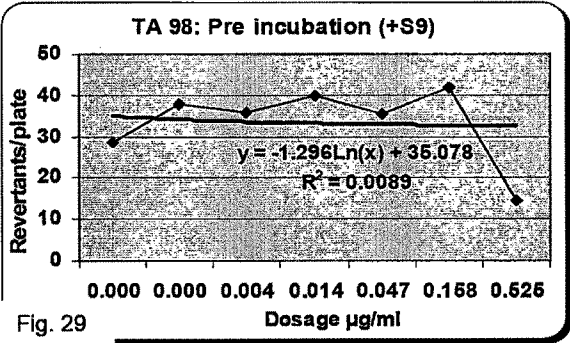


Fig. 29

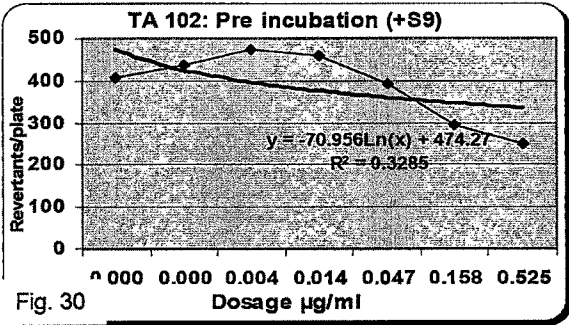


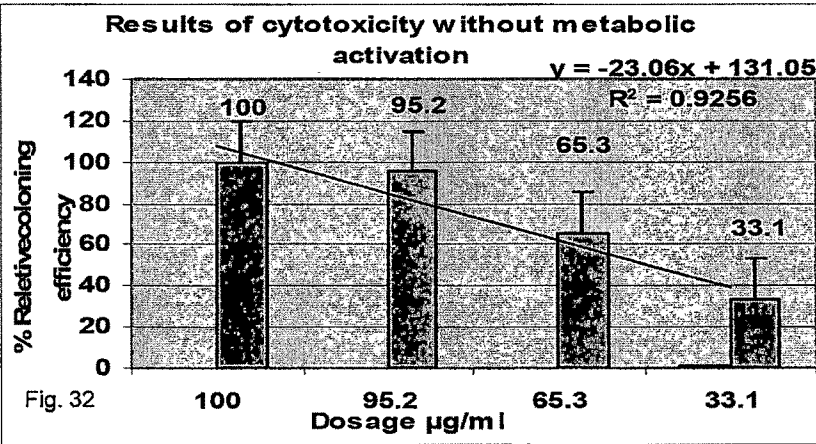
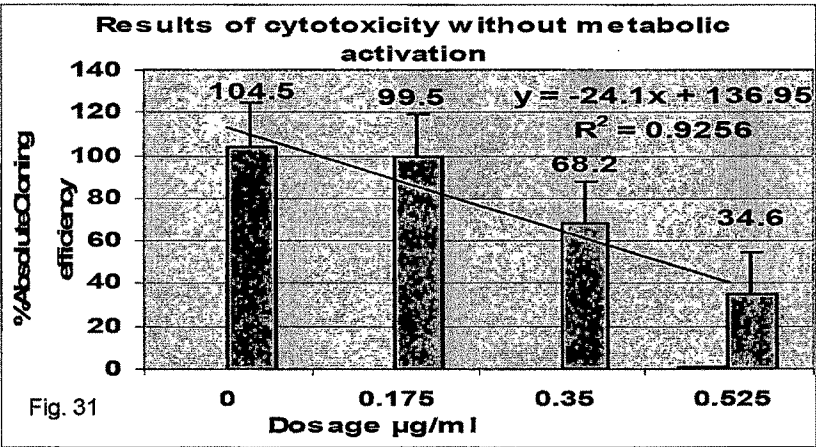
Fig. 30

FORWARD MUTATION ASSAYS: DOSE SELECTION STUDY

TABLE: 5 SURVIVAL FREQUENCIES (Cytotoxicity)
Table: 5.1 (Without Metabolic Activation)

Survival frequency : Dose Selection (without metabolic activation, -S9)									
Dose levels (µg/ml)	No of cells seeded	No of colonies observed				ACE	%ACE	RCE	%RCE
		R1	R2	Mean	SD				
VC(0)	100	115	94	104.5	14.849	1.045	104.5	1	100
0.175	100	94	105	99.5	7.7782	0.995	99.5	0.952	95.2
0.35	200	151	122	136.5	20.506	0.682	68.2	0.653	65.3
0.525	205	50	92	71.0	29.698	0.346	34.6	0.331	33.1

Note: VC = Vehicle Control, N = Number of Cells seeded, ACE = Absolute Clonning Efficiency, RCE = RelativeClonning Efficiency



FORWARD MUTATION ASSAYS: MAIN STUDY

TABLE: 5 SURVIVAL FREQUENCIES (BEFORE MUTANT SELECTION)

Table: 5.2 (Without Metabolic Activation)

Survival frequency of the cells before selection without metabolic activation (-S9)									
Dose levels (µg/ml)	No of cells seeded	No of colonies observed				ACE	%ACE	RCE	%RCE
		R1	R2	Mean	SD				
NC(0)	200	207	215	211	5.66	1.055	105.50	1.27	127.11
VC(0)	200	193	139	166	38.18	0.830	83.00	1.00	100.00
0.175	200	119	105	112	9.90	0.560	56.00	0.67	67.47
0.35	300	262	232	247	21.21	0.823	82.33	0.99	99.20
0.525	400	28	32	30	2.83	0.075	7.50	0.09	9.04
PC(10 µl EMS)	200	124	134	129	7.07	0.645	64.50	0.78	77.71

Table: 5.3 (With Metabolic Activation)

Survival frequency of the cells before selection with metabolic activation (+S9)									
Dose levels (µg/ml)	No of cells seeded	No of colonies observed				ACE	%ACE	RCE	%RCE
		R1	R2	Mean	SD				
NC(0)	200	162	137	149.5	17.68	0.748	74.75	1.00	99.67
VC(0)	200	157	143	150	9.90	0.750	75.00	1.00	100.00
0.175	200	173	185	179	8.49	0.895	89.50	1.19	119.33
0.35	300	143	126	134.5	12.02	0.448	44.83	0.60	59.78
0.525	200	112	101	106.5	7.78	0.533	53.25	0.71	71.00
PC (12 µg B(a)P)	200	168	103	135.5	45.96	0.678	67.75	0.90	90.33

Note: NC = Negative Control, VC = Vehicle Control, PC = Positive Control,
ACE = Absolute Clonning Efficiency, RCE = Relative Clonning Efficiency, EMS =
Ethy Methane Sulphonate and B (a) P = Benzo-a -pyrene

TABLE 6: MUTATION FREQUENCY WITH SURVIVAL FREQUENCY (Without Metabolic Activation)
Table: 6.1 (Without Metabolic Activation)

Mutation frequency and survival frequency of the cells without metabolic activation (-S9)												
Dose levels (µg/ml)	N	No of colonies observed				ACE	Cells/ml counted	Volume of cells added (ml)	No. of Cells Plated	No. of Clonable Cells	O	MF
		R1	R2	Mean	SD							
NC(0)	200	183	165	174	12.73	0.870	285000	0.5	142500	123975	3	4.84
VC(0)	200	121	137	129	11.31	0.645	227500	0.5	113750	73369	1	2.73
0.175	200	122	106	114	11.31	0.570	250000	0.5	125000	71250	2	5.61
0.35	200	109	120	114.5	7.78	0.573	368750	0.4	147500	84444	1	2.37
0.525	400	222	224	223	1.41	0.558	153750	2.0	307500	171431	0	0.00
PC (10µl EMS)	403	260	244	252	11.31	0.625	65000	2.9	188500	117871	38	64.48

Table: 6.2 (With Metabolic Activation)

Mutation frequency and survival frequency of the cells with metabolic activation (+S9)													
Dose levels (µg/ml)	N	No of colonies observed				ACE		Cells/ml counted	Volume of cells added (ml)	No. of Cells Plated	No. of Clonabl e Cells	O	MF
		R1	R2	Mea n	SD	NA							
NC(0)	200	211	221	216	7.07	1.080		277500	1.0	277500	299700	4.00	2.67
VC(0)	200	142	113	127.5	20.51	0.638		193750	1.0	193750	123516	6.00	9.72
0.175	200	113	125	119	8.49	0.595		363750	0.5	181875	108216	0.00	0.00
0.35	200	129	116	122.5	9.19	0.613		557500	0.8	446000	273175	2.00	1.46
0.525	207	255	238	246.5	12.02	1.191		103750	2.0	207500	247095	3.00	2.43
PC (12µg BaP)	300	236	343	289.5	75.66	0.965		358750	0.5	179375	173097	29.00	33.51

Note: NC = Negative Control, VC = Vehicle Control, PC = Positive Control, N = Number of Cells seeded, O = No. of Colonies observed on selective plates, ACE = Absolute Cloning Efficiency, EMS = Ethy Methane Sulphonate, B(a)P = Benzo-a -pyrene, MF = Mutation Frequency /10⁶ cells, n = 5.

Gene Mutation Assays

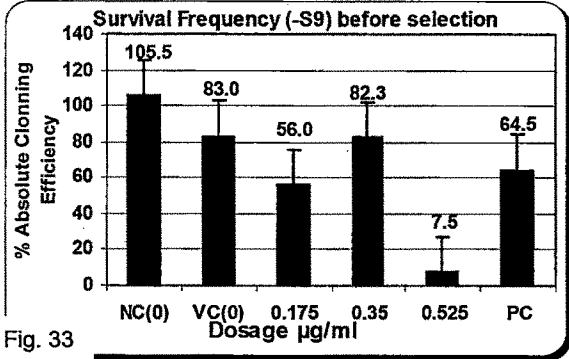


Fig. 33

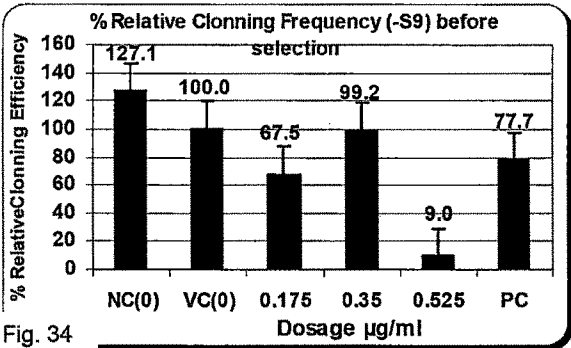


Fig. 34

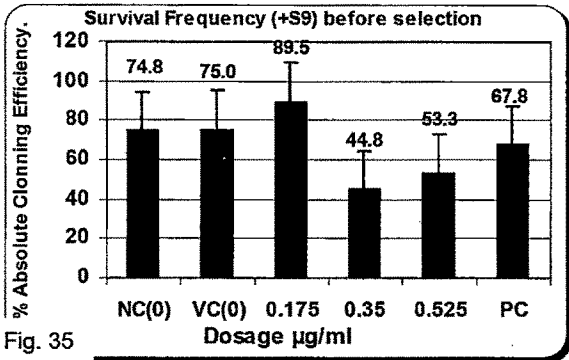


Fig. 35

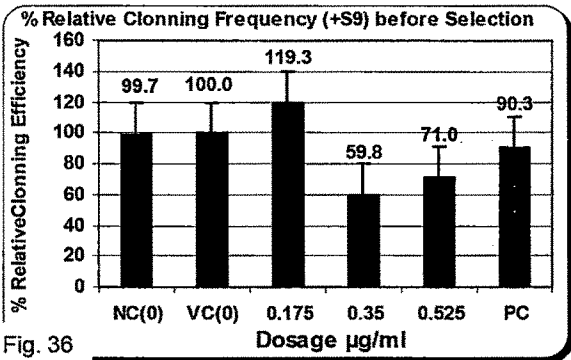


Fig. 36

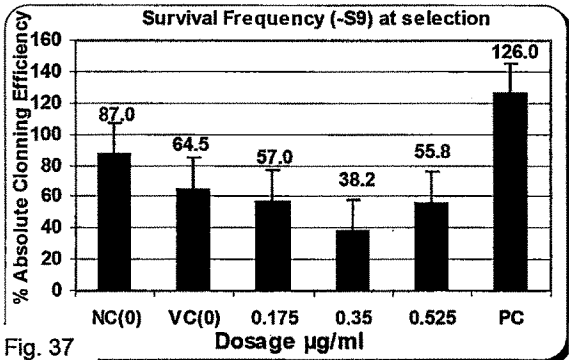


Fig. 37

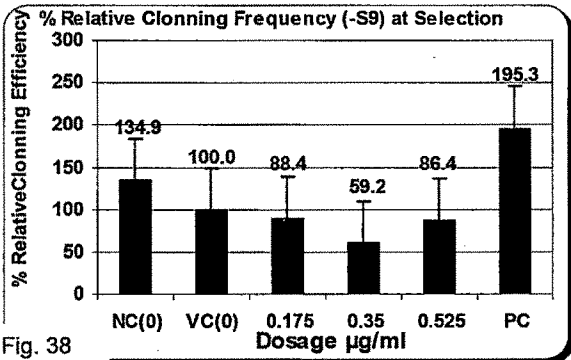
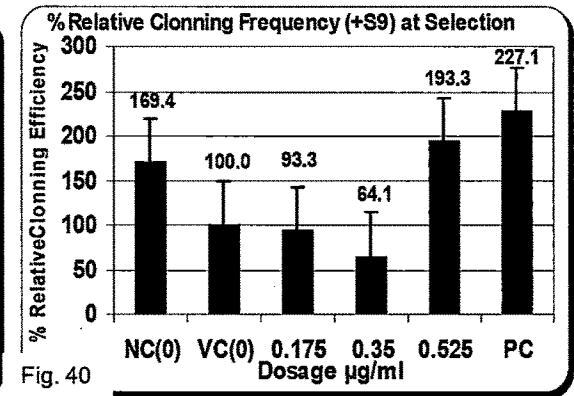
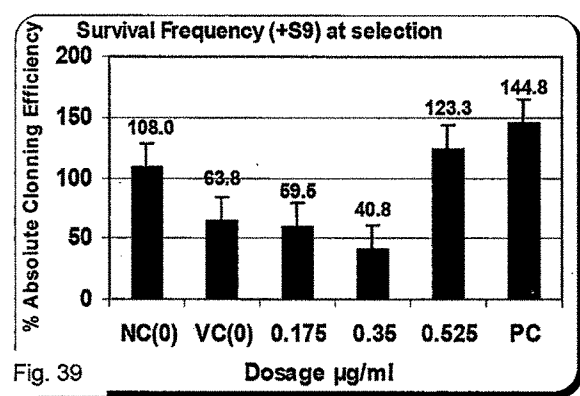


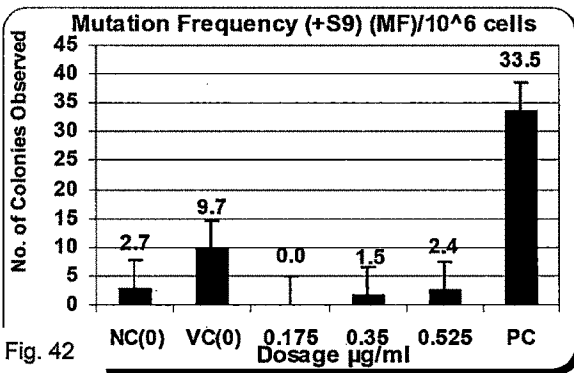
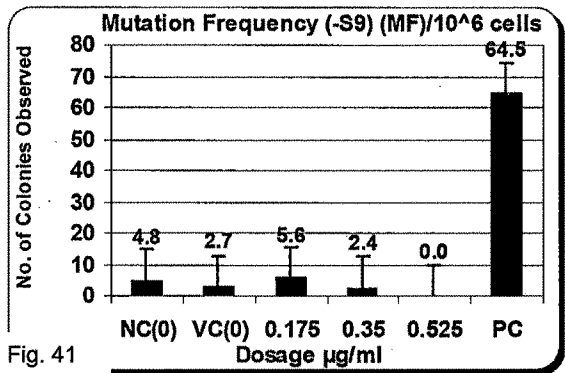
Fig. 38

