Chemail: A Endpoint: PCT Tail DNA Transformation: id Organ: Stomach
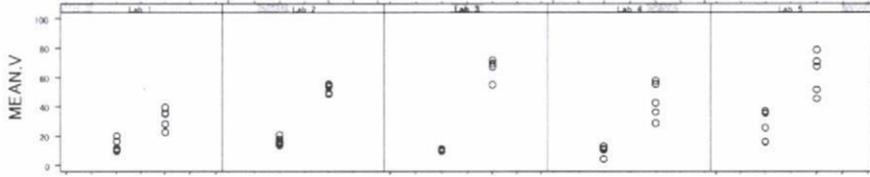

Fig. 2-SA

Table 2-SA

Chemical: A
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Vehicle					EMS			
		N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
1	id(MEAN_V)	5	13.6	4.4	9.9	20.1	5	32.2	6.8	22.6	39.8
2	id(MEAN_V)	5	16.8	2.8	13.8	20.9	5	52.6	3.2	48.8	55.6
3	id(MEAN_V)	5	10.8	0.7	9.7	11.4	5	66.6	6.7	54.9	72
4	id(MEAN_V)	5	10.1	3.3	4.4	12.9	5	44.2	12.3	28.8	57.5
5	id(MEAN_V)	5	26	10.1	15.9	37	5	62.9	13.8	45.7	78.9

Chemical: A Endpoint: PCT Tail DNA Transformation: id Organ: Stomach
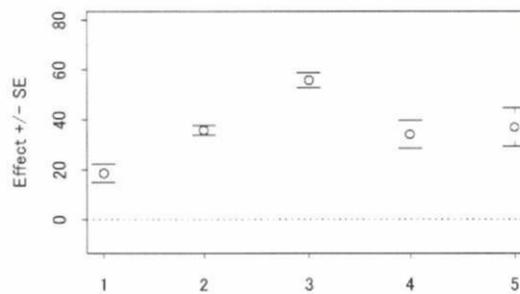

Fig. 3-SA

Chemical: A
Organ: Stomach Parameter: PCT tail DNA
Transformation: id Link: id

Table 3-SA

Lab.	Variable	Effect	SE	L95%	U95%	Method						
						Pooled			Satterthwaite			
						df	P-value	J T	df	P-value	J T
1	id(MEAN_V)	18.58	3.63	10.22	26.95	8	9.00E-04	1	6.85	0.001	1
2	id(MEAN_V)	35.83	1.91	31.43	40.23	8	6.70E-08	1	7.83	8.60E-08	1
3	id(MEAN_V)	55.78	3.03	48.8	62.76	8	7.70E-08	1	4.09	4.30E-05	1
4	id(MEAN_V)	34.09	5.7	20.94	47.23	8	3.30E-04	1	4.57	0.003	1
5	id(MEAN_V)	36.9	7.64	19.29	54.52	8	0.001	1	7.34	0.002	1

For the calculation for CIs the pooled method were used.

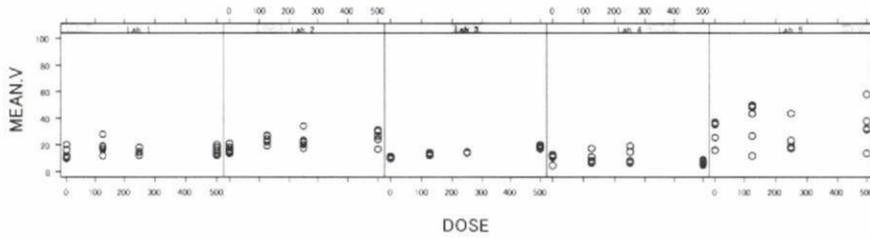# Chemail: A Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 4-SA

Table 4-SA

Chemical: A
Organ: Stomach Endpoint: Tail length
Transformation: id Link: id

Lab.	Variable	Vehicle						Low						Medium						High					
		Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max
1	id(MEAN.V)	0	5	0	0	0	0.1	125	5	0.1	0	0	0.1	250	5	0.1	0	0	0.1	500	5	0.1	0	0	0.1
2	id(MEAN.V)	0	5	84.8	12.1	71.3	104	125	5	81.6	9.3	73.1	95.8	250	5	87.3	8.9	77.4	97.6	500	5	75.7	11.4	60	88.3
3	id(MEAN.V)	0	5	65.2	12.3	51.3	80.2	125	5	92.2	12.8	75.4	105	250	5	79	20.7	49.3	103	500	5	102	19.1	71.5	119
4	id(MEAN.V)	0	5	35.8	5.2	29.8	41.3	125	5	40.7	7.7	32.9	51.9	250	5	41.8	12.7	26.7	55.2	500	5	33.4	3.4	29.5	37.6
5	id(MEAN.V)	0	5	54.6	18.2	34.7	83.9	125	5	59.4	10.9	49.4	77.9	250	5	55.2	16.9	39.7	83	500	5	63.3	16.9	43.1	88.6

