3.1.3. Study report

The study report from each testing facility will at least include the following information:

3.1.3.1. Test substance and positive/negative controls

Identification; CAS number; supplier; lot number; physical nature and purity; physiochemical property relevant to the conduct of the study, if known; justification for choice of vehicle; and solubility and stability of the substances in the solvent/vehicle, if known.

3.1.3.2. Test animals

Species/strain used; number, age and sex of animals; source, housing conditions, quarantine and acclimation procedure, and animal identification and group assignment procedure; individual weight of the animals on the day of receipt, at the end of the acclimation period, and before administration (at the time of grouping), including body weight range, mean and standard deviation for each group; and choice of tissue(s) and justification.

3.1.3.3. Reagents to prepare reagent solutions

Identification; supplier; lot number; and time limit for usage if known.

3.1.3.4. Test conditions

Data from range-finding study, if conducted; rationale for dose level selection; details of test substance preparation; details of the administration of the test substance; rationale for route of administration; methods for verifying that the test substance reached the general circulation or target tissue, if applicable; details of food and water quality; detailed description of treatment and sampling schedules; method of measurement of toxicity, including histopathology; detailed methods of single cell preparation; method of slide preparation, including agarose concentration, lysis conditions, alkali conditions and pH, alkali unwinding time and temperature, electrophoresis conditions (pH, V/cm, mA, and temperature at the start of unwinding and the start and the end of electrophoresis) and staining procedure; criteria for scoring comets and number of comets analyzed per slide, per tissue and per animal; evaluation criteria; criteria for considering studies as positive, negative or equivocal.

3.1.3.5. Results

Signs of toxicity, including histopathology in the appropriate tissue(s) if applicable; individual and mean/median values for DNA migration (and ranges) and % cells with low molecular weight DNA and % hedgehogs in individual tissue, animal, and group;

concurrent positive and negative control data; and statistical evaluation.

3.1.3.6. Discussion of the results and/or conclusion, as appropriate.

4. ARCHIVES AND REVIEW

The study report and all raw data (including slide samples and image data) from this study will be retained according to the SOP in each testing facility. All raw data will be submitted to the management team for review if required.

5. REFERENCES

Burlinson B, et al., 4th International Workgroup on Genotoxicity Testing: result of the in vivo comet assay workgroup (in preparation).

Collins AR, et al., Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis, 14, 1733-1735, 1993.

Hartmann A, et al., Recommendation for conducting the *in vivo* alkaline Comet assay. Mutagenesis, 18(1), 45-51, 2003.

Lovell DP, G Thomas G, R Dubow., Issues related to the experimental design and subsequent statistical analysis of in vivo and in vitro comet studies. Teratog Carcinog Mutagen. 19(2), 109-119, 1999.

Olive PL, et al., Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cell using the "comet" assay. Radiat. Res., 122, 86-94, 1990.
Tice RR et al., Single cell gel/Comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen., 35, 206-221, 2000.

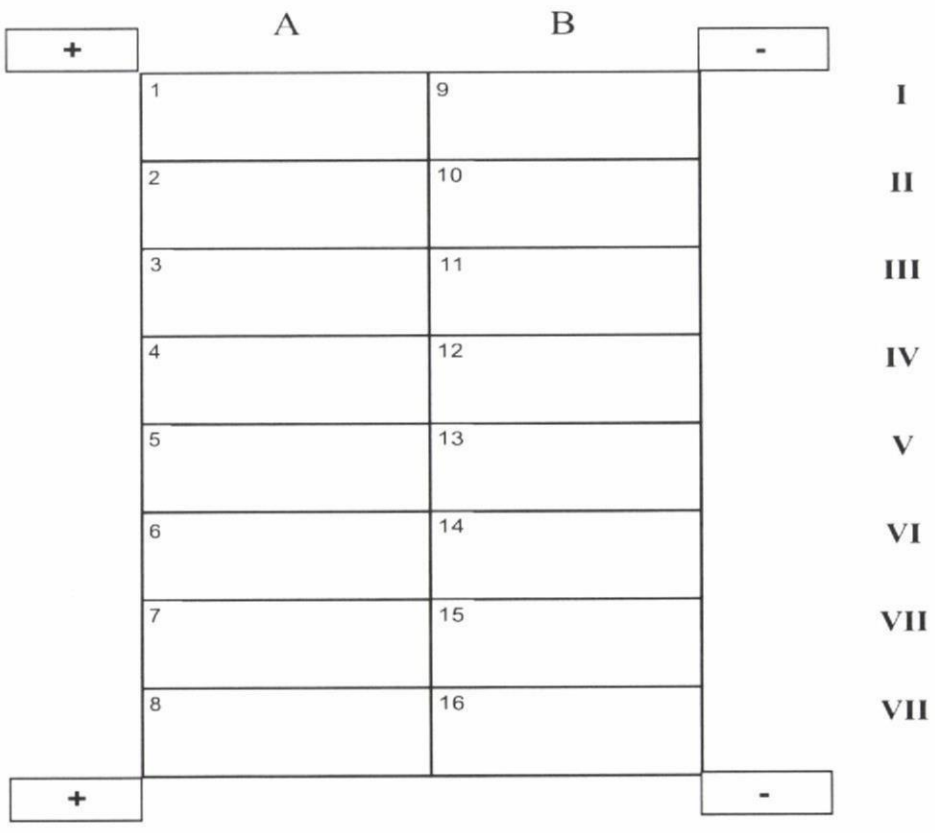
Wiklund SJ, E Agurell., Aspects of design and statistical analysis in the Comet assay. Mutagenesis 18(2):167-175, 2003.