Forward Mutation Assay – Data Graphs-II

Forward Mutation Assays



Mutation Frequencies



CHROMOSOMAL ABERRATION TESTS

TABLE- 7: DOSE SELECTION ASSAYS CHROMOSOMAL ABERRATION

Table -7.1 Mitotic Index

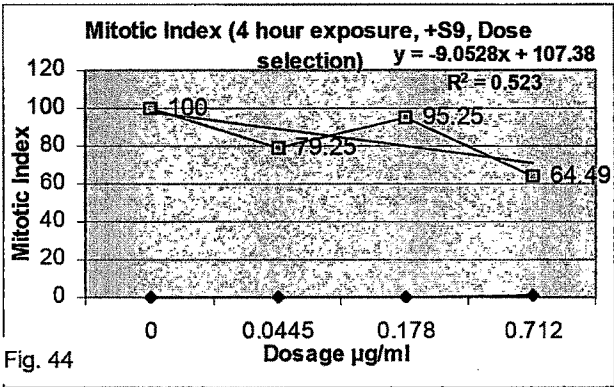
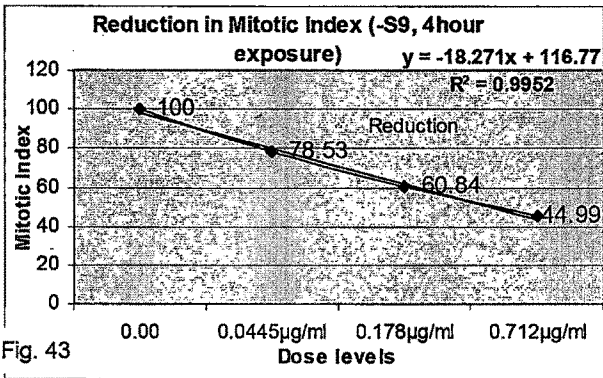
Without Metabolic activation (4.0 hour Exposure, 2 hour colchicine 0.4µg/ml)						
Dose group	Dose Levels µg/ml	Mitotic Index		Mean MI	Relative MI	% Reduction
		Replicate 1	Replicate 2			
NC	0.00	4.3912	3.920	4.1556**	NA	NA
VC	0.00	1.998	2.4496	2.5691	100	NA
T1	0.045	1.959	2.076	2.0175	78.53	21.47
T2	0.178	1.764	1.3623	1.56315	60.84	39.16
T3	0.712	1.1522	1.1596	1.1559*	44.99	55.01
$y = -18.271x + 116.77, R^2 = 0.9952, r = 0.997597$						

Table -7.2 Mitotic Index

With Metabolic activation (4.0 hour Exposure, 2 hour colchicine 0.4µg/ml)						
Dose group	Dose Levels µg/ml	Mitotic Index		Mean MI	%Relative MI	% Reduction
		Replicate 1	Replicate 2			
NC	0.00	1.6553	2.453	2.05415	NA	NA
VC	0.00	2.2705	2.606	2.4382	100	NA
T1	0.045	1.994	2.078	2.036	79.25	20.75
T2	0.178	2.645	2.249	2.447	95.25	4.75
T3	0.712	1.8578	1.4559	1.65685	64.49	35.51
$y = -9.0528x + 107.38, R^2 = 0.523, r = 0.7231$						

Note: NC = Negative Control, VC = Vehicle Control, T1 – T3 are treatment concentrations, MI = Mitotic Index, n = 2, * = $p \leq 0.05$ and ** $p \leq 0.01$

Data Graphs
Chrosomal Aberration Assays (Dose Selection)



CHROMOSOMAL ABERRATION

TABLE -8 MAIN STUDIES (MITOTIC INDEX AND CHROMOSOMAL ABERRATIONS)

Table - 8.1: Short-Term Exposure Mitotic Indexes (-S9)

Mitotic Index: Main Study Without metabolic activation (4h exposure)								
Dose		M	No. of cells		% Mitotic index	mean %mi	% Relative MI	Increase
Group	levels µg/ml		observed	Total				
NCR1	0	59	1050	1109	5.3201	4.6729	92.85	NA
NCR2	0	44	1049	1093	4.0256			
VCR1	0	56	1000	1056	5.3030	5.0325	NA	NA
VCR2	0	51	1020	1071	4.7619			
T1R1	0.175	96	1016	1112	8.6331	8.1487	161.92	61.92
T1R2	0.175	84	1012	1096	7.6642			
T2R1	0.350	46	1021	1067	4.3112	6.7447	134.02	34.02
T2R2	0.350	105	1039	1144	9.1783			
T3R1	0.525	83	1084	1167	7.1123	5.2835	104.99	4.99
T3R2	0.525	37	1034	1071	3.4547			
PCR1	MMC (0.5)	52	1046	1098	4.7359	4.5820	91.05	-8.95
PCR2	MMC (0.5)	48	1036	1084	4.4280			

Note: NC = Negative Control, VC = Vehicle Control, PC = Positive Control,
M = Metaphases, MMC = Mitomycin-C, R = Replicate and n = 2, Values are not
significant at any of the tested concentrations.

Chapter 3

TABLE -8 MAIN STUDIES (MITOTIC INDEX AND CHROMOSOMAL ABERRATIONS)

Table - 8.2: Short-Term Exposure Mitotic Indexes (+S9)

Mitotic Index : Main Study With metabolic activation (4h exposure)								
Dose		M	No. of cells		%Mitotic Index	Mean %MI	% Relative MI	Increase
Group	Levels µg/ml		Observed	Total				
NCR1	0	112	1006	1118	10.0179	10.7850	115.84	NA
NCR2	0	131	1003	1134	11.5520			
VCR1	0	109	1071	1180	9.2373	9.3101	100.00	NA
VCR2	0	102	1041	1143	8.9239			
VCR3	0	110	1016	1126	9.7691			
T1R1	0.175	196	1174	1370	14.3066	14.8305**	156.06	56.06
T1R2	0.175	195	1075	1270	15.3543			
T2R1	0.350	115	1002	1117	10.2954	9.8161	103.29	3.29
T2R2	0.350	107	1039	1146	9.3368			
T3R1	0.525	106	1088	1194	8.8777	9.0589	95.32	4.68
T3R2	0.525	107	1051	1158	9.2401			
PCR1	Cyp(25)	85	1012	1097	7.7484	6.6962*	70.46	29.54
PCR2	Cyp(25)	78	1304	1382	5.6440			

Note: NC = Negative Control, VC = Vehicle Control, PC = Positive Control,
M = Metaphases, Cyp = Cyclophosphamide, R = Replicate n = 2,
* = $p \leq 0.05$ and ** $p \leq 0.01$

Table - 8.3: Short-Term Exposure Structural Chromosomal Aberrations

Chromosomal Aberration without metabolic activation (4 hour)																				
Dose levels µg/ml	M	Gap		Break		DL	Fragment		R	Ex.	Int.	Dc.	Trs. /inv.	Total Aberration		Aberrant Metaphases		% Ab Cell WT gap	Mean % AB. Cell	S. D.
		C	CI	C	CI		ACF	F						with gaps	WT gaps	with gap	WT gap			
0	100	0	0	2	0	0	1	0	0	0	0	0	0	3	3	3	3	2.00	1.41	
0	100	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1			
0	100	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0.50	0.71	
0	100	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	1			
0.175	100	1	3	0	1	1	0	3	1	1	0	1	1	13	9	10	9	5.50	4.95	
0.175	100	1	0	0	0	2	0	3	0	1	0	3	0	10	9	3	2			
0.350	59	0	1	0	1	0	2	0	0	0	0	0	0	4	3	3	2	3.19	0.71	
0.350	100	0	1	0	1	0	0	1	0	2	0	1	0	6	5	4	3			
0.525	100	1	0	0	0	1	1	3	0	1	0	3	0	10	9	9	8	6.50	2.12	
0.525	100	3	5	2	1	0	0	2	0	0	0	1	0	14	6	9	5			
MMC (0.5)	50	2	4	6	4	2	2	12	0	4	2	1	0	39	33	37	29	56.00	1.41	
MMC (0.5)	50	1	1	4	2	3	5	18	2	8	2	1	0	47	45	43	27			

Note: M = Number of metaphases, C = Chromatid, CI = Chromosomal, Del. = Deletion, ACF = Acentric Fragment, F = Fragments, R = Ring chromosome, Ex. = Exchanges, Int. = Interchanges, DL = Deletion, Int. = Interchange, WT = Without, Ab. = Aberrant, Dc. = Dacentrics, Trs. = Transitions, Inv. = Inversions, S.D. = Standard Deviations, MMC = Mitomycin-C.

Short-Term Exposure Structural Chromosomal Aberrations

Chromosomal Aberration with metabolic activation (4 hour)																				
Dose levels µg/ml	M	Gap		Break		Del.	Fragment		R	Ex.	Int.	Dic.	Trs./ inv.	Total Aberration		Aberrant Metaphases		% Ab Cell WT gap	Mean % ab Cell	S. D.
		C	CI	C	CI		ACF	F						with gap	WT gap	with gap	WT gap			
NC	100	0	0	0	1	0	0	3	0	0	0	0	0	4	4	4	4	4	4.50	0.71
NC	100	1	1	2	0	0	0	0	1	0	1	2	8	6	6	5	5			
VC	100	1	0	1	0	0	0	0	4	0	0	1	0	11	10	10	8	8	5.00	2.65
VC	100	0	0	1	0	0	0	0	2	0	0	0	0	3	3	3	3	3		
VC	100	0	0	0	1	2	1	0	0	0	0	0	0	4	4	4	4	4	7.50	2.12
0.175	100	0	0	1	0	0	2	1	0	2	0	1	0	7	7	6	6	6		
0.175	100	0	0	1	0	1	0	0	2	2	2	1	0	9	9	9	9	9	10.00	9.90
0.350	100	0	0	0	0	0	0	1	0	1	0	1	0	3	3	3	3	3.0		
0.350	100	0	0	1	3	3	2	7	1	4	1	0	2	24	24	24	17	17	11.45	4.24
0.525	100	0	0	3	2	0	2	2	1	0	0	0	0	10	10	10	10	10		
0.525	31	0	0	0	0	0	0	3	0	1	0	0	0	4	4	4*	4	12.9	26.00	2.83
Cyp(25)	50	1	1	2	0	2	1	2	1	10	2	4	3	29	27	18	15	30		
Cyp(25)	50	3	0	1	0	0	2	0	2	8	3	6	2	27	24	15	11	22		

Note: M = Number of metaphases, C = Chromatid, CI = Chromosomal, Del. = Deletion, ACF = Acentric Fragment, F = Fragments, R = Ring Chromosome, DI. = Deletion, Int. = Interchange, WT = Without, Ab. = Aberrant, Ex. = Exchanges, Inter. = Interchanges, Dic. = Dicentric, Trs. = Transitions, Inv. = Inversions, S.D. = Standard Deviations, Cyp = Cyclophosphamide and * = ps 0.05.

Table - 8.5: Short-Term Exposure Numerical Chromosomal Aberrations

Numerical Aberrations (-S9) 4 hour exposure						
Dose levels µg/ml	Ploidy					
	Aneuploidy	Polyploidy	ER	Total Ploidy	Mean	SD
0	0	3	0	3	2.00	1.41
0	0	1	0	1		
0	0	2	0	2	1.50	0.71
0	0	1	0	1		
0.175	0	1	0	1	1.00	0.00
0.175	0	1	0	1		
0.350	0	2	0	2	2.00	0.00
0.350	0	2	0	2		
0.525	0	1	0	1	1.00	0.00
0.525	0	1	0	1		
MMC (0.5)	0	4	0	4	5.50	2.12
	0	7	0	7		

Note: E.R = Endoreduplication, S.D. = Standard Dviations,
MMC = Mitomycin-C.

Chapter 3

Table - 8.6: Short-Term Exposure Numerical Chromosomal Aberrations

Numerical Aberration (+S9) 4 hour exposure						
Dose levels	Ploidy					
µg/ml	Aneuploidy	Polyploidy	ER	Total Ploidy	Mean	SD
NC	0	6	0	6	4.50	2.12
NC	0	3	0	3		
VC	0	14	0	14	11.00	3.61
VC	0	12	0	12		
VC	0	7	0	7		
0.175	0	4	0	4	5.50	2.12
0.175	0	7	0	7		
0.350	0	5	0	5	8.00	4.24
0.350	0	11	0	11		
0.525	0	14	0	14	10.00	5.66
0.525	0	6	0	6		
Cyp(25)	6	4	1	11	10.5	0.71
Cyp(25)	4	6	0	10		

Note: NC = Note Negative Control, VC = Vehicle Control, PC = Positive Control, ER = Endoreduplication, Cyp = Cyclophosphamide.

Table- 9.2: Numerical Aberrations

Numerical Aberrations : (-S9) Trial-I (Long Term Exposure)						
Dose levels	Aneuploidy	Polyploidy	ER	Total Ploidy	Mean	SD
NC	0	7	0	7	6.20	2.95
NC	0	8	0	8		
NC	0	7	0	7		
NC	0	1	0	1		
NC	0	8	0	8		
VC	0	3	0	3	5.00	1.87
VC	0	6	0	6		
VC	0	3	0	3		
VC	1	6	0	7		
VC	1	5	0	6		
0.175	0	8	0	8	5.40	2.41
0.175	1	5	0	6		
0.175	0	7	0	7		
0.175	1	3	0	4		
0.175	0	2	0	2		
0.350	0	17	0	17	9.40	4.34
0.350	0	6	0	6		
0.350	0	8	0	8		
0.350	0	8	0	8		
0.350	0	8	0	8		
0.525	0	6	2	8	7.00	1.41
0.525	0	7	0	7		
0.525	0	5	0	5		
0.525	0	8	0	8		
EMS (0.1µl/ml)	0	13	0	13	12.50	0.71
	0	12	0	12		

TABLE -10:LONG-TERM EXPOSURE (TRIAL-II) With Excess colchicines

Trial-II (Chromosomal Aberration without metabolic activation), with excess colchicine																
Dose levels	□/ml	M	Gap			Break			DI.	Fragment		R	Ex.	Int.	Dic.	Trs./inv.
			C	CI	C	C	CI	C		ACF	F					
NC (0.00)		100	1	0	1	0	1	0	0	0	1	1	0	0	1	0
NC (0.00)		100	0	0	1	0	1	0	1	0	2	0	1	0	1	0
VC (0.00)		100	1	1	1	1	1	0	0	1	0	0	1	0	0	0
VC (0.00)		100	1	0	1	4	1	1	1	1	1	0	0	0	0	1
0.175		100	1	1	0	1	0	1	0	5	2	0	0	0	0	0
0.175		100	0	0	0	0	0	0	0	7	1	0	0	0	3	1
0.175		100	0	0	0	0	0	0	0	0	1	0	0	0	0	2
0.175		100	0	7	3	6	1	4	1	4	1	1	0	0	1	0
MMC 0.5)		50	3	0	4	4	2	1	7	1	7	1	0	0	3	0
MMC (0.5)		27	0	2	1	1	5	5	0	5	0	1	0	0	3	0
													Total Aberration		Aberrant Metaphases	
													with gaps	without gaps	with gap	WT gap
													5	4	5	4
													6	6	5	5
													6	4	4	4
													10	9	8	7
													10	8	8	7
													12	12	11	11
													3	3	3	3
													19	17	18	15
													25	22	14	13
													18	16	10	10
													Aberrant cell WT gap		Mean % aberrant Cell	
													4		4.50	
													5			
													4		3.67	
													7		2.12	
													7			
													11		9.00	
													3.0			
													15			
													26		31.52	
													37.04		2.12	

Note: NC = Negative Control, VC = Vehicle Control, MMC = Mitomycin-C, DI. = Deletion, Int. = Interchange, WT = Without

Data Graphs
Chrosomal Aberration Assays
Chromosomal Aberration

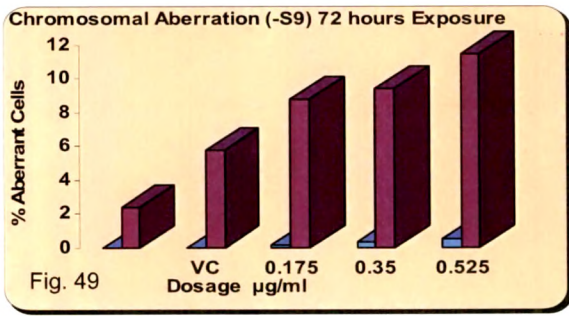
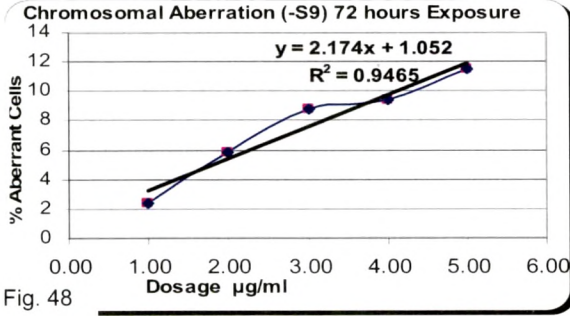
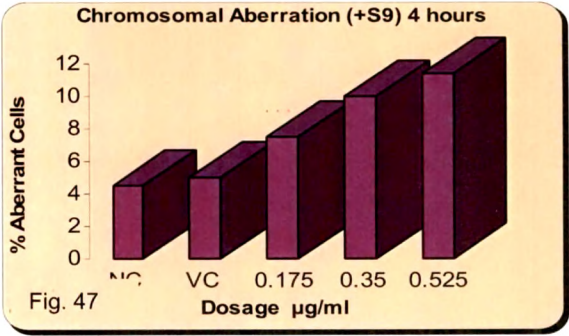
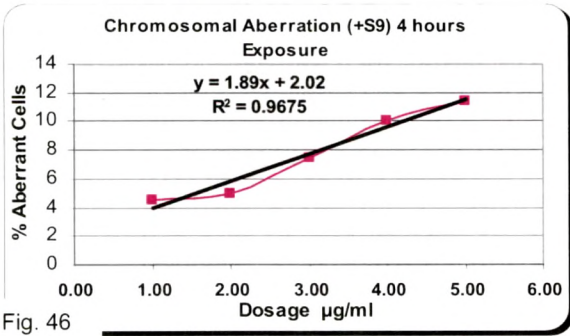
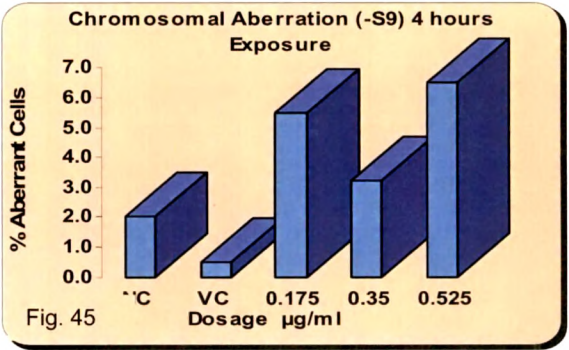
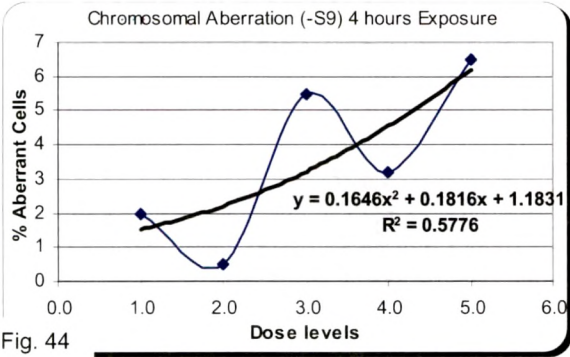