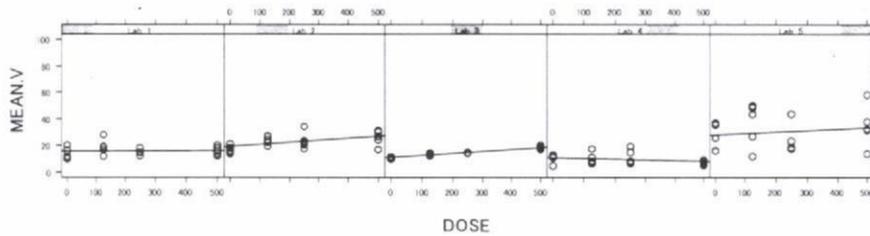# Chemical: A Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 5-SA

Table 5-SA

Chemical: A
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Slope	SE	L95%	U95%	P-value	J_LI
1	id(MEAN V)	0.0015	0.005	−0.01	0.0127	0.789	0
2	id(MEAN V)	0.0151	0.006	0.0024	0.0277	0.022	1
3	id(MEAN V)	0.0142	0.001	0.0122	0.0163	2.00E-11	1
4	id(MEAN V)	−0.006	0.005	−0.016	0.0043	0.247	0
5	id(MEAN V)	0.0106	0.017	−0.025	0.0459	0.535	0

Chemical: A Endpoint: PCT Tail DNA Transformation: id Organ: Stomach
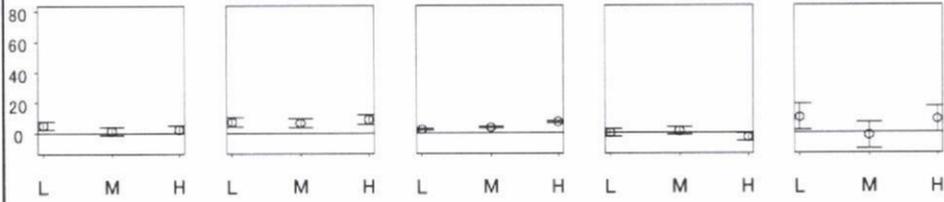

Fig. 6-SA

Table 6-SA

Chemical: A
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	V – L					V – M					V – H					J D	J L
		Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD		
1	id(MEAN V)	5.21	16	2.65	0.079	0.033	1.32	16	2.65	0.545	0.312	2.3	16	2.65	0.385	0.199	0	1
2	id(MEAN V)	7.24	16	3.04	0.038	0.015	6.6	16	3.04	0.055	0.023	9	16	3.04	0.012	0.005	1	1
3	id(MEAN V)	2.44	16	0.52	3.10E-04	1.10E-04	3.73	16	0.52	2.90E-06	1.00E-06	7.31	16	0.52	3.00E-10	9.00E-11	1	1
4	id(MEAN V)	-0.21	16	2.53	0.778	0.532	1.09	16	2.53	0.575	0.336	-2.96	16	2.53	0.974	0.87	0	0
5	id(MEAN V)	10.02	16	8.66	0.274	0.132	-1.83	16	8.66	0.819	0.582	8.71	16	8.66	0.33	0.165	0	0

One side tests were performed

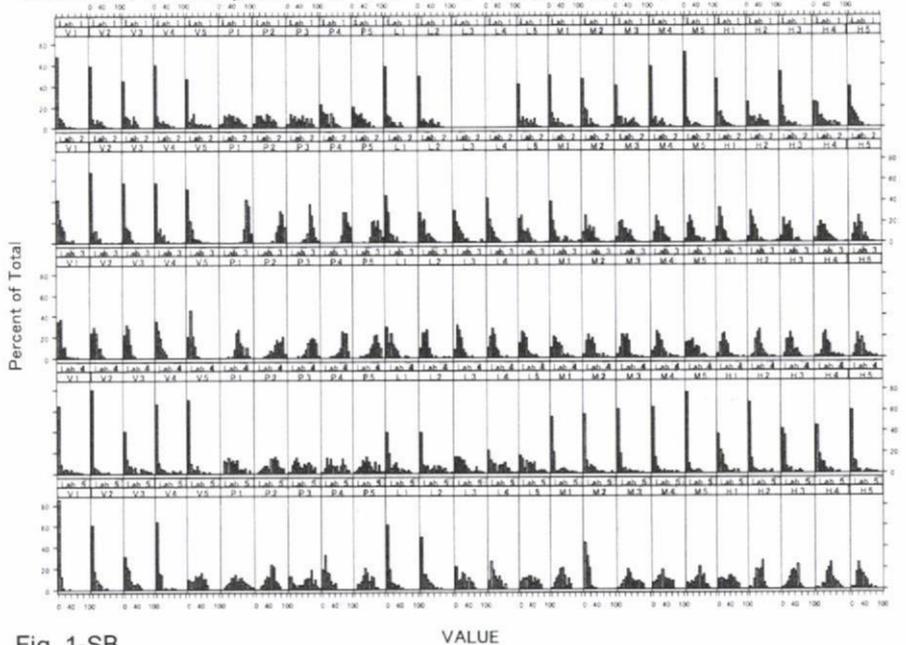## Chemical: B , Endpoint: PCT tail DNA, Transformation: id, Organ: Stomach

Fig. 1-SB

VALUE

Table 1-SB

Chemical: B
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id

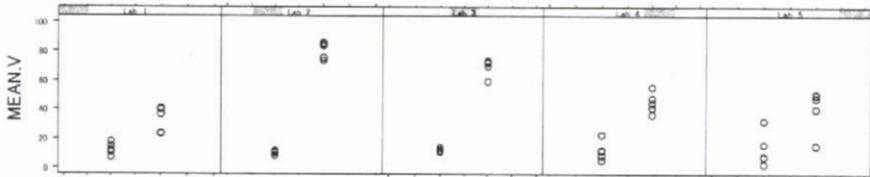Chemail: B Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 2-SB