Attachment 1:

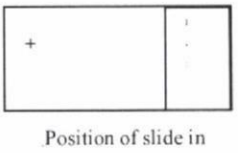
SLIDES UNWINDING & ELECTROPHORESIS RECORDING SHEET

Electrophoresis Run #				**Initials & Date**
Approximate alkaline electrophoresis buffer volume in chamber				
Unwinding				
Time	Total	Start	End	
Buffer Temperature				
Electrophoresis				
Running time	Total	Start	End	
Volts				
Milliamperes				
Buffer Temperature				
Thermometer No.				
Electrophoresis chamber No.				
Power supply No.				

Diagram Electrophoresis Chamber



RED(+) 11 **BLACK(-)**



Position of slide in

INTERNATIONAL VALIDATION OF THE *IN VIVO* RODENT ALKALINE COMET ASSAY FOR THE DETECTION OF GENOTOXIC CARCINOGENS
- Study Plan for 3rd phase validation Study (DRAFT) -

Issued by: the Validation Management Team (VMT)
Date: March X, 2008

A. PURPOSE OF THIS DOCUMENT

This document is provided trial by trial as a supplement of study protocol to clarify the purpose, the schedule, and the specific notes of each trial of an international validation study to evaluate the ability of the *in vivo* rodent alkaline Comet.

B. STUDY TITLE

3rd phase validation study of international validation of the *in vivo* rodent alkaline Comet assay for the detection of genotoxic carcinogens (abbreviation: 3rd phase validation study of *in vivo* Comet assay)

C. BACKGROUND AND PURPOSE OF THIS STUDY

In the 2nd phase validation study of *in vivo* Comet assay, following problems were clarified: 1) EMS treatment induced positive responses for the liver in all (five) leading laboratories thorough three independent experiments, but failed to produce positive results for the stomach in each one of three experiments conducted in two leading laboratories; and 2) large variation of Effects (difference of an average of Estimate between a negative control group and an EMS treatment group) were observed among five testing facilities. In addition, one and three of five laboratories showed large within-laboratory variation of the Effect in the liver and in the stomach, respectively.

One of success criteria of the 2nd phase validation study of *in vivo* Comet assay was to obtain positive results in all positive control groups in all testing facilities. Thus the above problems indicate that the comet assay protocol-version 12 may not be suitable as it is for the further validation studies, at least for the stomach. Based on discussion with the members of VMT, leading laboratories and consultation team including statisticians at Atagawa meeting (March 13-14), the comet assay protocol has been revised to version 13 intended to solve above problems. In addition,

tentative criteria on data acceptability applied in the laboratory selection process for the future validation (caution: to be determined and described in this supplementary protocol later) have been established considering the data from the 2nd phase validation study of *in vivo* Comet assay. It is also necessary to investigate whether or not the tentative data-acceptance criteria can be applied to judge data reliability in the future validation studies.

In this 3rd phase validation study of *in vivo* Comet assay, two or three coded test compounds will be assayed in leading laboratories in accordance with the Comet assay protocol-version 13. The first purpose is to examine reproducibility and robustness of positive control results with EMS when experiments are conducted in accordance with the Comet assay protocol-version 13, and this means to examine acceptability of the Comet assay protocol-version 13 for further validation studies. The second purpose is to investigate whether or not the tentative data-acceptance criteria are suitable to judge reliability of data.

D. SCHEDULE

~March 31, 2008: Fixation of this supplementary protocol in VMT

~April 15, 2008: Delivery of protocol-version 13 and supplementary protocol to testing facilities

~April 30, 2008: Delivery of test compounds; Fixation of study protocol in each testing facility

May~August, 2008:Experimental period (Data on each test compound will be submitted to VMT ASAP when available)

August 31, 2008: Deadline of all data submission to VMT

~October 31, 2008: Finalization of data analysis

E. SPECIFIC NOTES

1. SUCCESS CRITERIA

1-1. To obtain positive results in all positive control groups in all testing facilities.

1-2. To confirm that data from all testing facilities can satisfy the tentative data-acceptance criteria.

2. OTHERS

2-1. Dose selection of three coded test compounds

The dose levels of all compounds will be directed by VMT. VMT will inform an appropriate individual within the organization who is not involved in the study, and

then the individual will inform you of the dose levels.

2-2. Solvent/vehicle

VMT will inform the solvent/vehicles for all compounds later.