Table- 10.1: Numerical Aberrations

Numerical Aberration (-S9) with excess colchicine						
Dose levels	Ploidy					
µg/ml	Aneuploidy	Polyploidy	ER	Total Ploidy	Mean	SD
NC (0.00)	4	6	0	10	7.00	4.24
NC (0.00)	3	1	0	4		
VC (0.00)	1	4	0	5	5.00	0.00
VC (0.00)	0	5	0	5		
0.175	2	2	3	7	13.75	6.70
0.175	10	5	4	19		
0.175	8	12	0	20		
0.175	0	6	3	9		
MMC(0.5)	2	6	3	11	7	5.66
MMC(0.5)	3	0	0	3		

Note: NC = Negative Control, VC = Vehicle Control, MMC = Mitomycin-C.

IN VIVO ASSAYS

Result Summary Tables

Table: 11 Symptoms of toxicity (Range Finding Study)

Dose (mg/kg body weight)	Mortality		Symptoms Morphological	Gross pathology
	Male	Female		
100	Died (1/1)	Died (1/1)	Both male and female Abdominal breathing and convulsions, catalapsy and death	Male and female Liver: mottling (+++) Lung : congestion (++) Kidney : mottling (+)
40	Died (1/1)	Died (1/1)	Both male and female Abdominal breathing and convulsion, catalapsy and death	Male and female Liver: mottling (+) Lung : congestion (+) Kidney : mottling (+) only male
20 (day-1)	Died (0/1)	Died (0/1)	Male Abdominal breathing was observed only in male animal, which got recovered after 4 hrs. Female The animal was devoid of symptoms.	-
20 (day-2)	Died (2/3)	Died (0/1)	Male (before death) Exaggerated response to sound stimuli, Clonic tonic convulsion of fore and hind limbs, Catalapsy, Salivation. Lacrimation, Convulsion, Writhing. Dilation of pupil. Male Moderate abdominal breathing was observed Female Normal.	male Liver: mottling (++) Lung : congestion (+) Kidney : mottling (+)
10 (day-1)	Died (1/1)	Died (0/1)	Male Salivation and death Female Normal.	male Liver: mottling (+) Lung : congestion (+) Kidney: -

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TABLE: 12: MAIN STUDY (GROSS PATHOLOGICAL OBSERVATION AT NECROPSY)

Animal No.	Dose mg/Kg body weight	sex	External observation	organ	Gross pathological observation
1-8	0.0	Male and female	-	-	-
9-16	4.0	Male and female	-	-	-
17-20	8.0	male	Abdominal breathing, Tremors, Salivation	Lung(Right cranial lobe) Liver Kidney	Mild congestion - -
			Abdominal breathing, Tremors, Salivationand nasal discharge	Lung (all the lobes) Liver Kidney	Congestion Mottling (mild) -
			Hyper activity Abdominal breathing	Lung ° Liver Kidney	- mottling(mild) -
21-24		female	Tremor, writhing, salivation finally bleeding from mouth	Lung Liver Kidney	- - -

TABLE: 13 – WEEKLY BODY WEIGHT

Table: 13.1 Mice Weekly Body Weight (Male)

WEEK	GROUP	MEAN	SD	SE	p-value significance
WEEK-1	G1	40.39	3.49	1.75	-
	G2	39.71	3.13	1.56	0.767
	G3	40.77	1.20	0.60	0.847
WEEK -2	G1	40.30	3.26	1.63	-
	G2	41.84	1.88	0.94	0.468
	G3	42.12	1.87	0.93	0.430
WEEK-3	G1	42.59	2.34	1.17	-
	G2	42.64	1.14	0.57	0.971
	G3	42.16	1.89	0.95	0.829
WEEK-4	G1	43.00	3.91	1.95	-
	G2	42.57	0.82	0.41	0.840
	G3	40.27	3.39	1.69	0.418

Table: 13.2 Mice Weekly Body Weight (Female)

WEEK	GROUP	MEAN	SD	SE	p-value significance
WEEK-1	G1	35.66	4.42	2.21	-
	G2	36.46	2.76	1.38	0.797
	G3	32.82	1.40	0.70	0.380
WEEK -2	G1	35.23	4.11	2.05	-
	G2	36.02	2.86	1.43	0.802
	G3	34.97	0.94	0.47	0.901
WEEK-3	G1	38.22	4.49	2.25	-
	G2	38.77	1.86	0.93	0.859
	G3	36.45	1.12	0.56	0.554
WEEK-4	G1	36.90	4.17	2.08	-
	G2	37.11	1.93	0.97	0.936
	G3	36.02	1.54	0.77	0.734

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), SD = Standard deviation and SE = standard error.

TABLE: 14-HEMATOLOGY

TABLE: 14.1 ANALYSIS RESULTS OF HEMATOLOGY (MALE)

	GROUP	MEAN	SD	SE	p-value significance
WBC	G1	8.25	1.61	0.80	-
	G2	5.25	0.97	0.48	0.088
	G3	5.30	1.31	0.66	0.082
RBC	G1	8.03	0.50	0.25	-
	G2	8.63	0.15	7.33	0.064
	G3	8.35	0.35	0.17	0.216
Hb	G1	13.15	1.02	0.51	-
	G2	14.68	0.32	0.16	0.060
	G3	13.20	1.67	0.83	0.946
HCT	G1	39.73	2.10	1.05	-
	G2	42.85	0.49	0.25	0.083
	G3	43.90	1.53	0.76	0.018*
MCV	G1	-49.50	1.44	0.72	-
	G2	-49.68	0.99	0.50	0.758
	G3	-49.68	1.56	0.78	0.844
MCH	G1	-16.35	0.54	0.27	-
	G2	-17.00	0.34	0.17	0.080
	G3	-16.08	1.08	0.54	0.624
MCHC	G1	33.05	0.93	0.46	-
	G2	34.25	0.51	0.25	0.038*
	G3	33.48	0.26	0.13	0.507
PLT	G1	1337.00	275.13	137.56	-
	G2	1050.00	176.31	88.16	0.159
	G3	1121.50	157.75	78.88	0.393

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), WBC = white blood cells, RBC = red blood cells, Hb = Haemoglobin, HCT = hemetocrit, MCV = mean corpuscular volume, SD = Standard Deviation, SE = Standard Error, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, PLT = platelets, * $p \leq 0.05$.

Table: 14.2 Individual Animal Values (Male Animals)

Haematological Analysis of Whole Blood in Male Animals								
Animal No.	WBC (103/mm3)	RBC (106/mm3)	Hb (g/dl)	HCT (%)	MCV (mm3)	MCH (pg)	MCHC (g/dl)	PLT (103/mm)
1	8.8	8.22	13	39.7	-48.3	-15.8	32.7	1551
2	6.5	8.3	14.2	42.1	-50.7	-17.1	33.7	1074
3	7.5	8.32	13.6	40.1	-48.2	-16.3	33.9	1597
4	10.2	7.28	11.8	37	-50.8	-16.2	31.9	1126
MEAN	8.25	8.03	13.15	39.725	-49.5	-16.35	33.05	1337
SD	1.61	0.50	1.02	2.10	1.44	0.54	0.93	275.13
Ref. Range- 4th Wk.	2.61± 0.88	8.34 ± 0.62	11.65 ± 0.93	35.45 ± 3.02	42.48 ± 0.99	13.95 ± 0.32	32.91± 0.34	214.2 ± 15.46
Ref.range- 12th wk	5.32-6.23	8.98-10.59	14.06-14.74	1.59-43.37	40.89-42.66	14.04-14.48	33.46-34.96	10.73-12.19
9	4.2	8.56	14.2	42.3	-49.4	-16.6	33.6	1267
10	5.8	8.63	14.8	42.6	-49.4	-17.1	34.7	1120
11	6.3	8.83	14.9	43.1	-48.8	-16.9	34.6	900
12	4.7	8.49	14.8	43.4	-51.1	-17.4	34.1	913
MEAN	5.25	8.6275	14.675	42.85	-49.675	-17	34.25*	1050
SD	0.97	0.15	0.32	0.49	0.99	0.34	0.51	176.31
Ref. Range- 4th Wk.	2.61± 0.88	8.34 ± 0.62	11.65 ± 0.93	35.45 ± 3.02	42.48 ± 0.99	13.95 ± 0.32	32.91± 0.34	214.2 ± 15.46
Ref.range- 12th wk	5.32-6.23	8.98-10.59	14.06-14.74	1.59-43.37	40.89-42.66	14.04-14.48	33.46-34.96	10.73-12.19
17	6.8	8.47	14.1	42	-49.6	-16.6	33.6	985
18	6	8.78	15.1	45.6	-51.9	-17.2	33.1	1266
19	4.1	8.11	11.7	44.5	-48.6	-14.7	33.5	985
20	4.3	8.02	11.9	43.5	-48.6	-15.8	33.7	1250
MEAN	5.3	8.345	13.2	43.9*	-49.675	-16.075	33.475	1125.5
SD	1.31	0.35	1.67	1.53	1.56	1.08	0.26	157.75
Ref. Range- 4th Wk.	2.61± 0.88	8.34 ± 0.62	11.65 ± 0.93	35.45 ± 3.02	42.48 ± 0.99	13.95 ± 0.32	32.91± 0.34	214.2 ± 15.46
Ref.range- 12th wk	5.32-6.23	8.98-10.59	14.06-14.74	1.59-43.37	40.89-42.66	14.04-14.48	33.46-34.96	10.73-12.19

Note: WBC = white blood cells, RBC = red blood cells, Hb = Haemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, SD = Standard Deviation, SE = Standard Error, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, PLT = platelets, * p ≤ 0.05.

TABLE: 14.3 ANALYSIS RESULTS OF HEMATOLOGY (FEMALE)

	GROUP	MEAN	SD	SE	P-VALUE SIGNIFICANCE
WBC	G1	5.20	1.49	0.74	-
	G2	4.88	1.44	0.72	0.352
	G3	4.85	1.00	0.50	0.438
RBC	G1	8.47	0.35	0.17	-
	G2	8.55	0.58	0.29	0.827
	G3	8.02	0.42	0.21	0.024*
Hb	G1	14.25	0.62	0.31	-
	G2	14.38	1.24	0.62	0.874
	G3	14.23	0.98	0.49	0.961
HCT	G1	42.13	0.78	0.39	-
	G2	42.33	3.17	1.59	0.905
	G3	42.15	1.00	0.50	0.952
MCV	G1	-49.78	1.13	0.56	-
	G2	-49.50	0.59	0.30	0.754
	G3	-51.63	0.83	0.42	0.015*
MCH	G1	-8.38	16.85	8.43	-
	G2	-16.85	0.40	0.20	0.383
	G3	-9.13	17.15	8.58	0.060
MCHC	G1	33.83	0.91	0.46	-
	G2	33.95	0.51	0.25	0.855
	G3	34.00	1.23	0.62	0.689
PLT	G1	842.75	225.61	112.80	-
	G2	1109.00	76.83	38.42	0.161
	G3	1125.50	81.62	40.81	0.136

Note: WBC = White blood cells, RBC = red blood cells, Hb = Haemoglobin, HCT = hemetocrit, MCV = mean corpuscular volume, SD = Standard Deviation, SE = Standard Error, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, PLT = platelets, * $p \leq 0.05$.

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TABLE: 14.4 INDIVIDUAL ANIMAL VALUES (FEMALE ANIMALS)

HAEMATOLOGICAL ANALYSIS OF WHOLE BLOOD IN FEMALE ANIMALS								
ANIMAL NO.	WBC (103/MM3)	RBC (106/MM3)	Hb (G/DL)	HCT (%)	MCV (MM3)	MCH (PG)	MCHC (G/DL)	PLT (103/MM)
5	6.8	8.15	13.5	41.4	-50.8	-16.6	32.6	882
6	5.4	8.94	15	43.1	-48.2	-16.8	34.8	1121
7	3.2	8.51	14.4	42.4	-49.8	16.9	34	792
8	5.4	8.27	14.1	41.6	-50.3	-17	33.9	576
MEAN	5.2	8.4675	14.25	42.125	-49.775	-8.375	33.825	842.75
SD	1.49	0.35	0.62	0.78	1.13	16.85	0.91	225.61
REF. RANGE-4TH WK.	2.47± 0.80	9.34 ± 0.52	12.99 ± 0.57	37.96 ± 2.00	40.66 ± 0.70	13.92 ± 0.40	34.24 ± 0.54	260.40 ± 2718
REF.RANGE-12TH WK	5.26-6.92	10.35-11.05	14.82-15.89	44.38-47.32	42.17-43.39	14.13-14.69	33.57-34.03	10.82-14.21
13	6	9.09	15.6	45.1	-49.6	-17.2	34.6	1090
14	5.9	8.68	14.9	43.7	-50.3	-17.2	34.1	1071
15	2.9	8.69	14.3	42.7	-49.1	-16.5	33.5	1053
16	4.7	7.72	12.7	37.8	-49	-16.5	33.6	1222
MEAN	4.875	8.545	14.375	42.325	-49.5	-16.85	33.95	1109
SD	1.44	0.58	1.24	3.17	0.59	0.40	0.51	76.83
REF. RANGE-4TH WK.	2.47± 0.80	9.34 ± 0.52	12.99 ± 0.57	37.96 ± 2.00	40.66 ± 0.70	13.92 ± 0.40	34.24 ± 0.54	260.40 ± 2718
REF.RANGE-12TH WK	5.26-6.92	10.35-11.05	14.82-15.89	44.38-47.32	42.17-43.39	14.13-14.69	33.57-34.03	10.82-14.21
21	6.3	7.94	13.9	41.9	-52.8	-17.5	33.2	1210
22	4.5	8.57	15.6	43.6	-50.9	-18.2	35.8	1014
23	4	8.01	13.3	41.3	-51.6	16.6	33.2	1144
24	4.6	7.56	14.12	41.8	-51.2	-17.4	33.8	1134
MEAN	4.85	8.02	14.23	42.15	-51.625	-9.125	34	1125.5
SD	1.00	0.42	0.98	1.00	0.83	17.15	1.23	81.62
REF. RANGE-4TH WK.	2.47± 0.80	9.34 ± 0.52	12.99 ± 0.57	37.96 ± 2.00	40.66 ± 0.70	13.92 ± 0.40	34.24 ± 0.54	260.40 ± 2718
REF.RANGE-12TH WK	5.26-6.92	10.35-11.05	14.82-15.89	44.38-47.32	42.17-43.39	14.13-14.69	33.57-34.03	10.82-14.21

Note: WBC = White blood cells, RBC = red blood cells, Hb = Haemoglobin, HCT = hemetocrit, MCV = mean corpuscular volume, SD = Standard Deviation, SE = Standard Error, MCH = Mean Corpuscular Haemoglobin, * p ≤ 0.05.