Table 2-SB

Chemical: B
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Vehicle					EMS			
		N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
1	id(MEAN V)	5	12.6	4.1	7.2	17.9	5	32.8	8.7	23.2	40.8
2	id(MEAN V)	5	10	1.6	8.2	11.7	5	80.8	5.7	73.8	85.9
3	id(MEAN V)	5	12	1.1	11	13.8	5	69.6	5.9	59.3	73.4
4	id(MEAN V)	5	12.2	6.5	5.6	22.8	5	45.1	7.1	36.7	55.5
5	id(MEAN V)	5	13.5	11.6	2.9	32.4	5	41.1	14.7	15.7	50.8

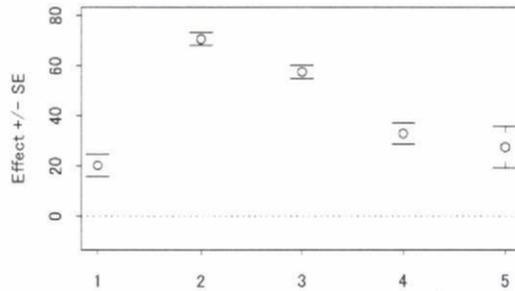# Chemical: B Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 3-SB

Chemical: B
Organ: Stomach Parameter: PCT tail DNA
Transformation: id Link: id

Table 3-SB

Lab.	Variable	Effect	SE	L95%	U95%	Pooled			Satterthwaite		
						df	P-value	J T	df	P-value	J T
1	id(MEAN V)	20.23	4.31	10.28	30.17	8	0.002	1	5.65	0.004	1
2	id(MEAN V)	70.81	2.63	64.75	76.87	8	4.00E-09	1	4.6	3.10E-06	1
3	id(MEAN V)	57.61	2.69	51.4	63.82	8	2.40E-08	1	4.27	1.70E-05	1
4	id(MEAN V)	32.95	4.3	23.03	42.87	8	6.00E-05	1	7.95	6.20E-05	1
5	id(MEAN V)	27.58	8.4	8.22	46.94	8	0.011	1	7.58	0.012	1

For the calculation for CIs the pooled method were used

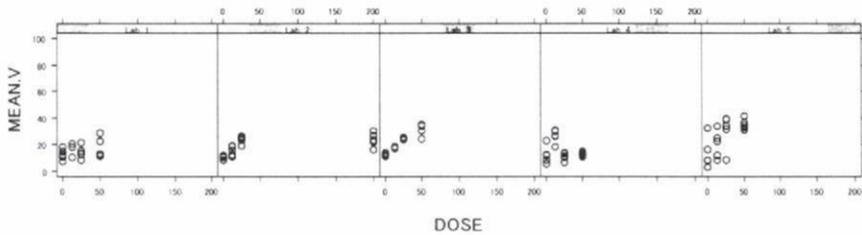# Chemail: B Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 4-SB

Table 4-SB

Chemical: B
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Vehicle						Low						Medium						High					
		Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max
1	id(MEAN V)	0	5	12.6	4.1	7.2	17.9	12.5	3	16.3	5.3	10.4	20.6	25	5	14.2	4.8	8.3	21.4	50	5	17.7	7.6	11.2	28.8
2	id(MEAN V)	0	5	10	1.6	8.2	11.7	12.5	5	15.2	3.6	10.8	18.8	25	5	23.7	2.9	18.9	26.2	200	5	23.8	5.4	16.2	30.4
3	id(MEAN V)	0	5	12	1.1	11	13.8	12.5	5	18	0.8	17	18.6	25	5	24.5	0.8	23.5	25.5	50	5	31.7	4.5	24.3	35.1
4	id(MEAN V)	0	5	12.2	6.5	5.6	22.8	12.5	5	26.4	5	18.4	30.7	25	5	11	2.9	6.6	14	50	5	12.7	1.5	10.8	14.7
5	id(MEAN V)	0	5	13.5	11.6	2.9	32.4	12.5	5	20.2	10.3	8.2	33.8	25	5	30.4	12.7	8.3	39.3	50	5	35.3	4	31.1	41.5

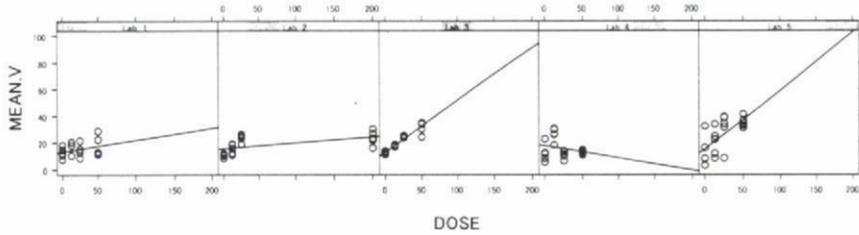Chemical: B Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 5-SB