Figs and tables

2008/03/13

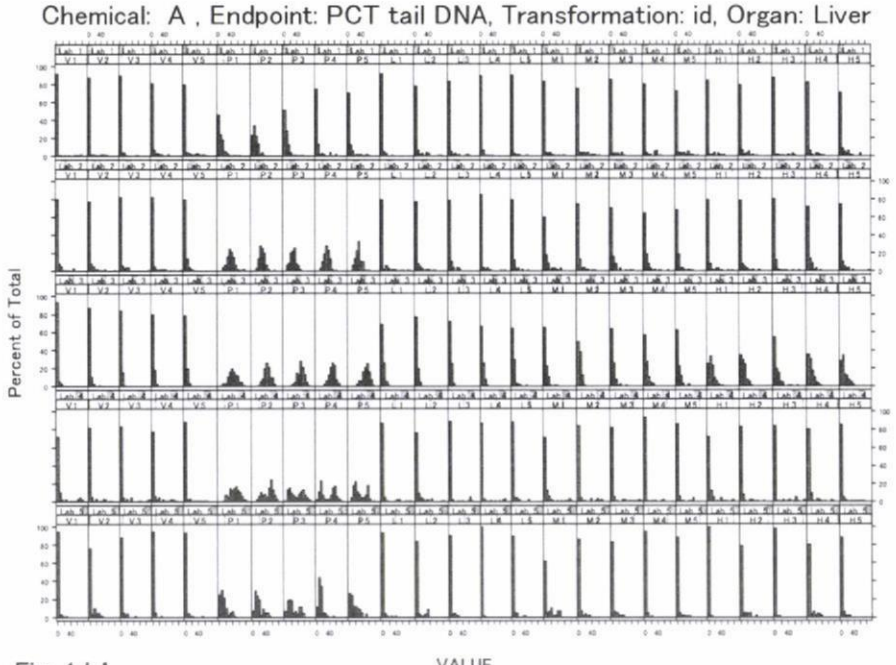


Fig. 1-LA

Table 1-LA

Chemical: A
Organ: Liver Endpoint: PCT tail DNA
Transformation: id

	Group																																	
	Vehicle							EMS						Low						Medium						High									
Lab	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max
1	0	100	4.5	0	16.1	0	78.4	200	100	9.1	6.1	10.6	0.2	62.5	12.5	100	1.6	0	4.4	0	31.8	25.0	100	4.5	0	11.8	0	65	50.0	100	5	0.1	12.1	0	54.8
2	0	100	4.8	0	12.8	0	58.5	200	100	12.8	10.8	8.1	1.8	37.1	12.5	100	8.1	0.2	14	0	77.8	25.0	100	7.8	0.2	14.1	0	66.1	50.0	100	5.2	0.2	10.7	0	55.3
3	0	100	2.4	0	8.2	0	57.8	200	100	9.3	5.2	9.6	1	58.2	12.5	100	5.2	0.2	12.1	0	69.5	25.0	100	3.8	0.1	8.9	0	61.4	50.0	100	4	0	14.5	0	68.5
4	0	100	4.2	0	10.1	0	54	200	100	8.8	2	12.7	0	63.4	12.5	100	2	0	2.9	0	41.8	25.0	100	3.9	0.1	12.6	0	53.1	50.0	100	4.8	0.2	11.5	0	53
5	0	100	5	0.1	15.5	0	87.3	200	100	8.2	2.3	14	0	83.4	12.5	100	2.9	0.1	8.8	0	55.1	25.0	100	3.8	0.2	16.3	0	83.6	50.0	100	7.3	0.8	13.8	0	85.6
1	0	100	4.5	1.6	10.9	0	55	200	100	37.9	36.1	11.5	15.5	75.2	12.5	100	7.4	0.6	16.1	0	82.8	25.0	100	9.8	3.7	12.8	0	55.9	50.0	100	8.1	0.8	13.2	0	79.3
2	0	100	6.4	0.7	13.3	0	85.6	200	100	30.2	31	9.4	13.9	70.3	12.5	100	5.4	0.5	11.9	0	57.8	25.0	100	9.9	1.9	18.6	0	82.4	50.0	100	5.6	1.4	10.6	0	57.9
3	0	100	4.7	1	11	0	90.6	200	100	28.1	27.7	10.3	8.7	54.2	12.5	100	4.2	0.7	7.7	0	34.7	25.0	100	6	2.2	10.8	0	86.7	50.0	100	5.2	2	11.2	0	77.5
4	0	100	6.2	0.6	15.5	0	90	200	100	34.5	34.2	8.9	15.2	62.6	12.5	100	4.9	0.9	11.6	0	77.1	25.0	100	7.1	3	11.7	0	80.7	50.0	100	5.8	2.6	7.1	0	30.5
5	0	100	3	0.5	5.3	0	27.4	200	100	32.2	30.5	9.4	11.6	64.7	12.5	100	4.1	1.1	8.3	0	53.8	25.0	100	5.8	2.5	10.1	0	54	50.0	100	8.3	1.8	12	0	57.7
1	0	100	1.6	0.8	2.8	0	13.6	200	100	43.5	42.2	13.9	7.7	75.5	12.5	100	4.6	3.5	4	0	18.4	25.0	100	5.5	2.9	8.4	0	38.8	50.0	100	12	10.8	8.5	0.5	44.8
2	0	100	2.6	1.4	4.2	0	21.8	200	100	47.8	47.8	10.7	23.4	69.3	12.5	100	3.7	2.7	3.7	0	16.3	25.0	100	5.8	5.4	4.8	0.1	22	50.0	100	9.7	9.3	7.5	0	31.6
3	0	100	2.3	1.5	2.8	0	14.8	200	100	52.7	53.2	10.5	30.2	75.6	12.5	100	3.8	2.3	3.8	0	15.9	25.0	100	5.1	3.6	7	0	48.8	50.0	100	8.8	4.8	11.4	0	70.7