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TABLE: 15 ANALYSIS RESULTS OF DIFFERENTIAL LEUCOCYTE COUNTS

TABLE: 15.1 (MALE ANIMALS) 1ST DAY AFTER EXPOSURE

PARAMETER	GROUP	MEAN	SD	SE	p - value of significance
LYMPHOCYTES	G1	79.00	6.98	3.49	-
	G2	74.25	11.95	5.98	0.514
	G3	75.00	5.29	0.65	0.671
NEUTROPHILS	G1	16.75	4.99	2.50	-
	G2	23.00	11.97	5.99	0.501
	G3	22.75	5.97	2.98	0.308
MONOCYTES	G1	1.50	1.29	0.65	-
	G2	1.75	2.36	1.18	0.886
	G3	1.25	0.96	0.48	0.761
EOSINOPHILS	G1	2.75	1.50	0.75	-
	G2	1.00	0.82	0.41	0.133
	G3	1.00	1.15	0.58	0.133

TABLE: 15.2 INDIVIDUAL ANIMAL VALUES DLC COUNTS (MALE ANIMALS)

1ST DAY AFTER EXPOSURE DIFFERENTIAL LEUCOCYTE COUNT (MALE ANIMALS)						
MICE NO	LYMPHOCYTE COUNT	NEUTROPHIL	MONOCYTE	EOSINOPHIL	BASOPHIL	TOTAL CELLS
1	73	22	1	4	0	100
2	84	13	2	1	0	100
3	73	20	3	4	0	100
4	86	12	0	2	0	100
MEAN	79	16.75	1.5	2.75	0	100
SD	6.98	4.99	1.29	1.50	0.00	0.00
REF.RANGE	78.01-84.71	13.13-19.67	1.0-2.0	1.0-2.0	0-1	—
9	86	12	0	2	0	100
10	60	39	0	1	0	100
11	82	16	2	0	0	100
12	69	25	5	1	0	100
MEAN	74.25	21	1.75	1	0	100
SD	11.95	11.97	2.36	0.82	0.00	0.00
17	76	24	0	0	0	100
18	72	27	1	0	0	100
19	82	14	2	2	0	100
20	70	26	2	2	0	100
MEAN	75	22.75	1.25	1	0	100
SD	5.29	5.97	0.96	1.15	0.00	0.00

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), SD = Standard Deviation, SE = Standard Error.

Chapter 3

TABLE: 15 ANALYSIS RESULTS OF DIFFERENTIAL LEUCOCYTE COUNTS
TABLE: 15.3 (FEMALE ANIMALS) 1ST DAY AFTER EXPOSURE

PARAMETER	GROUP	MEAN	SD	SE	p-value significance
LYMPHOCYTES	G1	82.50	1.29	0.65	-
	G2	79.75	9.11	4.55	0.591
	G3	75.50	6.35	3.18	0.155
NEUTROPHILS	G1	14.25	2.75	1.38	-
	G2	17.25	8.42	4.21	0.536
	G3	20.50	3.70	1.85	0.127
MONOCYTES	G1	1.25	0.96	0.48	-
	G2	2.00	1.63	0.82	0.486
	G3	2.00	1.41	0.71	0.319
EOSINOPHILS	G1	2.00	0.82	0.41	-
	G2	1.00	0.82	0.41	0.182
	G3	2.00	1.63	0.82	1.000

TABLE: 15.4 INDIVIDUAL ANIMAL VALUES DLC COUNTS (FEMALE ANIMALS)

1ST DAY AFTER EXPOSURE DIFFERENTIAL LEUCOCYTE COUNT (FEMALE ANIMALS)						
MICE NO	LYMPHOCYTE COUNT	NEUTROPHIL	MONOCYTE	EOSINOPHIL	BASOPHIL	TOTAL CELLS
5	82	16	0	2	0	100
6	81	17	1	1	0	100
7	84	11	2	3	0	100
8	83	13	2	2	0	100
MEAN	82.5	14.25	1.25	2	0	100
SD	1.29	2.75	0.96	0.82	0.00	0.00
REF.RANGE	83.5-92.32	6.6-14.25	1.0-4.0	0-1	0	—
13	67	29	2	2	0	100
14	88	9	2	1	0	100
15	84	15	0	1	0	100
16	80	16	4	0	0	100
MEAN	79.75	17.25	2	1	0	100
SD	9.11	8.42	1.63	0.82	0.00	0.00
21	78	19	1	2	0	100
22	79	19	2	0	0	100
23	66	26	4	4	0	100
24	79	18	1	2	0	100
MEAN	75.5	20.5	2	2	0	100
SD	6.35	3.70	1.41	1.63	0.00	0.00

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), SD = Standard Deviation, SE = Standard Error.

TABLE: 16 ANALYSIS RESULTS OF DIFFERENTIAL LEUCOCYTE COUNTS
Table: 16.1 (Male animals) 1st week after exposure

Parameter	GROUP	MEAN	SD	SE	p-value significance
Lymphocytes	G1	77.50	6.61	3.30	-
	G2	70.75	6.13	3.07	0.279
	G3	50.00	11.60	5.80	0.031*
Neutrophils	G1	21.00	6.58	3.29	-
	G2	26.00	6.32	3.16	0.369
	G3	48.25	12.31	6.16	0.037*
Monocytes	G1	1.00	0.82	0.41	-
	G2	0.25	0.50	0.25	0.215
	G3	1.00	0.82	0.41	1.000
Eosinophils	G1	0.50	0.58	0.29	-
	G2	3.00	1.83	0.91	0.063
	G3	0.75	0.96	0.48	0.638

Table: 16.2 Individual Animal Values DLC counts (Male Animals)

1st week after repetative exposure DLC counts (Male animals)						
Mice No	Lymphocyte count	Neutrophil	Monocyte	Eosinophil	Basophil	Total cells
1	69	29	2	0	0	100
2	77	22	0	1	0	100
3	79	20	1	0	0	100
4	85	13	1	1	0	100
mean	77.5	21	1	0.5	0	100
SD	6.61	6.58	0.82	0.58	0.00	0.00
9	69	30	0	1	0	100
10	74	24	0	2	0	100
11	77	18	1	4	0	100
12	63	32	0	5	0	100
mean	70.75	26	0.25	3	0	100
SD	6.13	6.32	0.50	1.83	0.00	0.00
17	45	53	1	1	0	100
18	67	30	1	2	0	100
19	47	53	0	0	0	100
20	41	57	2	0	0	100
mean	50*	48.25*	1	0.75	0	100
SD	11.60	12.31	0.82	0.96	0.00	0.00

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), SD = Standard Deviation, SE = Standard Error, DLC = differential Leucocyte Count, * p ≤ 0.05.

TABLE: 17-REPEATED TREATMENT BIOCHEMICAL TEST (Male)

Table: 17.1- Biochemical Test (Male)

	GROUP	MEAN	SD	SE	p-value significance
GLUCOSE	G1	145.85	15.38	8.88	-
	G2	152.95	13.69	7.91	0.651
	G3	149.65	8.03	4.64	0.756
ALT	G1	53.52	21.11	14.22	-
	G2	43.91	19.19	11.08	0.473
	G3	47.16	19.76	11.41	0.721
AST	G1	109.36	57.89	8.49	-
	G2	59.76	18.95	10.94	0.965
	G3	61.17	13.62	7.87	0.917
CRE	G1	0.45	0.41	0.23	-
	G2	0.20	0.03	1.93	0.371
	G3	0.19	0.03	1.60	0.369
BUN	G1	18.77	1.15	0.66	-
	G2	17.64	2.49	1.44	0.275
	G3	16.67	2.31	1.34	0.270
ALB	G1	2.61	0.28	0.16	-
	G2	2.96	0.15	0.01	0.057
	G3	2.62	0.30	0.18	0.919
CHE	G1	3111	195.61	-	-
	G2	3554	741.68	-	0.465
	G3	3616.25	633.04	-	0.314

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), ALT = alanine aminotransferase, AST = aspartate aminotransferase, CRE = creatinine, BUN = blood urea nitrogen, ALB = albumin, CHE = choline esterase, SD = Standard Deviation, SE = Standard Error.

CHOLINESTRASE ASSAYS

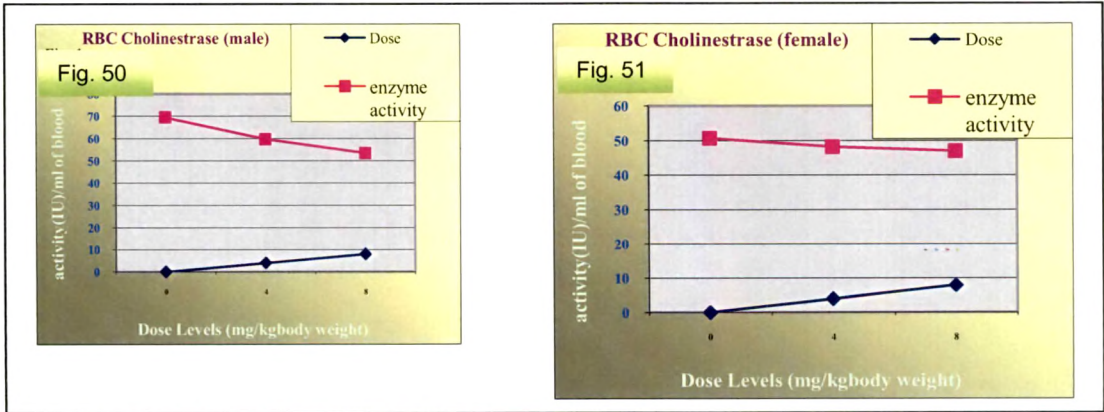


TABLE: 17- REPEATED TREATMENT BIOCHEMICAL TEST (MALE)

Table: 17.2 BIOCHEMICAL TEST (MALE)

REPEATED TREATMENT BIOCHEMICAL TEST (MALE)							
Animal No.	Glucose	ALT	AST	CRE	BUN	ALB	CHE
1	136	28.3	40.63	0.31	19.67	2.62	3332
2	161.1	75.65	151.63	0.18	19.32	2.83	2816
3	160.5	36.99	65.39	0.15	16.8	2.84	3063
4	125.8	73.12	179.79	1.14	19.3	2.16	3233
MEAN	145.85	53.515	109.36	0.445	18.7725	2.6125	3111
SD	15.38	21.11	57.89	0.41	1.15	0.28	195.61
Ref. Range-4th Wk.	132.7± 20.7	89.9±34.1	92.1±36.7	0.2±0.0	19.7±2.8	2.4±0.1	—
Ref. Range-12th wk	106.94-161.06	32.86-42.24	43.78-106.22	0.12-0.30	18.43-23.37	—	—
9	149.9	24.13	44.12	0.18	18.32	3.15	3104
10	164.5	25.37	40.89	0.16	20.07	2.99	4482
11	131.8	61.73	87.98	0.25	13.47	2.98	4030
12	165.6	64.4	66.04	0.2	18.69	2.73	2600
MEAN	152.95	43.9075	59.7575	0.1975	17.6375	2.9625	3554
SD	13.69	19.19	18.95	0.03	2.49	0.15	741.68
Ref. Range-4th Wk.	132.7± 20.7	89.9±34.1	92.1±36.7	0.2±0.0	19.7±2.8	2.4±0.1	—
Ref. Range-12th wk	106.94-161.06	32.86-42.24	43.78-106.22	0.12-0.30	18.43-23.37	—	—
17	162	80.09	78.36	0.18	19.73	2.83	3494
18	140.6	34.82	60.53	0.24	15.85	2.91	3617
19	151	44.36	65.32	0.17	17.64	2.62	4566
20	145	29.36	40.45	0.18	13.44	2.13	2788
MEAN	149.65	47.1575	61.165	0.1925	16.665	2.6225	3616.25
SD	8.03	19.76	13.62	0.03	2.31	0.30	633.04
Ref. Range-4th Wk.	132.7± 20.7	89.9±34.1	92.1±36.7	0.2±0.0	19.7±2.8	2.4±0.1	—
Ref. Range-12th wk	106.94-161.06	32.86-42.24	43.78-106.22	0.12-0.30	18.43-23.37	—	—

Note: ALT = alanine aminotransferase, AST = aspartate aminotransferase,
CRE = creatinine, BUN = blood urea nitrogen, ALB = albumin,
CHE = choline esterase, SD = Standard Deviation.

Table: 17 REPEATED TREATMENT BIOCHEMICAL TEST
Table: 17.3 BIOCHEMICAL TEST (FEMALE)

PARAMETERS	GROUP	MEAN	SD	SE	p-value significance
GLUCOSE	G1	123.73	25.64	14.81	-
	G2	140.63	12.65	7.31	0.41
	G3	178.63	12.32	7.11	0.06
ALT	G1	40.42	6.07	3.51	-
	G2	27.44	5.68	3.28	0.026
	G3	28.81	4.74	2.73	0.07*
AST	G1	70.57	9.15	5.28	-
	G2	54.47	5.7	3.29	0.09
	G3	52.83	4.19	2.42	0.10
CRE	G1	0.28	0.07	4.31	-
	G2	0.21	0.05	2.78	0.37
	G3	0.15	0.03	1.47	0.039*
BUN	G1	19.66	4.43	2.56	-
	G2	14.98	2.15	1.24	0.18
	G3	16.88	1.35	0.78	0.40
ALB	G1	3.31	0.12	6.868E	-
	G2	3.16	0.39	0.23	0.58
	G3	3.11	0.32	0.19	0.36
CHE	G1	5439.75	998.07	576.23	-
	G2	5525.00	670.24	386.96	0.89
	G3	5748.75	446.16	257.59	0.54

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), ALT = alanine aminotransferase, AST = aspartate aminotransferase, CRE = creatinine, BUN = blood urea nitrogen, ALB = albumin, SD = Standard Deviation, SE = Standard Error, CHE = choline esterase, *p ≤ 0.05.

Table: 17 REPEATED TREATMENT BIOCHEMICAL TEST
Table: 17.4 BIOCHEMICAL TEST (FEMALE)

Biochemical Analysis Individual Animals Data (Female)							
Animal No.	Glucose	ALT	AST	CRE	BUN	ALB	CHE
5	135.8	47.02	58.86	0.34	24.8	3.13	5649
6	84.9	45.86	65.14	0.15	19.24	3.3	6944
7	119.5	33.51	75.84	0.31	21.82	3.46	4907
8	154.7	35.27	82.42	0.31	12.78	3.35	4259
MEAN	123.725	40.415	70.565	0.2775	19.66	3.31	5439.75
SD	25.64	6.07	9.15	0.07	4.43	0.12	998.07
Ref. Range-4th Wk.	80.9±14.92	85.3±7.9	88.2±20.32	0.23±0.08	20.8±3.7	2.85±0.27	—
Ref. Range-12th wk	133.44-177.56	34.11-98.99	68.98-103.02	0.1-0.23	15.45-18.35	—	—
13	153.4	37.05	58.63	0.16	12.64	3.32	6303
14	135.1	23.49	48.05	0.25	17.29	3.45	5458
15	151.5	23.01	61.47	0.27	16.94	3.38	5853
16	122.5	26.2	49.74	0.17	13.04	2.48	4486
MEAN	140.625	27.4375	54.4725	0.2125	14.9775	3.1575	5525
SD	12.65	5.68	5.70	0.05	2.15	0.39	670.24
Ref. Range-4th Wk.	80.9±14.92	85.3±7.9	88.2±20.32	0.23±0.08	20.8±3.7	2.85±0.27	—
Ref. Range-12th wk	133.44-177.56	34.11-98.99	68.98-103.02	0.1-0.23	15.45-18.35	—	—
21	159.5	23.64	59.87	0.14	15.28	3.13	5280
22	182.8	35.75	51.61	0.12	18.95	3.27	6480
23	193.6	30.51	50.95	0.19	17.06	3.45	5557
24	178.6	25.35	48.88	0.15	16.24	2.58	5678
MEAN	178.625	28.8125*	52.8275	0.15*	16.8825	3.1075	5748.75
SD	12.32	4.74	4.19	0.03	1.35	0.32	446.16
Ref. Range-4th Wk.	80.9±14.92	85.3±7.9	88.2±20.32	0.23±0.08	20.8±3.7	2.85±0.27	—
Ref. Range-12th wk	133.44-177.56	34.11-98.99	68.98-103.02	0.1-0.23	15.45-18.35	—	—

Note: ALT = alanine aminotransferase, AST = aspartate aminotransferase, CRE = creatinine, BUN = blood urea nitrogen, SD = Standard Deviation, ALB = albumin, CHE = choline esterase, * p ≤ 0.05.

REPEATED DOSE MICRONUCLEUS ASSAY

TABLE 18: PCE TO TOTAL ERYTHROCYTES RATIO

Table: 18. PCE/TOTAL ERYTHROCUTES MALE AND FEMALE ANIMALS

Sex	GROUP	MEAN	SD	SE	p-value significance	Regration
Male	G1	0.60	3.14	1.57	-	Y = 0.0024x + 0.6128 r = 0.49
	G2	0.64	3.90	1.95	0.318	
	G3	0.62	1.50	7.52	0.438	
Female	G1	0.66	5.75	2.87	-	Y = -0.0163x + 0.6779 r = 0.87
	G2	0.65	4.83	2.41	0.960	
	G3	0.53	5.69	2.84	0.085	

Note: G1 – G3 are Control (G1) and treatment groups (G2, G3), PCE = Polychromatic Eruthrocytes, SD = Standard Deviations, SE = Standard Error, n = 4.