Table 5-SB   Chemical: B
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Slope	SE	L95%	U95%	P–value	J_LI
1	id(MEAN_V)	0.0896	0.066	-0.051	0.2302	0.196	0
2	id(MEAN_V)	0.0459	0.016	0.0122	0.0796	0.01	1
3	id(MEAN_V)	0.3926	0.03	0.3287	0.4564	2.00E-10	1
4	id(MEAN_V)	-0.09	0.092	-0.284	0.1039	0.342	0
5	id(MEAN_V)	0.4398	0.121	0.1859	0.6938	0.002	1

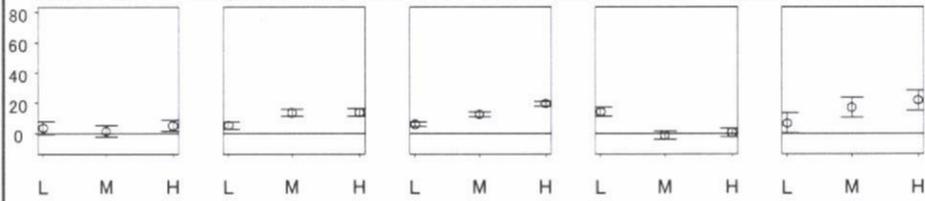Chemical: B Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 6-SB

Table 6-SB

Chemical: B
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	V - L					V - M					V - H					J D	J L
		Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD		
1	id(MEAN_V)	3.72	14	4.12	0.371	0.191	1.61	14	3.57	0.567	0.33	5.13	14	3.57	0.189	0.086	0	0
2	id(MEAN_V)	5.3	16	2.29	0.043	0.017	13.71	16	2.29	2.80E-05	9.70E-06	13.83	16	2.29	2.50E-05	8.80E-06	1	1
3	id(MEAN_V)	6	16	1.5	0.001	5.20E-04	12.51	16	1.5	4.60E-07	1.60E-07	19.69	16	1.5	8.00E-10	3.00E-10	1	1
4	id(MEAN_V)	14.26	16	2.79	1.50E-04	5.30E-05	-1.12	16	2.79	0.87	0.654	0.5	16	2.79	0.681	0.43	1	1
5	id(MEAN_V)	6.73	16	6.47	0.316	0.157	16.88	16	6.47	0.024	0.01	21.75	16	6.47	0.005	0.002	1	1

One side tests were performed

Chemical: C , Endpoint: PCT tail DNA, Transformation: id, Organ: Stomach
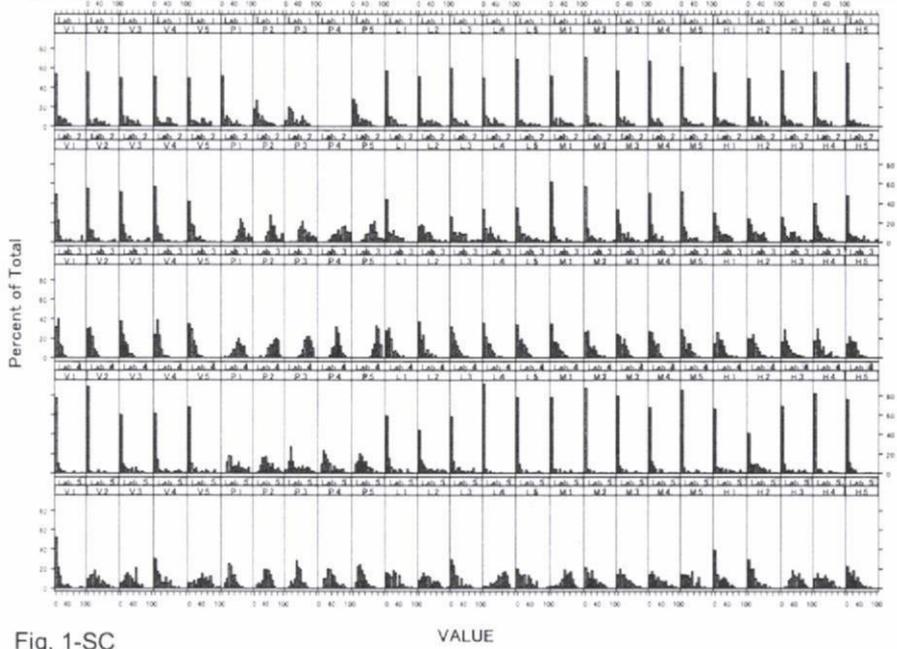

Fig. 1-SC

VALUE

Table 1-SC

Chemail: C   Endpoint: PCT Tail DNA   Transformation: id   Organ: Stomach

Fig. 2-SC

Table 2-SC

Chemical: C
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable		Vehicle					EMS			
		N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
1	id(MEAN_V)	5	16.1	2.9	11.8	19.4	4	21.7	6.3	15.1	29.9
2	id(MEAN_V)	5	14.3	2.5	10.7	17.7	5	66.5	3.8	62.6	71.7
3	id(MEAN_V)	5	11.1	0.7	10.1	11.8	5	67	9.3	56.1	78.4
4	id(MEAN_V)	5	10.2	4.6	4.5	15.2	5	37.8	6.5	32.4	46
5	id(MEAN_V)	5	27.3	12.7	11	44.1	5	36.2	6.4	27.9	42.9