4	0	100	3.7	2.2	3.6	0	17.3	200	100	51.2	51.4	9.2	28.8	70.7	12.5	100	4.8	3.6	4.6	0	18.7	25.0	100	8.2	4.4	8.5	0	22.8	50.0	100	10.2	8.9	8.2	0.1	39.2
5	0	100	3.5	2.4	3.3	0	18.1	200	100	54.7	55.7	10.6	27.9	78.5	12.5	100	3.2	4	6.3	0	47	25.0	100	5.9	3.5	7.4	0	41.2	50.0	100	11.9	9.1	10	0	43.3
1	0	100	11.8	1.9	22.8	0	85.7	200	100	68.1	48.7	15.4	13.8	83.2	12.5	100	5.7	0.5	14.8	0	82.2	25.0	100	9.2	2.3	8.9	0	82.4	50.0	100	8.5	1.2	17	0	79.8
2	0	100	8.9	0.4	19.2	0	71.4	200	100	50.3	54.3	16.1	1.3	81.1	12.5	100	6.5	0.6	13.8	0	65.4	25.0	100	7	0.5	13	0	70.2	50.0	100	4.3	0.5	12.1	0	71
3	0	100	6.8	0.8	18.2	0	93.1	200	100	38.1	26.7	22	0	88.5	12.5	100	6	0.6	11.2	0	72.9	25.0	100	8.6	0.5	20.9	0	88.7	50.0	100	9.2	0.6	21.2	0	89.2
4	0	100	8.2	0.7	17.8	0	76.5	200	100	38.6	41.3	21.4	5.4	75	12.5	100	8.5	0.5	18.3	0	79.2	25.0	100	3.2	0.4	10.6	0	61.1	50.0	100	8.2	0.9	15.6	0	80.7
5	0	100	4.3	0.6	12.3	0	64.5	200	100	34.2	28.5	18.2	9.3	78.1	12.5	100	5	0.4	8.1	0	51.5	25.0	100	4.8	1.2	9	0	55.2	50.0	100	5.2	0.8	14.1	0	74.1
1	0	100	1.4	0.2	3.8	0	24.7	200	100	13.8	10.7	11.7	0.9	49	12.5	100	1.4	0.1	4.8	0	37	25.0	100	1.1	1.1	12.3	0	46	50.0	100	0.6	0.1	1.4	0	10.3
2	0	100	5.8	0.4	9.9	0	38	200	100	18.7	14.6	12.1	3.7	50.8	12.5	100	4.8	0.3	10	0	35	25.0	100	2.8	0.1	8.8	0	34.9	50.0	100	4.6	0.3	9.4	0	43.9
3	0	100	2.6	0.1	6.4	0	47.8	200	100	24	19.5	17.2	0.3	59.7	12.5	100	2.1	0.2	5.2	0	30.6	25.0	100	3.2	0.1	7.5	0	36.9	50.0	100	0.6	0	1.8	0	12.8
4	0	100	0.8	0	3.2	0	26.8	200	100	12.1	11.1	7.8	0	54.2	12.5	100	0.8	0	1.8	0	9.5	25.0	100	1	0.1	2.8	0	20.2	50.0	100	5.1	0.3	10	0	36.4
5	0	100	1.5	0.1	5.7	0	56.8	200	100	19.1	11.8	12.1	0	51.7	12.5	100	2.2	0.3	6.2	0	42.6	25.0	100	2.8	0.3	8.8	0	40.3	50.0	100	2.6	0.2	5.9	0	23.8

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

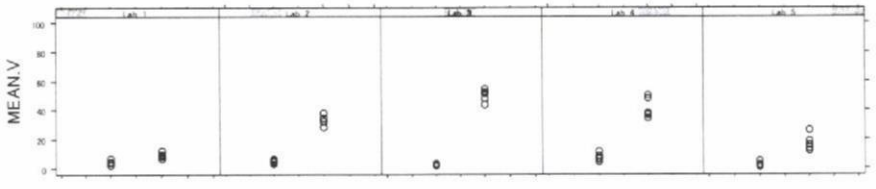


Fig. 2-LA

Table 2-LA

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

		Vehicle					EMS				
Lab.	Variable	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
1	id(MEAN_V)	5	4.5	1.6	2.4	7	5	9.1	2.1	6.8	12.3
2	id(MEAN_V)	5	5.1	1.3	3	6.2	5	33	3.6	28.1	37.9
3	id(MEAN_V)	5	2.7	0.6	1.8	3.3	5	50	4.4	43.5	54.7
4	id(MEAN_V)	5	7.5	2.8	4.3	11.8	5	41.5	7.2	34.3	50.3
5	id(MEAN_V)	5	2.3	1.7	0.9	5.3	5	17.3	5.4	12.1	26