TABLE: 19. BONE MARROW MICRONUCLEUS FREQUENCY

Table: 19.1 Micronucleus frequency mice bone marrow (Male Animals)

Micronucleus frequency mice bone marrow (Male Animals)							
Animal Number, Sex & Dose group	PCE	MN PCE	NCE	MN NCE	Total Erythrocytes (E)	% MN PCE among total	% MN NCE among total
A no 1-4, Male (0.0) mg/Kg Body weight	2621	3	1888	1	4513	0.066	0.022
	2581	3	1827	1	4412	0.068	0.023
	2666	3	1814	2	4485	0.067	0.045
	2527	1	1369	0	3897	0.026	0
Mean	2598.8	2.5	1724.5	1	4326.8	0.057	0.022
SD	59.11	1	239.19	0.82	289.65	0.021	0.018
A no 9-12, Male (4.0) mg/Kg Body weight	2740	12	1232	1	3985	0.301	0.025
	2542	4	1332	2	3880	0.103	0.052
	2507	7	1547	0	4061	0.172	0
	2528	6	1661	3	4198	0.143	0.071
Mean	2579.3	7.25	1443	1.5	4031	0.18	0.037
SD	108.13	3.4	195.94	1.29	133.8	0.086	0.031
A no 17-20, Male (8.0) mg/Kg Body weight	2548	9	1562	4	4123	0.218	0.097
	3210	16	1919	3	5148	0.311	0.058
	2615	15	1483	5	4118	0.364	0.121
	2940	12	1941	4	4897	0.245	0.082
Mean	2828.3	13	1726.3	4	4571.5	0.285**	0.09**
SD	306.72	3.16	237.64	0.82	530.76	0.066	0.027

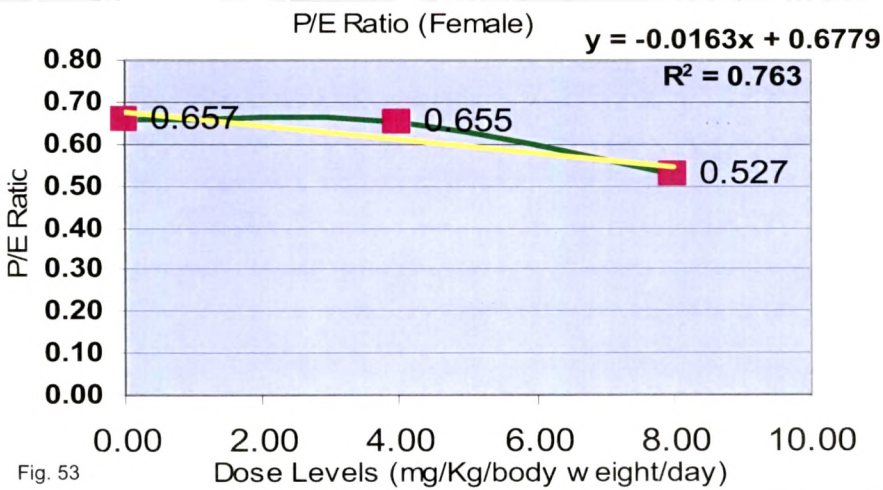
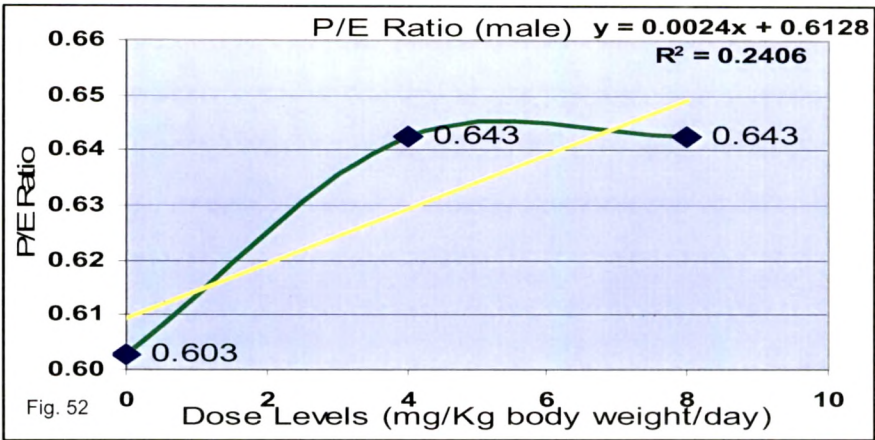
Note: PCE = Polychromatic Erythrocytes, NCE = Normochromatic Erythrocytes
MN PCE = Micronucleated Polychromatic Erythrocytes, % = Percent,
MN NCE = Micronucleated Normochromatic Erythrocytes, SD = Standard Deviations,
SE = Standard Error, n = 4, ** = Values are significant at 0.001.

Table 19.2 Micronucleus frequency mice bone marrow (Female Animals)

Micronucleus frequency mice bone marrow (Female Animals)							
Animal Number, Sex & Dose group	PCE	MN PCE	NCE	MN NCE	Total Erythrocytes (E)	% MN PCE among total	% MN NCE among total
A no 5-8, Female (4.0) mg/Kg Body weight	2780	2	1123	0	3905	0.051	0
	2505	2	1150	1	3658	0.055	0.027
	2658	2	1930	3	4593	0.044	0.065
	2721	6	1449		4176	0.144	0
Mean	2666	3	1413	1.33	4083	0.073	0.023
SD	118.33	2	374.99	1.53	400.44	0.047	0.031
A no 13-16, Female (4.0) mg/Kg Body weight	2581	12	1387	1	3981	0.301	0.025
	2565	10	1295	1	3871	0.258	0.026
	2805	11	1149	3	3968	0.277	0.076
	2511	3	1728	1	4243	0.071	0.024
Mean	2615.5	9	1389.75	1.5	4015.75	0.227	0.038
SD	129.83	4.08	245.87	1	159.25	0.106	0.025
A no 21-24, Female (8.0) mg/Kg Body weight	2823	11	3462	6	6302	0.175	0.095
	3423	14	3108	7	6552	0.214	0.107
	2706	10	2248	3	4967	0.201	0.06
	2620	11	1860	3	4494	0.245	0.067
Mean	2893	11.5	2669.50	4.75	5578.75	0.209**	0.082
SD	363	1.73	742.36	2.06	1003.53	0.029	0.022

Note: PCE = Polychromatic Erythrocytes, NCE = Normochromatic Erythrocytes
MN PCE = Micronucleated Polychromatic Erythrocytes, % = Percent
MN NCE = Micronucleated Normochromatic Erythrocytes, SD = Standard Deviations,
SE = Standard Error, n = 4, ** = Values are significant at 0.001.

Data Graphs



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Table: 20 Sperm Morphology Test

Dose mg/Kg body weight	Sperm Morphology											
	Head - Abnormality									Tail - Abnormality		
	N	Total Abnormal Sperm	% Head Ab.	Am.	banana shape	large head	small head	double head	without hook	Coiled	Folded	DT
0	1005	8	0.796	1	0	0	5	0	2	0	0	0
0	1253	5	0.399	1	1	0	0	0	3	1	0	0
0	1003	9	0.897	4	0	2	1	0	2	0	0	0
0	1061	3	0.283	1	0	0	0	0	2	0	0	0
4	1004	16	1.594	3	1	1	5	0	6	3	10	0
4	1046	16	1.530	5	0	2	3	0	6	3	17	0
4	1007	7	0.695	3	0	1	0	0	3	0	12	1
4	1002	16	1.597	6	1	0	1	1	7	1	16	1
8	1003	49	4.885	29	3	4	10	0	3	3	277	0
8	1009	39	3.865	19	4	6	7	0	3	3	176	0
8	1000	36	3.600	16	2	7	6	0	5	62	200	0
8	1001	67	6.693	27	8	9	12	0	11	50	95	0

Note: N = Number of Sperm Examined, % Head Ab. = % Head Abnormality Am. =Amorphous, DT = Double tailed

Table: 20.1

Dose mg/Kg body weight	Total No. of Sperm Examined	Total Abnormal Sperm	Relative fold values	%Abnormality	p- value
0	1080.5	6.25	NA	0.58	NA
4	1014.75	13.75	2.2*	1.36	0.03
8	1006	47.75	7.64**	4.75	0.0011

Note: n = 4, * p≤0.05 and ** = p≤0.01

Table: 21. MICRONUCLEI IN PERIPHERAL BLOOD
Table 21.1: (1ST DAY OF EXPOSURE)

Dose Group	Mice Number & Sex	Im. RBC	Matured RBC	MN RBC	Total RBCs	Ratio of mature RBC	MN RBC		% MN RBC
							Mean	SD	
0.0	1 M	21	1002	1	1024	0.979	1.25	0.50	0.122
	2 M	12	1022	2	1036	0.986			
	3 M	12	1047	1	1060	0.988			
	4 M	13	1017	1	1031	0.986			
	5 F	17	1029	1	1047	0.983	1.25	0.50	0.116
	6 F	8	1019	2	1029	0.99			
	7 F	7	1064	1	1072	0.993			
	8 F	13	1215	1	1229	0.989			
4.0	9 M	5	1020	2	1027	0.993	1.25	0.96	0.123
	10 M	5	1014	0	1019	0.995			
	11 M	10	1026	1	1037	0.989			
	12 M	19	1009	2	1030	0.98			
	13 F	6	1013	1	1020	0.993	1	0.00	0.098
	14 F	10	1024	1	1035	0.989			
	15 F	8	1021	1	1030	0.991			
	16 F	15	1026	1	1042	0.985			
8.0	17 M	7	1004	1	1012	0.992	1.25	1.26	0.117
	18 M	9	1019	3	1031	0.988			
	19 M	15	1223	0	1238	0.988			
	20 M	11	1025	1	1037	0.988			
	21F	12	1058	2	1072	0.987	2.25	0.50	0.213*
	22F	13	1087	2	1102	0.986			
	23F	11	1051	2	1064	0.988			
	24F	15	1038	3	1056	0.983			

Note: Im. RBC = Immature Red Blood Cells, MN RBC = Micronucleated Red Blood Cells, SD = Standard Deviations, n = 4, p ≤ 0.05.

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Table: 21. MICRONUCLEI IN PERIPHERAL BLOOD

Table – 21.2 : (1ST Week after EXPOSURE)

Dose Group	Mice Number & Sex	Immature RBC	Matured RBC	MN RBC	Total RBCs	Ratio of mature RBC	MN RBC		% MN RBC
							Mean	SD	
0.00	1 M	3	1013	0	1016	0.997	0.5	0.58	0.049
	2 M	2	1003	0	1005	0.998			
	3 M	3	1008	1	1012	0.996			
	4 M	6	1021	1	1028	0.993			
	5 F	9	1011	0	1020	0.991	1.25	1.26	0.118
	6 F	1	1186	3	1190	0.997			
	7 F	6	1002	1	1009	0.993			
	8 F	12	1041	1	1054	0.988			
4.0	9 M	6	1019	3	1028	0.991	2	0.82	0.191
	10 M	2	1043	2	1047	0.996			
	11 M	6	1104	1	1111	0.994			
	12 M	4	1033	2	1039	0.994			
	13 F	5	1035	1	1041	0.994	1.5	1.29	0.145
	14 F	5	1015	0	1020	0.995			
	15 F	2	1022	2	1026	0.996			
	16 F	7	1070	3	1080	0.991			
8.0	17 M	2	1182	1	1185	0.997	2	2.16	0.180
	18 M	23	1076	5	1104	0.975			
	19 M	3	1138	0	1141	0.997			
	20 M	10	1043	2	1055	0.989			
	21F	4	1007	4	1015	0.992	3.25	0.96	0.313
	22F	6	1060	2	1068	0.993			
	23F	9	1065	4	1078	0.988			
	24F	12	1019	3	1034	0.985			

Note: Im. RBC = Immature Red Blood Cells, MN RBC = Micronucleated Red Blood Cells, SD = Standard Deviations, n = 4.

Table: 21. MICRONUCLEI IN PERIPHERAL BLOOD
Table – 21.3: (2nd Week after EXPOSURE)

Dose Group	Mice Number & Sex	Immature RBC	Matured RBC	MN RBC	Total RBCs	MN RBC		MN RBC
						Mean	SD	
0.00	1 M	4	1030	1	1035	1.25	0.96	0.117
	2 M	5	1154	0	1159			
	3 M	5	1012	2	1019			
	4 M	4	1065	2	1071			
	5 F	8	1047	4	1059	2.5	1.29	0.230
	6 F	4	1068	3	1075			
	7 F	11	1105	1	1117			
	8 F	3	1120	2	1125			
4.0	9 M	10	1035	3	1048	1	1.41	0.095
	10 M	8	1040	0	1048			
	11 M	4	1149	1	1154			
	12 M	7	1001	0	1008			
	13 F	11	1043	3	1057	1.75	1.50	0.169
	14 F	9	1071	0	1080			
	15 F	26	1006	3	1035			
	16 F	20	1018	1	1039			
8.0	17 M	17	1020	2	1039	4	1.83	0.394*
	18 M	18	1046	5	1069			
	19 M	46	995	6	1047			
	20 M	23	1003	3	1029			
	21F	10	1005	2	1017	2.25	1.89	0.216
	22F	13	1036	5	1054			
	23F	18	1007	1	1026			
	24F	24	1115	1	1140			

Note: Im. RBC = Immature Red Blood Cells, MN RBC = Micronucleated Red Blood Cells, SD = Standard Deviations, n = 4, p ≤ 0.05.

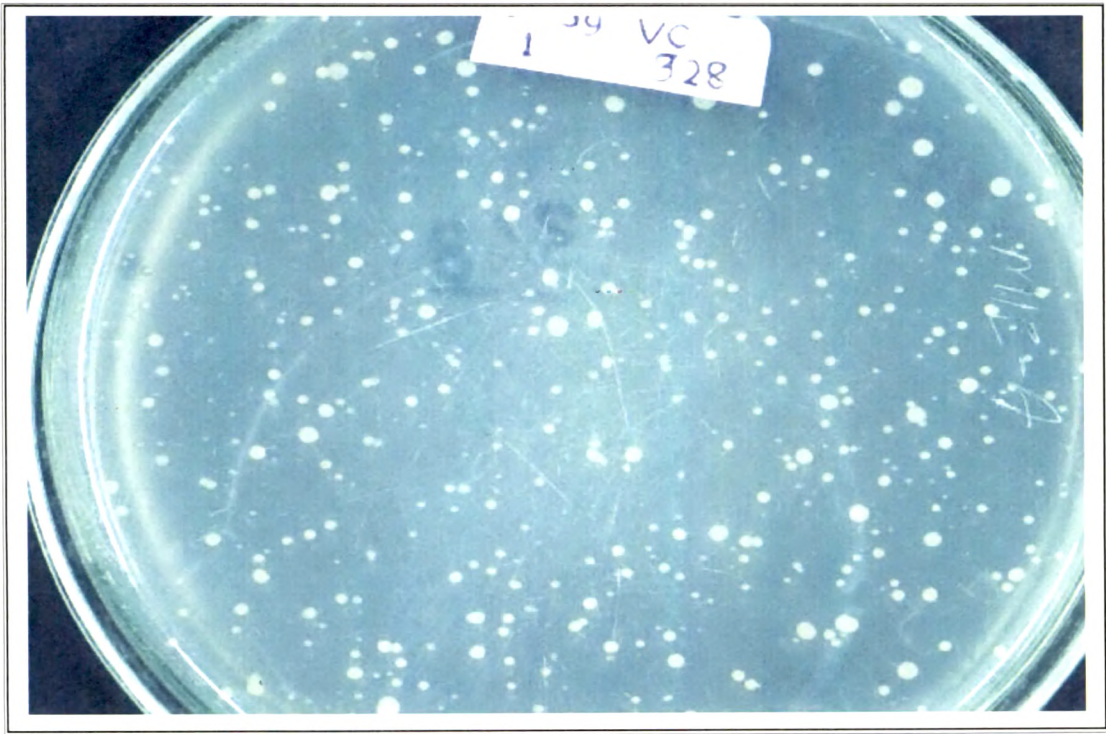


Figure 54: Ames Colonies; vehicle control group showing less number of colonies in the absence of metabolic activation



Fig: 55

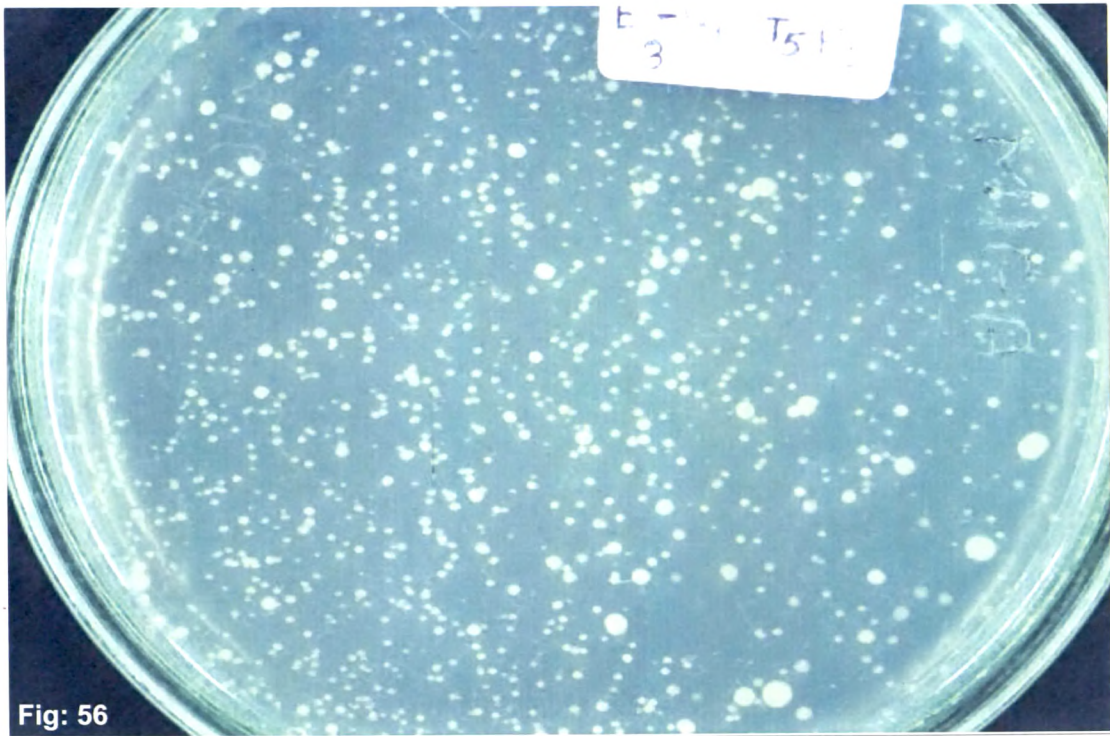


Fig: 56

Figure 55 & 56: Ames Colonies; Strain TA 102 showing a positive response in Endosulfan treated plates in the absence of metabolic activation.