Chemical: C   Endpoint: PCT Tail DNA   Transformation: id   Organ: Stomach
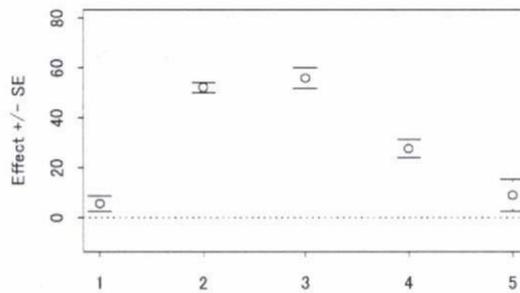
Fig. 3-SC

Chemical: C
Organ: Stomach Parameter: PCT tail DNA
Transformation: id Link: id

Table 3-SC

							Method					
							Pooled			Satterthwaite		
Lab.	Variable	Effect	SE	L95%	U95%	df	P-value	J T	df	P-value	J T
1	id(MEAN V)	5.63	3.11	-1.73	13	7	0.113	0	4.02	0.171	0
2	id(MEAN V)	52.22	2.05	47.49	56.95	8	6.00E-09	1	6.97	3.90E-08	1
3	id(MEAN V)	55.92	4.19	46.27	65.58	8	9.40E-07	1	4.04	1.70E-04	1
4	id(MEAN V)	27.66	3.57	19.42	35.9	8	5.50E-05	1	7.22	9.50E-05	1
5	id(MEAN V)	8.87	6.36	-5.78	23.53	8	0.2	0	5.9	0.213	0

For the calculation for CIs the pooled method were used.

Chemail: C Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 4-SC

Table 4-SC

Chemical: C
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

		Group																							
		Vehicle						Low						Medium						High					
Lab.	Variable	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max	Dose	N	Mean	SD	Min	Max
1	id(MEAN V)	0	5	16.1	2.9	11.8	19.4	125	5	13.8	3.9	10.2	19.6	250	5	11.6	4	7.6	17.7	500	5	13.9	2.1	11.1	16.2
2	id(MEAN V)	0	5	14.3	2.5	10.7	17.7	125	5	22.2	4.6	16.7	28	250	5	14.4	3.3	11.6	20.2	500	5	21.7	3.6	17.6	27.4
3	id(MEAN V)	0	5	11.1	0.7	10.1	11.8	125	5	13.7	0.3	13.4	14.1	250	5	15.7	1.1	14.2	16.6	500	5	19	0.5	18.3	19.6
4	id(MEAN V)	0	5	10.2	4.6	4.5	15.2	125	5	11.7	7.2	2.3	20.8	250	5	7.1	1.9	4.9	9.1	500	5	11.7	5.8	5.5	19.7
5	id(MEAN V)	0	5	27.3	12.7	11	44.1	87.5	5	30.9	14.7	17.5	55.6	175	5	30.2	10.9	22.9	49.4	350	5	26.7	13	16.9	47.6

Chemical: C Endpoint: PCT Tail DNA Transformation: id Organ: Stomach

Fig. 5-SC

Table 5-SCB Chemical: C
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Slope	SE	L95%	U95%	P-value	J LI
1	id(MEAN V)	−0.004	0.004	−0.013	0.0049	0.365	0
2	id(MEAN V)	0.0099	0.006	−0.003	0.0223	0.111	0
3	id(MEAN V)	0.0155	9.10E−04	0.0136	0.0174	2.00E−12	1
4	id(MEAN V)	0.0013	0.006	−0.012	0.0149	0.838	0
5	id(MEAN V)	−0.005	0.021	−0.049	0.0401	0.834	0

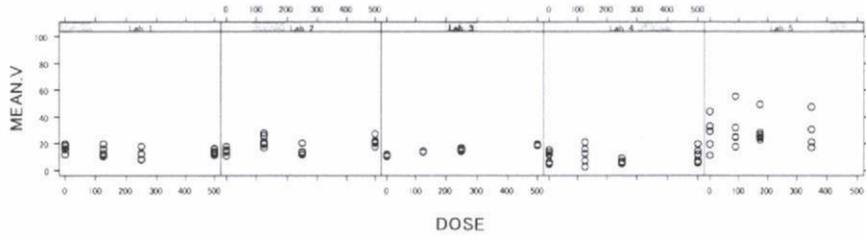## Chemical: C Endpoint: PCT Tail DNA Transformation: id Organ: Stomach
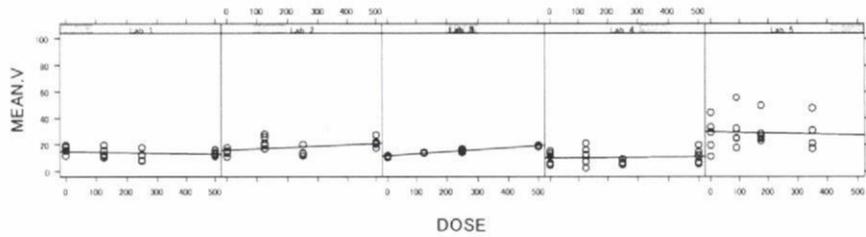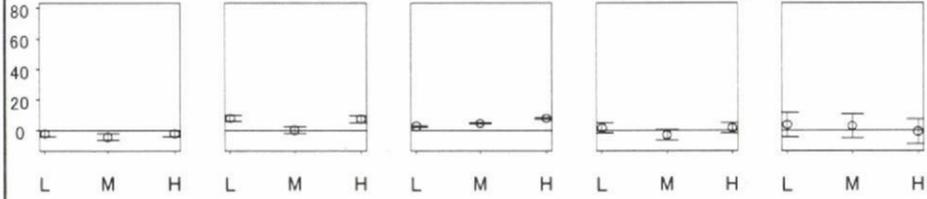