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

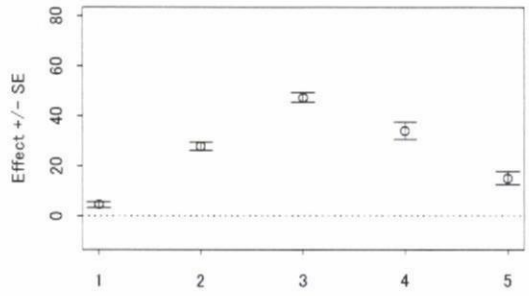


Fig. 3-LA

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

Table 3-LA

						Method					
						Pooled			Satterthwaite		
Lab.	Variable	Effect	SE	L95%	U95%	df	P-value	J T	df	P-value	J T
1	id(MEAN V)	4.56	1.17	1.86	7.25	8	0.005	1	7.59	0.005	1
2	id(MEAN V)	27.91	1.71	23.97	31.86	8	2.00E-07	1	5.05	1.40E-05	1
3	id(MEAN V)	47.27	1.99	42.68	51.87	8	1.10E-08	1	4.15	1.40E-05	1
4	id(MEAN V)	33.98	3.45	26.04	41.93	8	9.40E-06	1	5.18	1.50E-04	1
5	id(MEAN V)	15.03	2.56	9.14	20.93	8	3.70E-04	1	4.81	0.002	1

For the calculation for CIs the pooled method were used

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

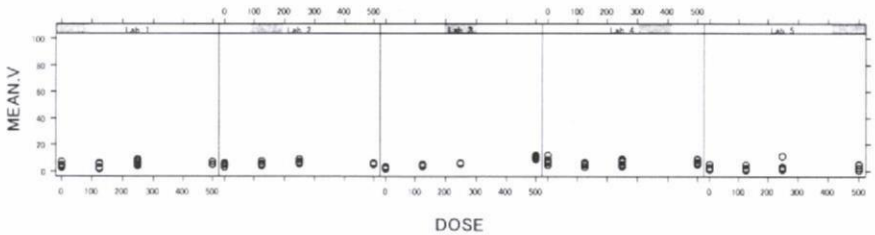


Fig. 4-LA

Table 4-LA

Chemical: A
Organ: Liver 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	4.5	1.6	2.4	7	125	5	3.6	2	1.6	6.1	250	5	6.1	2	3.8	8.8	500	5	5.5	1	4.8	7.3
2	id(MEAN V)	0	5	5.1	1.3	3	6.2	125	5	5.1	1.4	4.1	7.4	250	5	6.7	1.3	5.5	8.8	500	5	5.7	0.5	5.2	6.3
3	id(MEAN V)	0	5	2.7	0.6	1.8	3.3	125	5	4.4	0.7	3.6	5.2	250	5	5.8	0.3	5.5	6.2	500	5	10.5	1.4	8.8	12
4	id(MEAN V)	0	5	7.5	2.8	4.3	11.8	125	5	5.4	1.5	3	6.5	250	5	6.6	2.5	3.3	9.2	500	5	6.8	1.9	4.7	9.2
5	id(MEAN V)	0	5	2.3	1.7	0.9	5.3	125	5	2.2	1.5	0.6	4.6	250	5	4.2	4	1	11.1	500	5	2.6	2.2	0.6	5.1

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

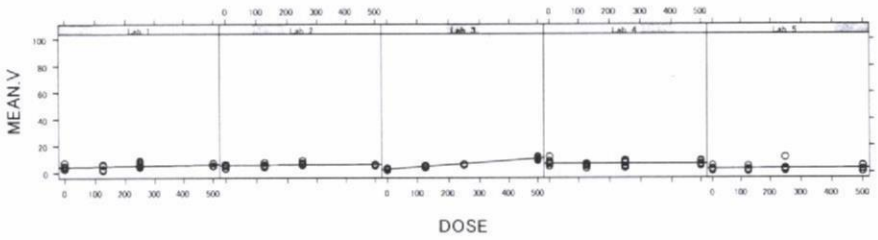


Fig. 5-LA

Table 5-LA Chemical: A
Organ: Liver Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Slope	SE	L95%	U95%	P-value	J_LI
1	id(MEAN_V)	0.003	0.002	−0.002	0.0076	0.198	0
2	id(MEAN_V)	0.0016	0.002	−0.002	0.0048	0.315	0
3	id(MEAN_V)	0.0156	0.001	0.0134	0.0178	1.00E−11	1
4	id(MEAN_V)	−2.10E−04	0.003	−0.006	0.0055	0.94	0
5	id(MEAN_V)	0.0012	0.003	−0.005	0.0076	0.697	0