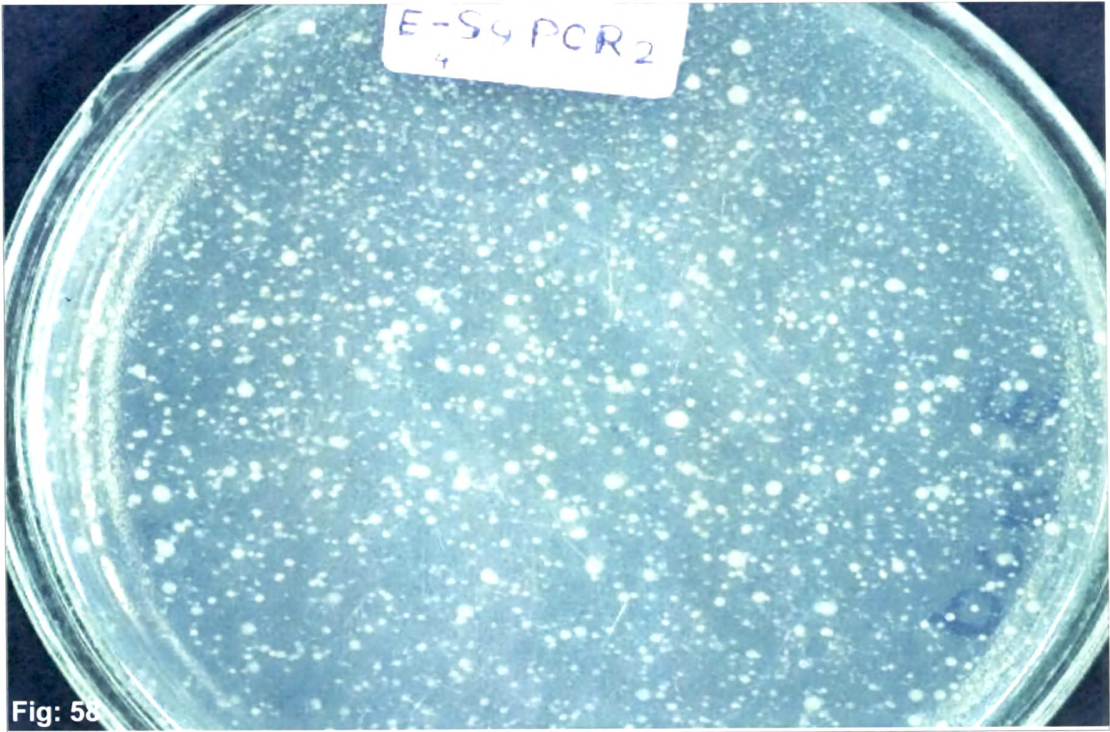
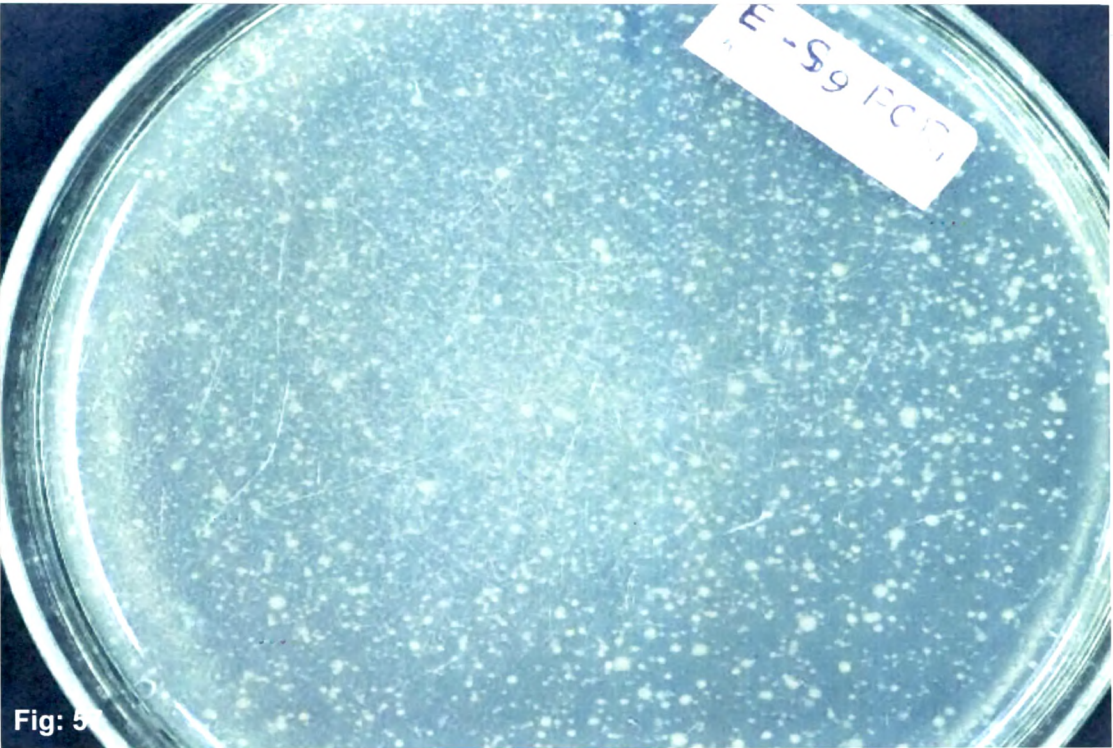


Figure 57 & 58: Ames Colonies;Strain TA 102 showing a positive response in plates treated with Mitomycin C (0.5 µg/plate)

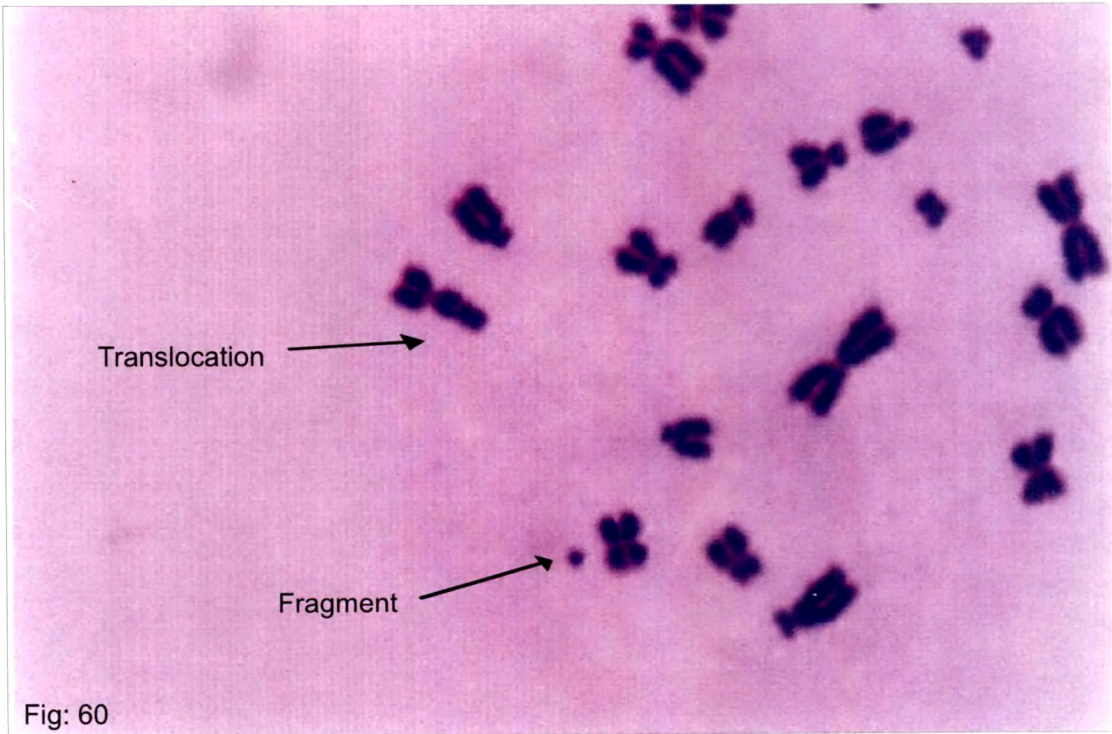
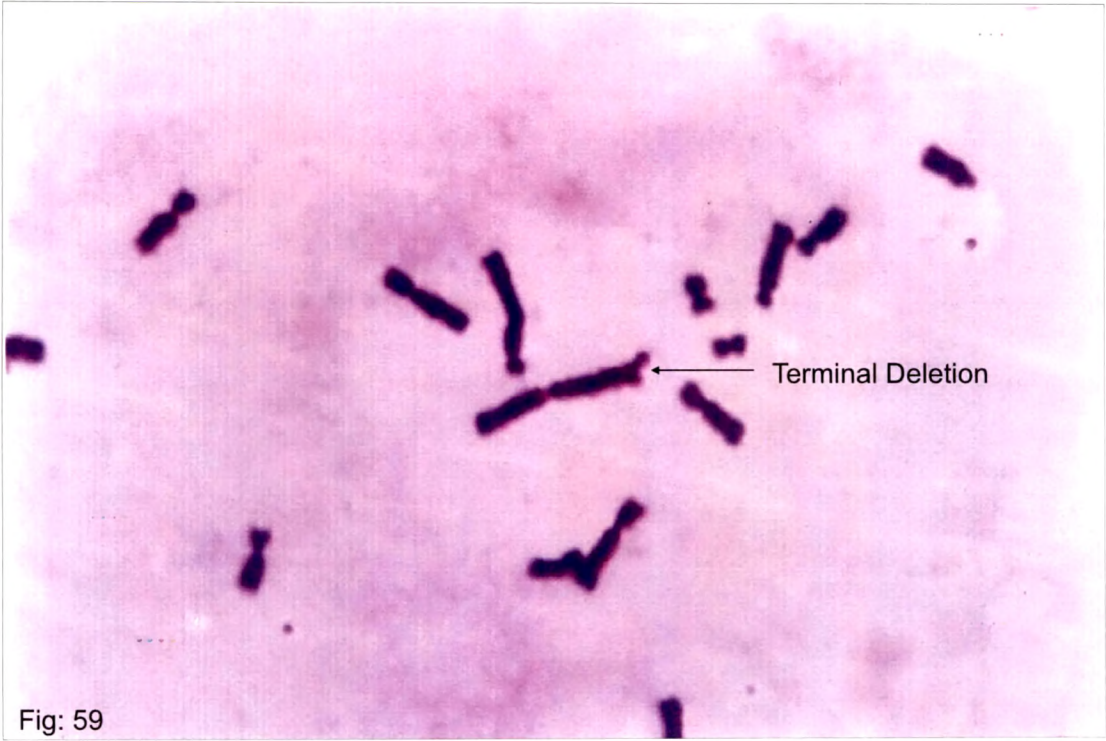


Figure 59 & 60: Chromosomal Aberration in Endosulfan treated groups.

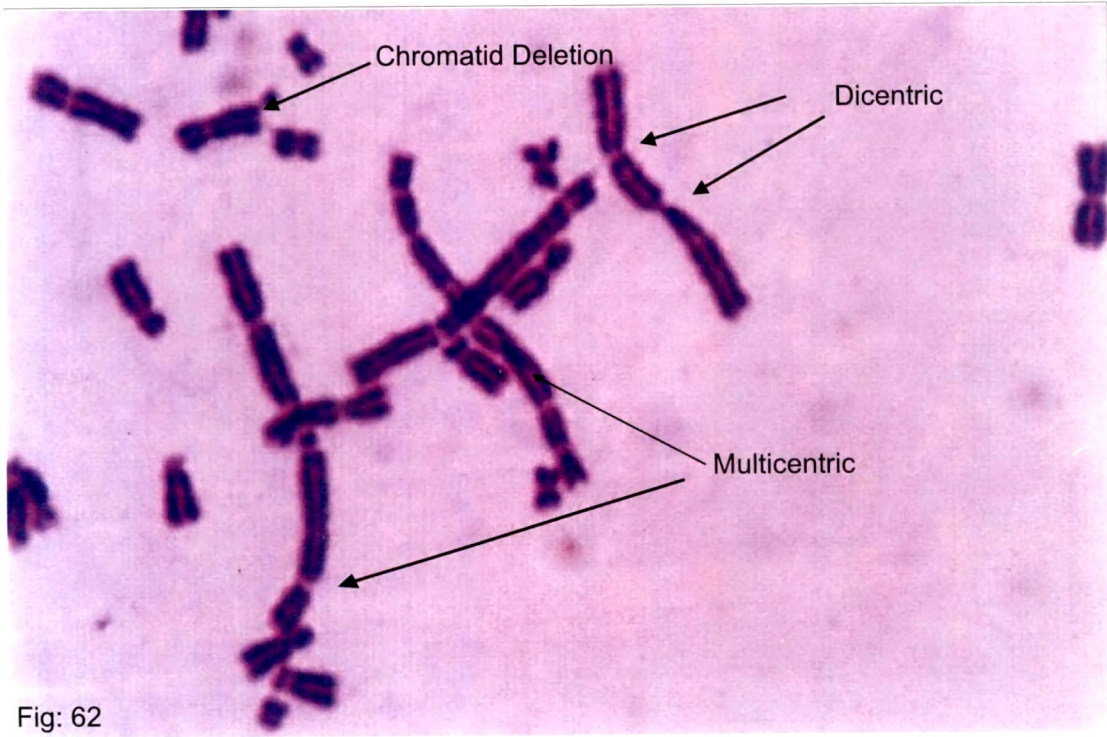
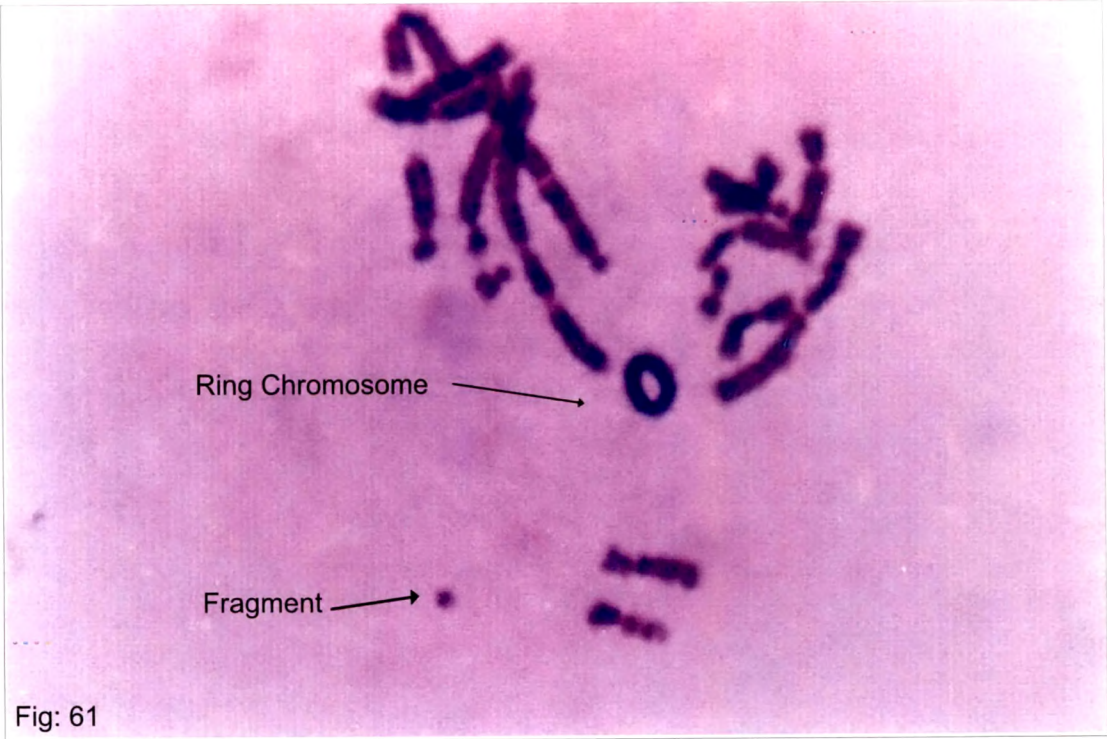


Figure 61 & 62: Chromosomal Aberrations in Endosulfan treated groups .