Fig. 6-SC

Table 6-SC

Chemical: C
Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

| Lab. | Variable | V – L | | | | | V – M | | | | | V – H | | | | | J D | J L |
		Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD		
1	id(MEAN_V)	-2.32	16	2.1	0.97	0.858	-4.52	16	2.1	0.998	0.977	-2.17	16	2.1	0.965	0.842	0	0
2	id(MEAN_V)	7.9	16	2.27	0.004	0.002	0.11	16	2.27	0.732	0.48	7.43	16	2.27	0.006	0.002	1	1
3	id(MEAN_V)	2.6	16	0.46	4.60E-05	1.60E-05	4.57	16	0.46	3.80E-08	1.30E-08	7.88	16	0.46	3.00E-11	4.00E-12	1	1
4	id(MEAN_V)	1.58	16	3.33	0.555	0.32	-3.07	16	3.33	0.955	0.815	1.52	16	3.33	0.564	0.327	0	0
5	id(MEAN_V)	3.62	16	8.15	0.569	0.331	2.9	16	8.15	0.608	0.363	-0.65	16	8.15	0.778	0.531	0	0

One side tests were performed.

Endpoint: PCT Tail DNA Transformation: id Organ: Stomach
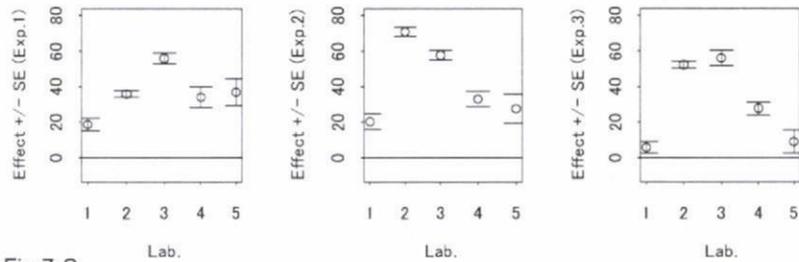
Fig 7-S

Table 7-S

Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

| | | | Lab. | | | | | | | | | |
| | | | 1 | | 2 | | 3 | | 4 | | 5 | |
Type	For Chem.	Variable	Effect	Variance	Effect	Variance	Effect	Variance	Effect	Variance	Effect	Variance
INTRA				53.4757		300.554		0		7.77E-07		163.705
EXP	A	id(MEAN_V)	18.581	13.1602	35.828	3.63801	55.78	9.15273	34.088	32.4868	36.903	58.3367
	B	id(MEAN_V)	20.228	18.5957	70.809	6.90883	57.61	7.24577	32.951	18.5164	27.582	70.483
	C	id(MEAN_V)	5.6343	9.69219	52.223	4.20841	55.924	17.5159	27.661	12.7567	8.8725	40.3883

Table 8-S

Organ: Stomach Endpoint: PCT tail DNA
Transformation: id Link: id

Variable	Component		Variance
id(MEAN_V)	Chem.(Lab.)		103.05
	Lab.		294.14
	Residual	1 A	13.16
		1 B	18.596
		1 C	9.6922
		2 A	3.638
		2 B	6.9088
		2 C	4.2084
		3 A	9.1527
		3 B	7.2458
		3 C	17.516
		4 A	32.487
		4 B	18.516
		4 C	12.757
		5 A	58.337
		5 B	70.483
		5 C	40.388