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

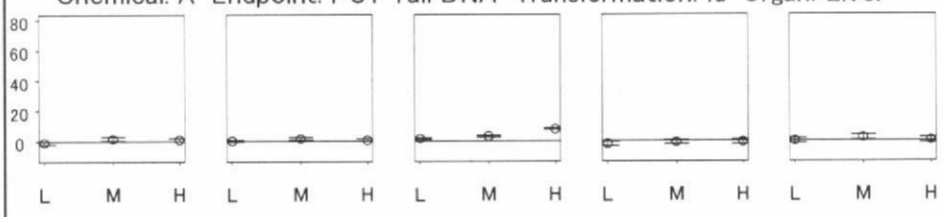


Fig. 6-LA

Table 6-LA

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

		V − L					V − M					V − H						
Lab.	Variable	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	J_D	J_L
1	id(MEAN_V)	−0.97	16	1.09	0.951	0.806	1.54	16	1.09	0.193	0.089	0.94	16	1.09	0.388	0.201	0	0
2	id(MEAN_V)	0.05	16	0.74	0.727	0.476	1.66	16	0.74	0.049	0.02	0.61	16	0.74	0.407	0.213	1	1
3	id(MEAN_V)	1.66	16	0.53	0.009	0.003	3.11	16	0.53	3.60E−05	1.30E−05	7.79	16	0.53	2.00E−10	6.00E−11	1	1
4	id(MEAN_V)	−2.06	16	1.41	0.988	0.919	−0.93	16	1.41	0.921	0.741	−0.68	16	1.41	0.888	0.683	0	0
5	id(MEAN_V)	−0.1	16	1.6	0.772	0.525	1.87	16	1.6	0.27	0.13	0.34	16	1.6	0.667	0.416	0	0