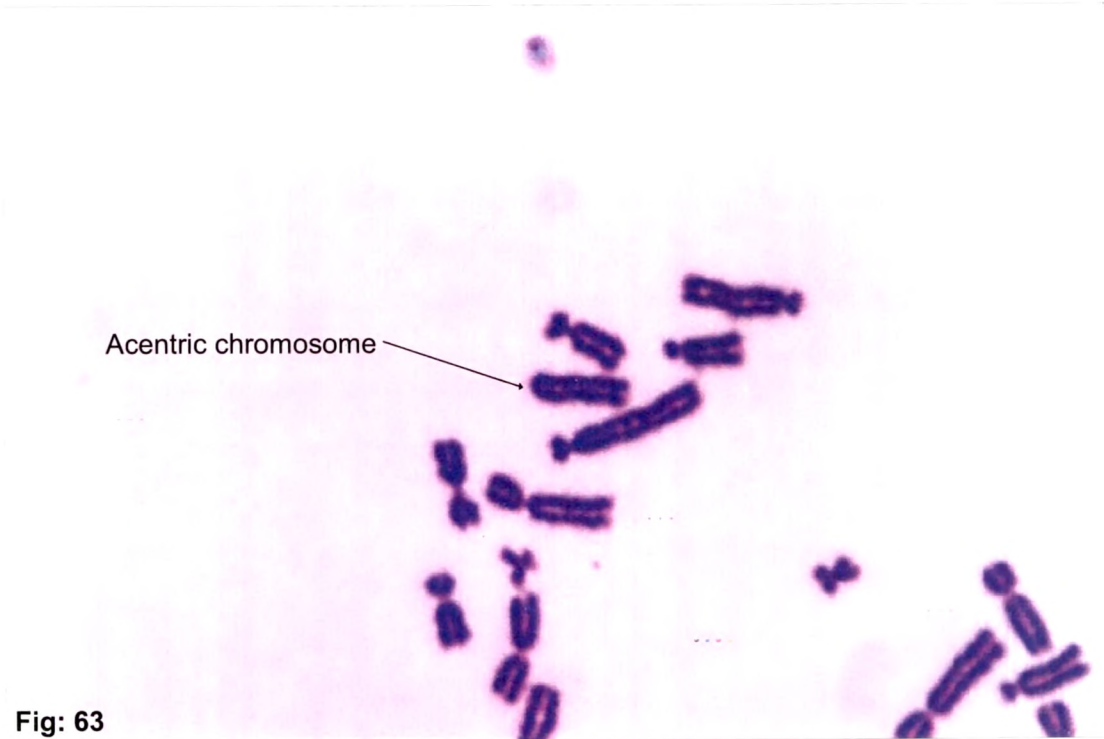


Fig: 63

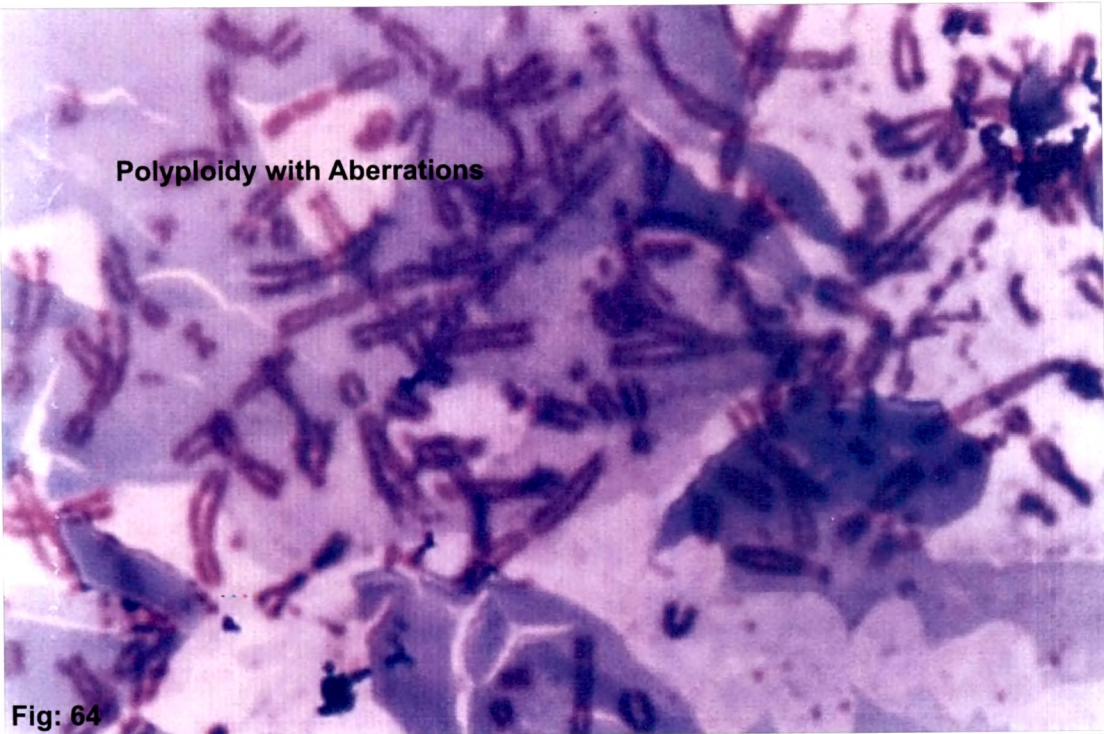


Fig: 64

Figure 63 & 64: Chromosomal Aberrations in Endosulfan treated groups.

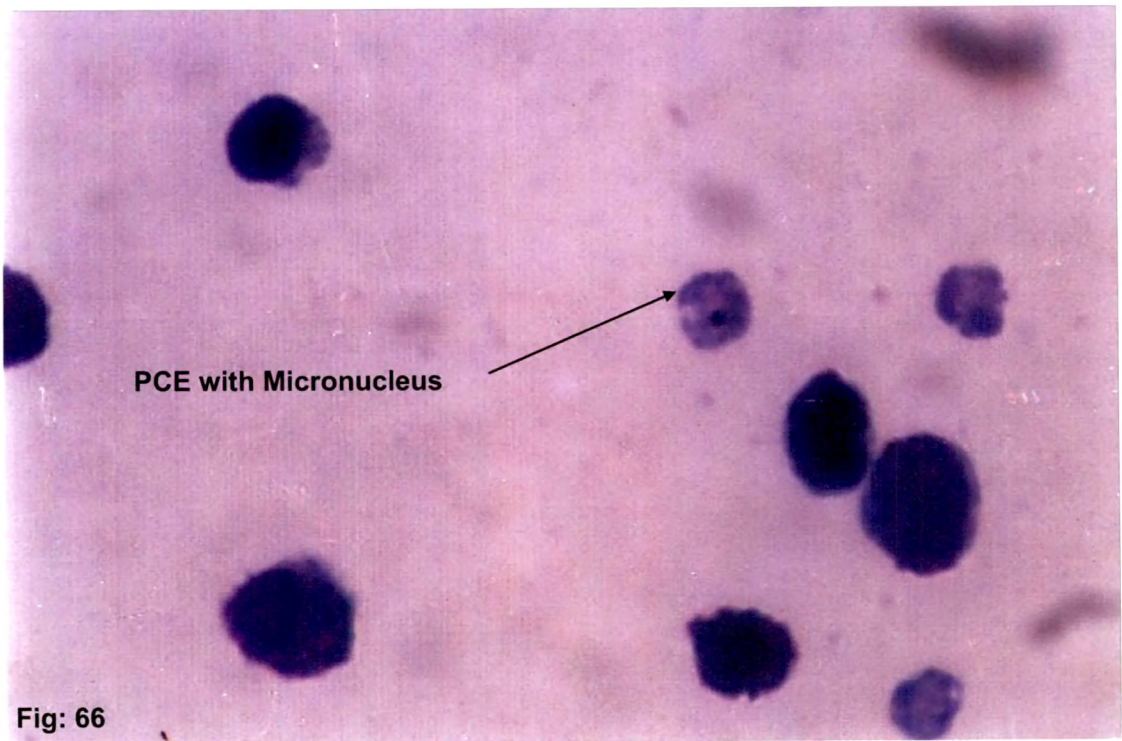
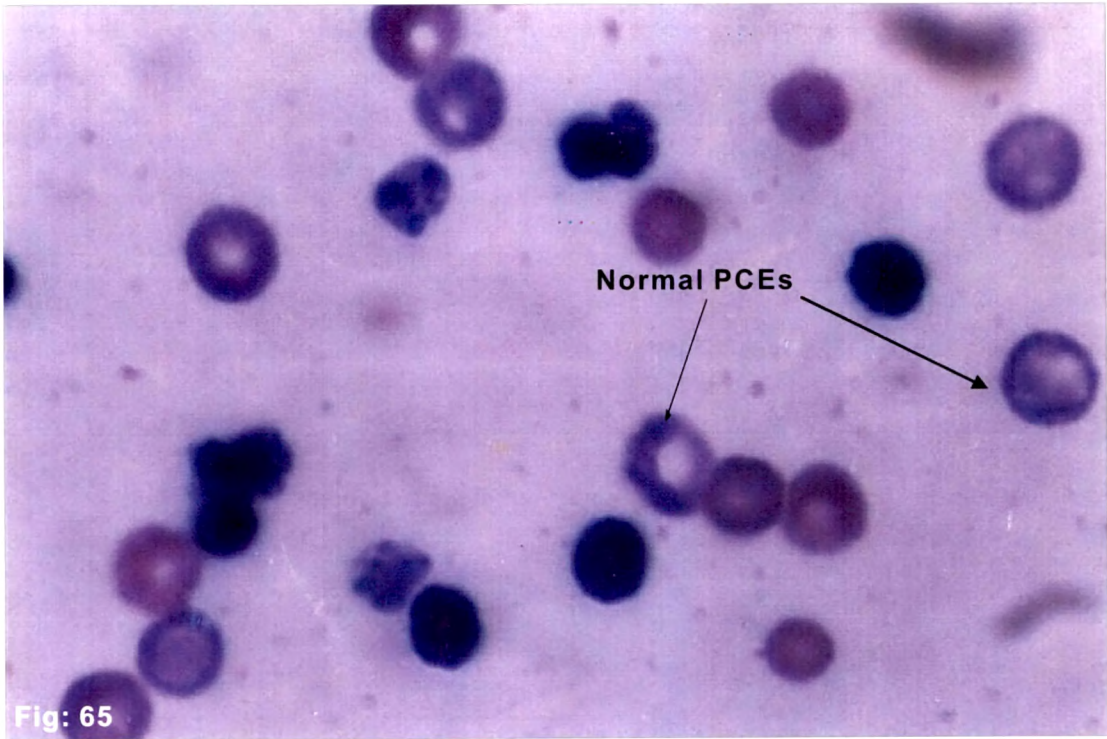


Figure 65 & 66: Polychromatic and Normochromatic Erythrocytes(PCE & NCE) in bone marrow. **Figure 23:** Micronucleated Polychromatic Erythrocytes.

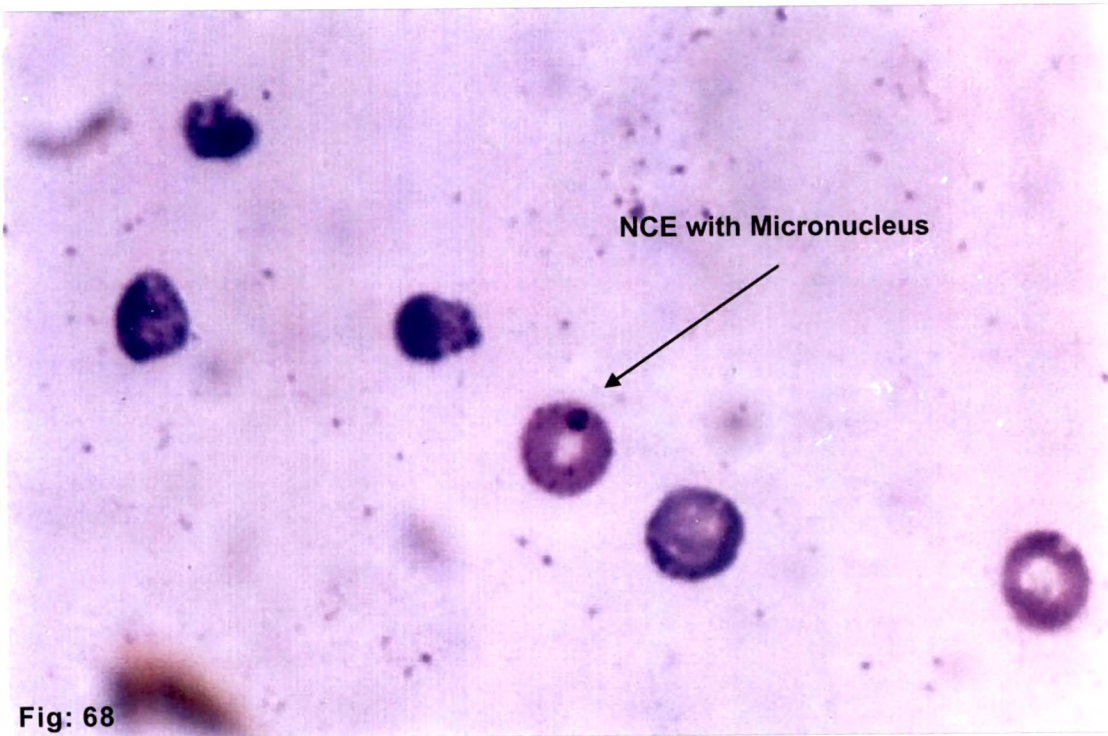
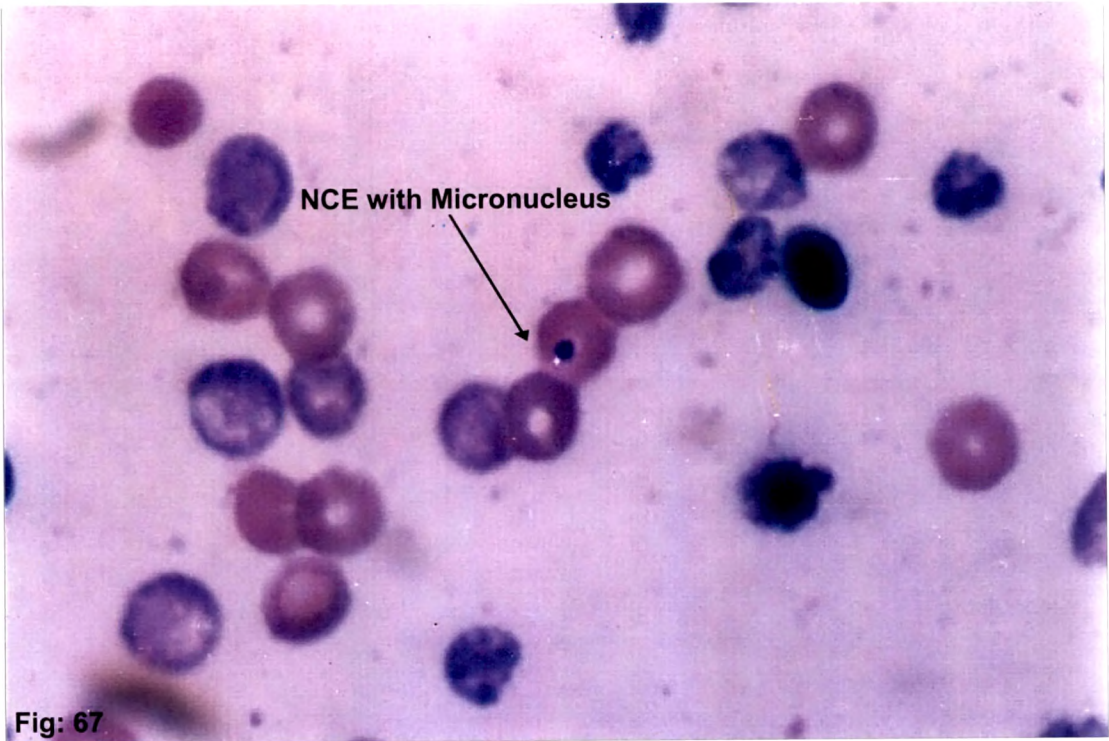


Figure 67 & 68: Normochromatic Erythrocytes (NCEs)with micronucleus.