Fig. 8
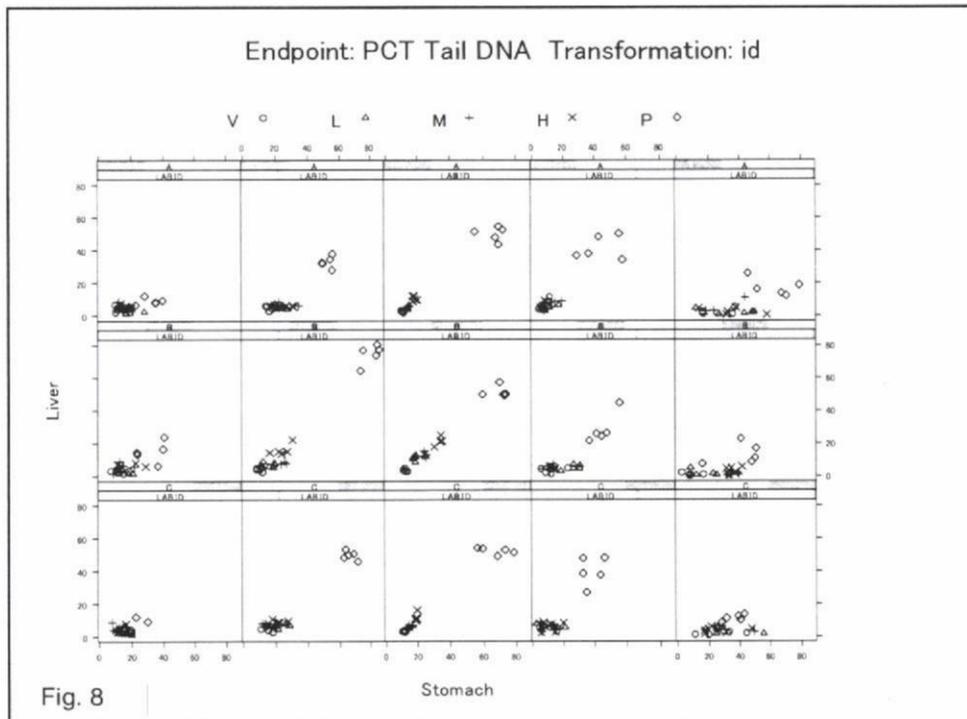
Table 9

Endpoint: PCT tail DNA
Transformation: id Link: id

Chemical	Variable	Lab.				
		1	2	3	4	5
A	id(MEAN V)	0.46	0.91	0.98	0.92	0.58
B	id(MEAN V)	0.72	0.98	0.98	0.87	0.5
C	id(MEAN V)	0.3	0.97	0.98	0.88	0.24

Fig. 9
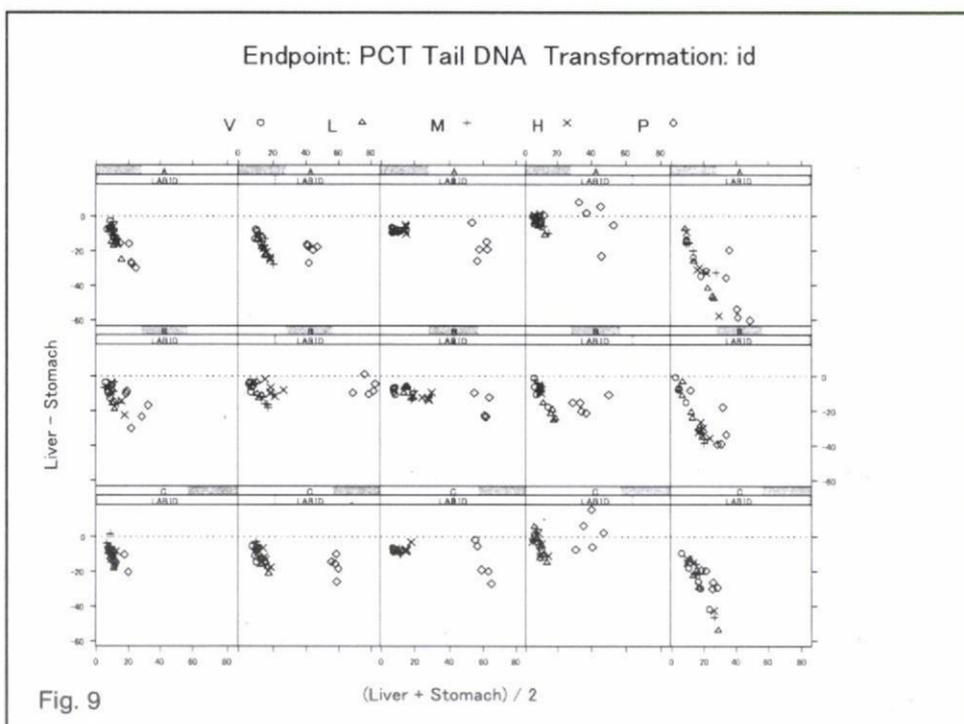
INTERNATIONAL PRE-VALIDATION STUDY OF THE IN VITRO ALKALINE COMET ASSAY

Results

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1

1. Ethymethansulfonate (EMS)

Cytotoxity Assays Results

TK6 cells after 4h Exposure to Ethymethansulfonate, without Metabolic Activation (-S9)

Group No.	EMS Treatment (µg/mL)	Daily Cell Growth[A] Measured by COULTER COUNTER			Relative Suspension Growth (%)
		Day 1	Day 2	Day 1 × Day 2	
1	DMSO	4.13	3.94	16.27	100.0
2	62.5	2.72	1.45	3.95	24.3
3	125	2.44	1.08	2.62	16.1
4	250	2.10	1.02	2.15	13.2
5	500	1.83	1.03	1.89	11.6
6	1000	1.46	1.01	1.47	9.1
7	2000	1.53	1.06	1.63	10.0

[A] Cell conc. on the day / Cell conc. on the day before

Day 1: After 24h from end of treatment

Day 2: After 48h from end of treatment

Group No.	EMS Treatment (µg/mL)	% Viabilty[B] Measured by Dye Exclusion Assay		
		Day 0	Day 1	Day 2
1	DMSO	97.5	97.8	100.0
2	62.5	100.0	97.1	91.7
3	125	100.0	100.0	40.6
4	250	96.9	98.1	50.0
5	500	97.3	79.5	18.8
6	1000	97.4	46.5	6.9
7	2000	96.7	23.5	0.0

[B] Number of unstained cells / number of total cells) x 100

Day 0: Just after treatment

Day 1: After 24h from end of treatment

Day 2: After 48h from end of treatment

3

Comet Assay Results

TK6 cells after 4h Exposure to Ethymethansulfonate, without Metabolic Activation (-S9)