One side tests were performed

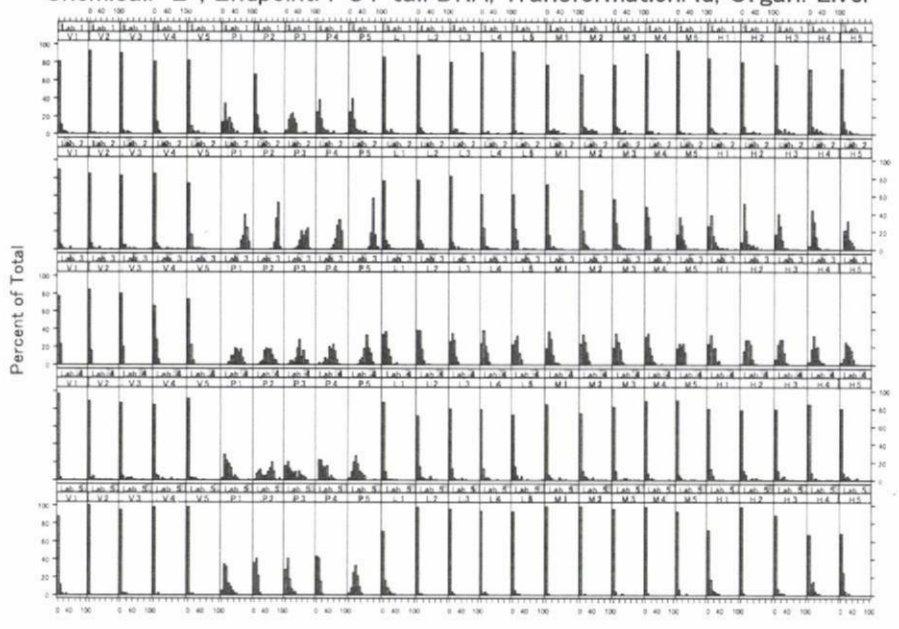


Fig. 1-LB

Table 1-LB

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

	Vehicle							Low							Medium							High						
Lab	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max
1	0	100	3.5	0	7.9	0	48.5	200	100	18.5	13.2	11.6	2.4	59.3	12.5	100	2.6	0.7	7.8	0	42	25	100	0.2	0.2	11.6	0	54.3	50	100	4.5	1.6	10.1	0	55.9
2	0	100	2.4	0	8.9	0	64.7	200	100	6.5	3.4	8.7	0.1	43.3	12.5	100	2	0.1	5.5	0	40.6	25	100	0.1	0.6	14.6	0	57.3	50	100	8.1	1.7	12.1	0	59.1
3	0	100	2	0	6.8	0	29.2	200	100	12.8	2.4	13.7	3.5	71.3	12.5	100	5.3	0.3	10.9	0	57	25	100	0.8	0.3	11.3	0	56.1	50	100	7.3	1.35	0	0	58.6
4	0	100	2.5	0	4.6	0	21.1	200	100	14.5	8.8	14.9	1.8	56.4	12.5	100	3.2	0.2	13.1	0	100	25	100	0.8	0.2	11	0	42.8	50	100	8.5	1.9	12.7	0.2	61.2
5	0	100	3.3	0.1	8.3	0	46.9	200	100	13.4	7.8	13	2.5	70.7	12.5	100	4	0	5	0	27.5	25	100	1.9	0.1	5.5	0	43.5	50	100	8.5	2.5	10.1	0	56.4
6	0	100	2.6	0.2	7.5	0	40.9	200	100	17.3	10	17.7	5.2	80.5	12.5	100	8.8	1.5	14.8	0	87.5	25	100	6.5	3.6	8.5	0.7	47.8	200	100	14.3	10	14.2	2.8	80.4
7	0	100	4	0.4	10.8	0	80.1	200	100	60.3	51.2	48.8	5.8	325	12.5	100	5.8	2.8	9.3	0.2	62.8	25	100	7.5	4.6	10.2	1.1	86.8	200	100	15.3	11.1	11.8	4.1	84.1
8	0	100	5.1	0.5	12.9	0	97.6	200	100	54.2	35.5	51.1	22.3	322.4	12.5	100	5.8	1.8	11.9	0.1	78.4	25	100	8.1	1.4	9.8	0.2	81.7	200	100	13.5	11.8	10.4	3.1	81.1
9	0	100	4.3	0.7	11.4	0	76.4	200	100	73.5	34.7	78	48.5	85	12.5	100	9	4.7	13.8	0.4	91.9	25	100	7.8	6.1	8.2	1	77	200	100	15.2	13.5	9.2	4.4	82.3
10	0	100	5.7	3.6	8.3	0	51.7	200	100	76.7	76.8	5.2	60.8	89	12.5	100	19	4.9	11.6	0.1	68.1	25	100	14.5	12	11.6	1.3	67.9	200	100	22.2	20.8	11	8.6	70.8
11	0	100	3.5	2.8	3.4	0	12.6	200	100	49.7	49.9	13.2	22.4	75.2	12.5	100	10.3	8.7	8.3	1.8	46.3	25	100	12.2	11.3	8.7	1.8	41.4	50	100	13.1	11.5	8.8	1.3	38.6
12	0	100	4.9	3.4	5.4	0	31.1	200	100	50.2	49.4	13.4	13.8	88	12.5	100	8.4	7.8	6.2	0	37.5	25	100	12.3	11.4	7.2	0.5	30	50	100	21.3	20.9	8.8	4.4	51.2
13	0	100	4	1.8	5.1	0	12.5	200	100	49.4	48	14.2	10.6	77.3	12.5	100	11.1	11.1	6.8	0	29	25	100	12.8	11.2	7.6	0.6	36.3	50	100	17.8	17	8.1	1.8	41.9
14	0	100	5	3.7	4.2	0.1	18.1	200	100	49.6	51.3	13.2	7.3	75.1	12.5	100	11.2	11	7.1	0	31.7	25	100	11.1	9.2	8.6	0.2	54.7	50	100	21.1	19.1	9.8	4.6	53.7
15	0	100	3.8	2.2	4.2	0	16.1	200	100	51.3	54.1	9.7	18.6	77.8	12.5	100	12.6	12.7	7.8	0	31.1	25	100	15.3	15.7	8.7	0.1	32.5	50	100	25	23.4	10.2	4.2	58.4
16	0	100	3.8	0.4	8	0	58	200	100	21.4	18.8	13.5	3.7	69.3	12.5	100	3	1.8	5.8	0	40.1	25	100	4.3	1.2	10.7	0	82	50	100	6.8	2.3	7.2	0	51.3
17	0	100	4.6	0.2	13	0	77.5	200	100	44.6	51.6	21.7	5	92.4	12.5	100	7.2	2.7	12.1	0	62.3	25	100	2.4	1.8	14.4	0	81.2	50	100	5.9	2	10.8	0.2	61.2
18	0	100	4.9	0.8	12.4	0	75.2	200	100	23.8	19.3	13.9	0	69.7	12.5	100	4.9	1.8	9	0	57	25	100	5.5	1	14.2	0	82.9	50	100	5.7	1.9	10.1	0.1	59.1
19	0	100	4.7	0.7	11.9	0	70.5	200	100	14.2	12.1	13.2	0	81.5	12.5	100	4.7	2.5	7.9	0	52.9	25	100	3.1	0.2	9.4	0	81.5	50	100	4.4	1.1	11.2	0	77.6
20	0	100	2.3	0.1	7	0	47	200	100	26.3	24	12.2	5.7	84.2	12.5	100	8.6	2.9	12.2	0	95.8	25	100	4.3	1.4	10.5	0	88.5	50	100	5.8	1.2	11.5	0.1	55.5
21	0	100	3.2	0.5	3.9	0	26	200	100	17	14.6	9.7	4.1	47.2	12.5	100	6.9	1.7	7.3	0	38.3	25	100	0.7	0.2	1.1	0	7.4	50	100	4.8	1.2	7.2	0	33.8
22	0	100	0.9	0	0.8	0	2.5	200	100	8.7	8.1	4.8	1.6	21	12.5	100	0.7	0.1	2	0	15.8	25	100	0.8	0	1.7	0	13.8	50	100	0.8	0	1.4	0	10.7
23	0	100	1.2	0.1	3.8	0	21.3	200	100	11	8.6	7.7	0.8	39.7	12.5	100	0.8	0.1	1.8	0	12.5	25	100	0.8	0.1	2.6	0	22.9	50	100	1.9	0.2	4.5	0	26.4
24	0	100	0.9	0.1	2.5	0	13	200	100	7.5	6.5	4.4	0.6	27.2	12.5	100	1.5	0.6	2.7	0	19.9	25	100	0.6	0.2	1.4	0	8.8	50	100	5.6	1.8	7.7	0	33.8
25	0	100	1.2	0.3	2.5	0	19	200	100	27.7	27.3	8.1	2.4	43	12.5	100	1.9	0.7	3.3	0	24.9	25	100	2	1.2	4	0	37.1	50	100	4.7	3.1	5.1	0.1	32.4