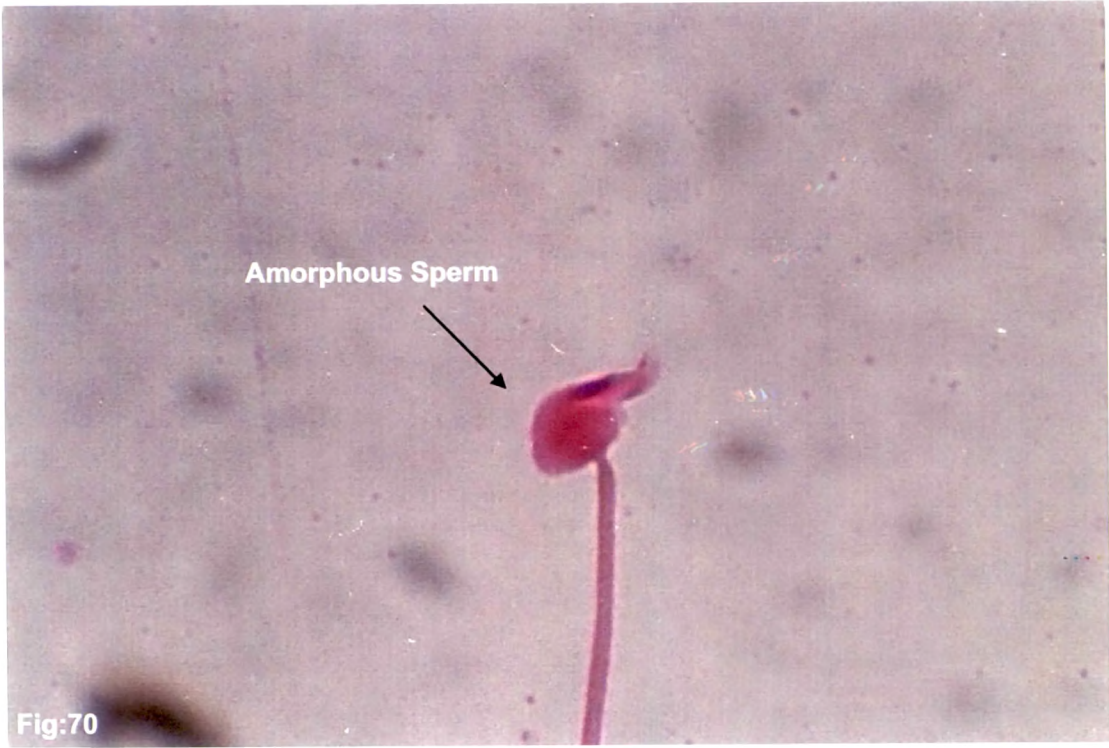
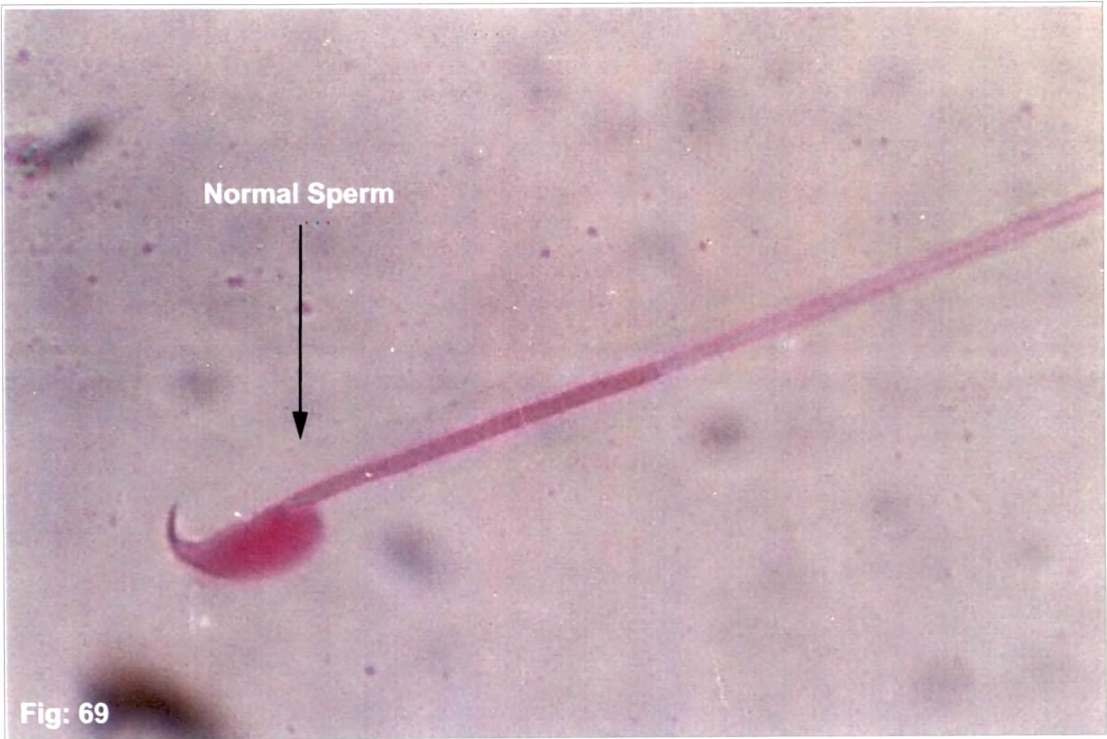


Figure 69 & 70: Sperm Morphology

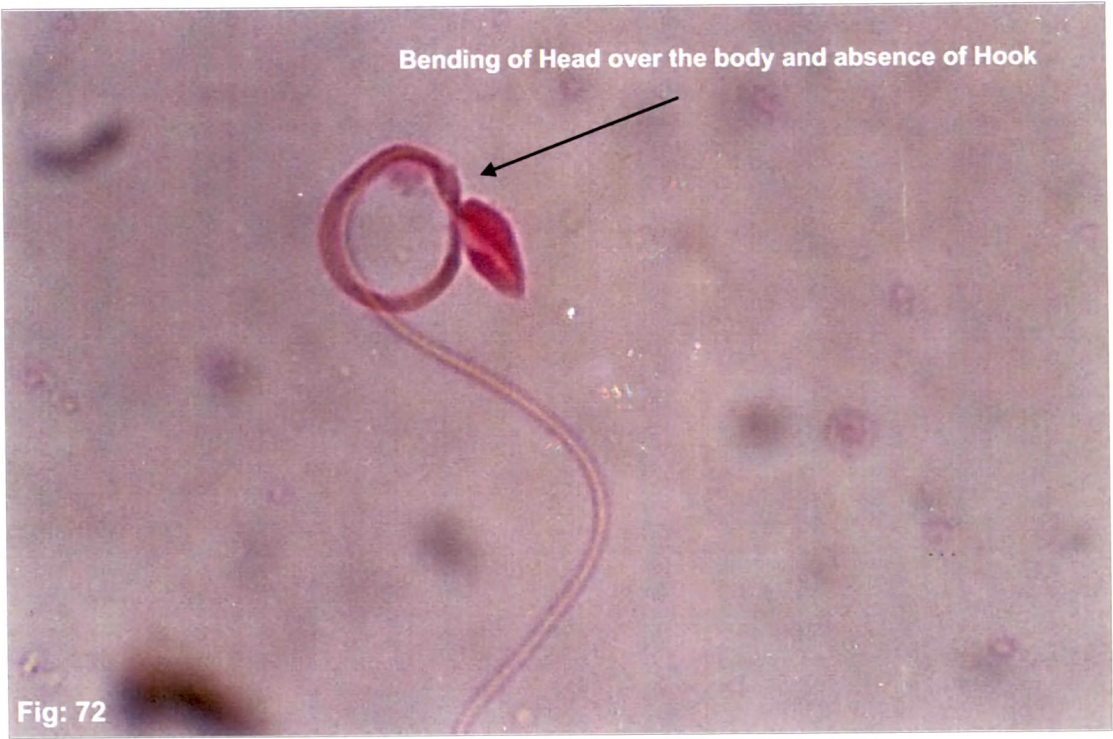


Figure 71 & 72: Abnormal Sperms

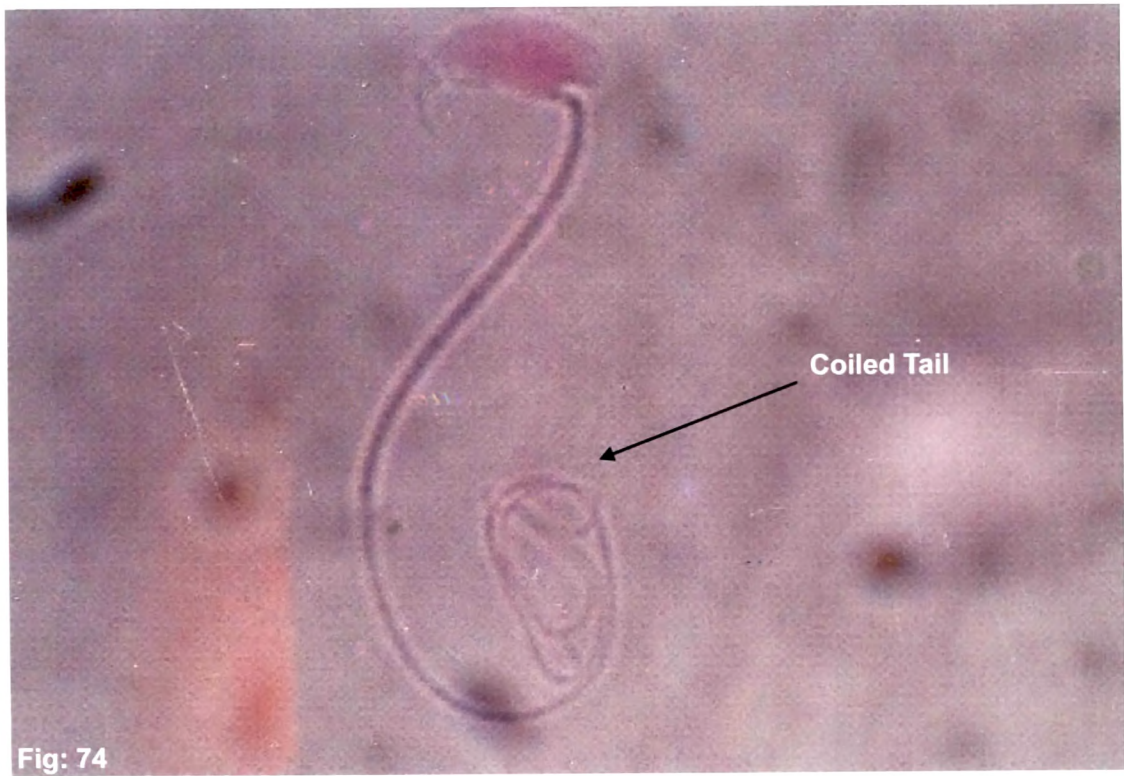
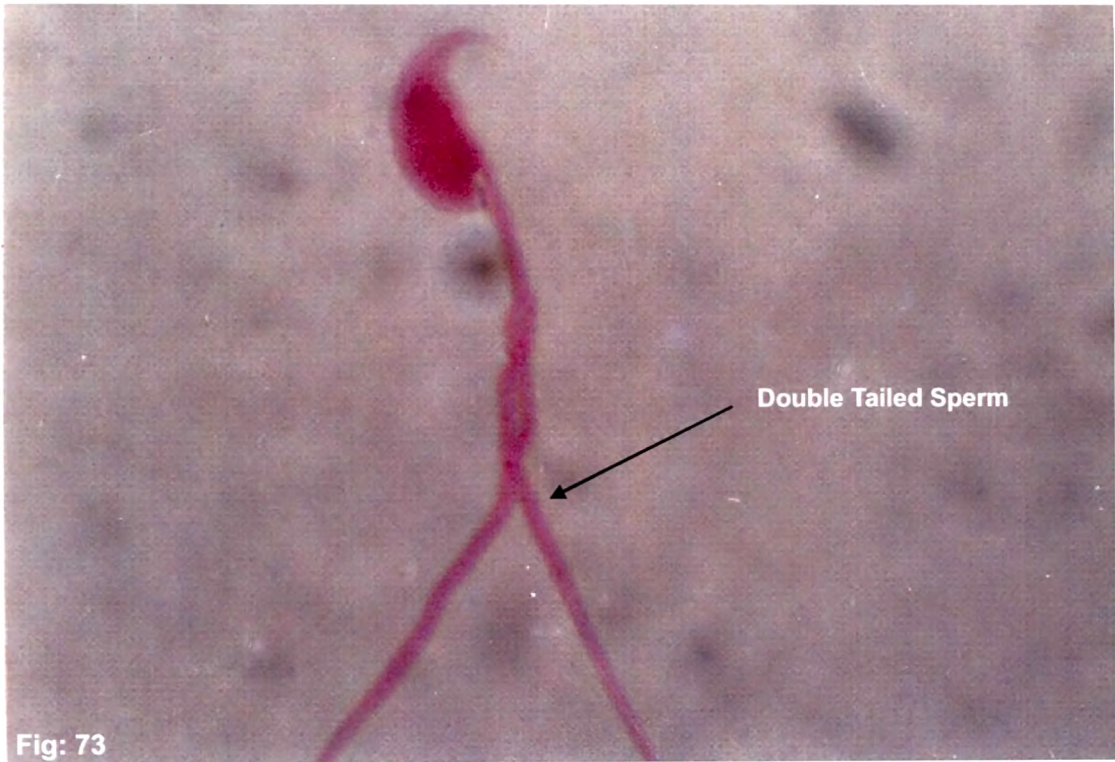


Figure 73 & 74 : Tail Abnormalities in Sperms

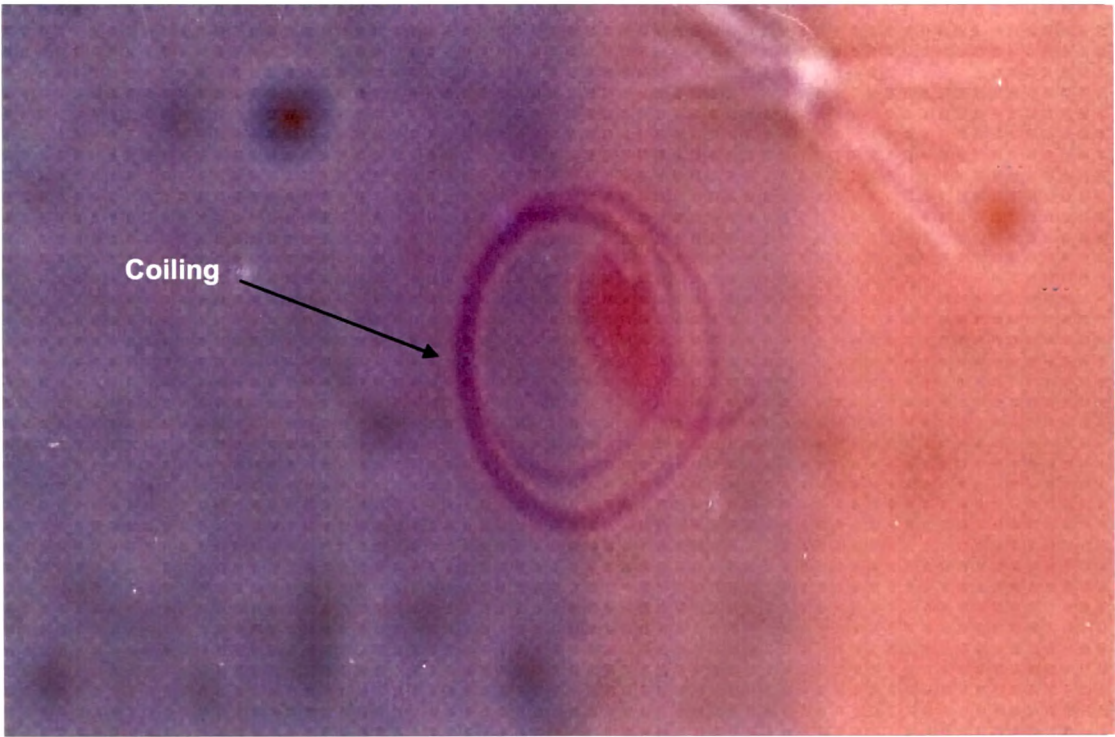


Figure 75: Abnormal Coiling in Sperms.