% Tail Intensity — Dose (ug/mL)
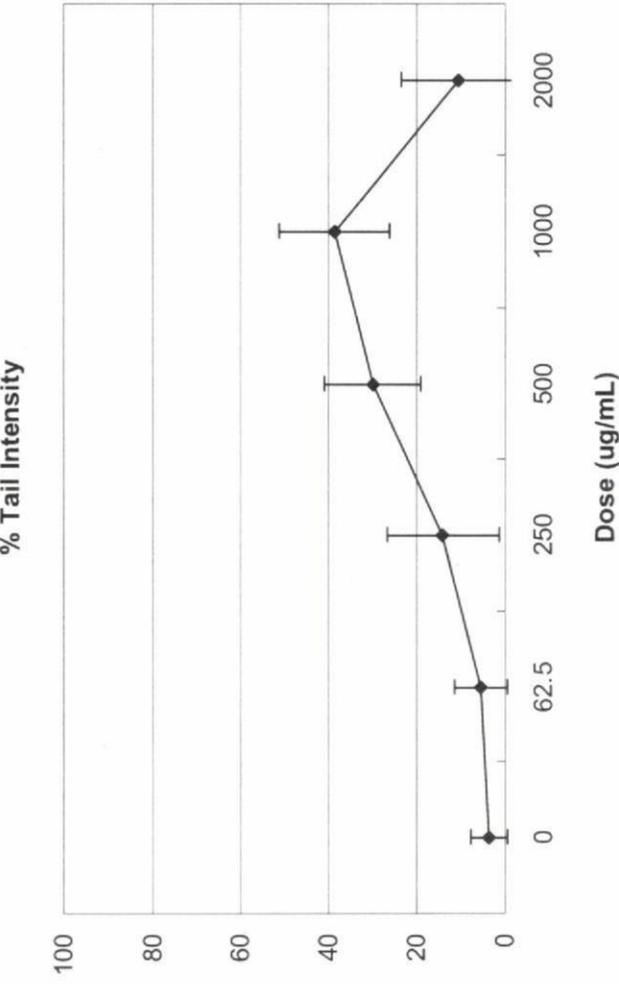
2000 µg/mL EMS
Hedgehog (small head)

Group No.	EMS Treatment (µg/mL)	Analyzed Cell No.	Data[A] from Comet Assay IV		
			Tail Moment	Tail Length	% Tail Intensity
1	DMSO	100	0.72	43.05	3.47
2	62.5	100	1.18	49.00	5.52
-	125	-*	-	-	-
3	250	100	3.21	56.45	14.01
4	500	100	8.32	75.24	30.19
5	1000	100	11.91	81.45	38.98
6	2000	100	3.41	74.04	10.37

[A] Mean of analyzed cells

* Not scored due to drop of agarose gel on slide

4

In vitro Micronucleus Assay Results

TK6 cells after 4h Exposure to Ethymethansulfonate, without Metabolic Activation (-S9)

Group No.	EMS Treatment (μg/mL)	Analyzed Cell No.	Number of Micronucleated cell	(% Incidence)
1	DMSO	1000	4	(0.4)
2	62.5	1000	68	(6.8)
3	125	1000	74	(7.4)
4	250	1000	84	(8.4)
5	500	720	49	(6.8)
6	1000	TOX	-	(-)
7	2000	TOX	-	(-)

TOX: Not scored due to cytotoxicity

Micronucleus assay was performed on Day 2.

5

2. Mitomycin C (MMC)

6

Cytotoxity Assays Results

TK6 cells after 4h Exposure to Mitomycin C, without Metabolic Activation (-S9)

Group No.	MMC Treatment (μg/mL)	Daily Cell Growth[A] Measured by COULTER COUNTER Day 1	Day 2	Day 1 × Day 2	Relative Suspension Growth (%)
1	Saline	3.72	3.82	14.20	100.0
2	0.125	2.18	2.54	5.54	39.0
3	0.25	1.43	1.61	2.32	16.3
4	0.5	1.16	1.29	1.50	10.6
5	1	1.00	0.93	0.93	6.6
6	2	0.89	0.88	0.78	5.5
7	4	0.87	0.95	0.83	5.8

[A] Cell conc. on the day / Cell conc. on the day before

Day 1: After 24h from end of treatment

Day 2: After 48h from end of treatment

Group No.	MMC Treatment (μg/mL)	% Viabilty[B] Measured by Dye Exclusion Assay Day 0	Day 1	Day 2
1	Saline	97.0	98.0	100.0
2	0.125	100.0	95.6	84.4
3	0.25	100.0	91.5	67.2
4	0.5	97.3	78.1	34.4
5	1	100.0	53.8	6.1
6	2	100.0	35.7	15.0
7	4	97.1	31.4	0.0

[B] Number of unstained cells / number of total cells) x 100

Day 0: Just after treatment

Day 1: After 24h from end of treatment

Day 2: After 48h from end of treatment

7

Comet Assay Results

TK6 cells after 4h Exposure to Mitomycin C, without Metabolic Activation (-S9)

% Tail Intensity

Group No.	MMC Treatment (µg/mL)	Analyzed Cell No.	Data[A] from Comet Assay IV		
			Tail Moment	Tail Length	% Tail Intensity
1	Saline	100	1.48	46.42	5.48
2	0.125	100	0.54	44.61	2.20
3	0.25	100	0.93	42.20	4.61
4	0.5	100	0.66	42.39	2.62
5	1	100	0.73	42.71	2.71
6	2	100	0.54	44.01	2.26
7	4	100	0.55	45.77	3.03

[A] Mean of analyzed cells

8