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

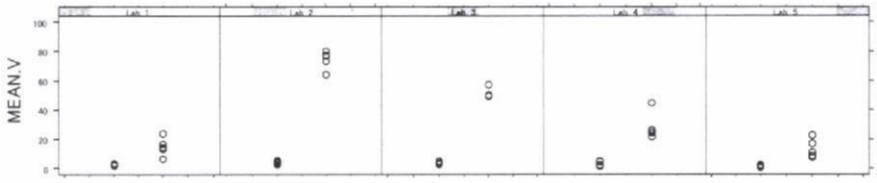


Fig. 2-LB

Table 2-LB

Chemical: B
Organ: Liver Endpoint: Olive tail moment
Transformation: id Link: id

		Vehicle					EMS				
Lab.	Variable	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
1	id(MEAN_V)	5	0	0	0	0	5	0	0	0	0
2	id(MEAN_V)	5	1.3	0.4	0.7	1.8	5	31.9	4.4	24.8	36.7
3	id(MEAN_V)	5	0.8	0.2	0.5	1.1	5	18.4	1.8	17.1	21.5
4	id(MEAN_V)	5	1.4	0.7	0.5	1.9	5	9.5	2.9	7.9	14.6
5	id(MEAN_V)	5	0.1	0.1	0	0.2	5	2	1.3	1	4

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

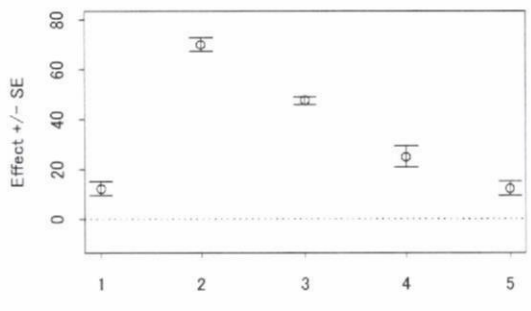


Fig. 3-LB

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

Table 3-LB

						Method					
						Pooled			Satterthwaite		
Lab.	Variable	Effect	SE	L95%	U95%	df	P-value	J T	df	P-value	J T
1	id(MEAN V)	12.22	2.81	5.73	18.7	8	0.002	1	4.08	0.012	1
2	id(MEAN V)	70.05	2.82	63.54	76.55	8	7.00E-09	1	4.28	8.60E-06	1
3	id(MEAN V)	47.57	1.57	43.94	51.19	8	2.00E-09	1	4.46	2.50E-06	1
4	id(MEAN V)	24.94	4.19	15.27	34.6	8	3.40E-04	1	4.25	0.003	1
5	id(MEAN V)	12.18	2.86	5.59	18.77	8	0.003	1	4.1	0.012	1

For the calculation for CIs the pooled method were used.

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

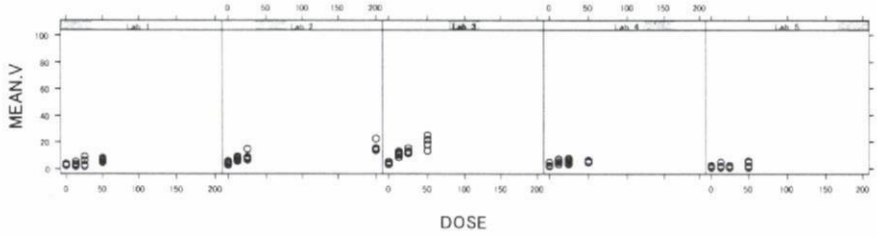


Fig. 4-LB

Table 4-LB

Chemical: B
Organ: Liver 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	2.8	0.6	2	3.5	12.5	5	3	1.5	1.4	5.3	25	5	5.1	3	1.7	9.1	50	5	6.5	1.4	4.5	8.3
2	id(MEAN_V)	0	5	4.3	1.2	2.6	5.7	12.5	5	7	1.5	5.6	9	25	5	9	3.2	6.5	14.5	200	5	16.1	3.5	13.5	22.2
3	id(MEAN_V)	0	5	3.7	0.8	2.9	5	12.5	5	10.9	1.6	8.4	12.9	25	5	12.7	1.6	11.1	15.3	50	5	19.7	4.5	13.1	25
4	id(MEAN_V)	0	5	3.5	1.6	1.3	4.9	12.5	5	5.2	1.6	3	7.2	25	5	5.1	1.6	3.1	7.4	50	5	5.2	0.7	4.4	5.9
5	id(MEAN_V)	0	5	1.2	0.7	0.3	2.3	12.5	5	1.9	1.7	0.7	4.9	25	5	1	0.7	0.6	2.1	50	5	3.5	2.2	0.6	5.6

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

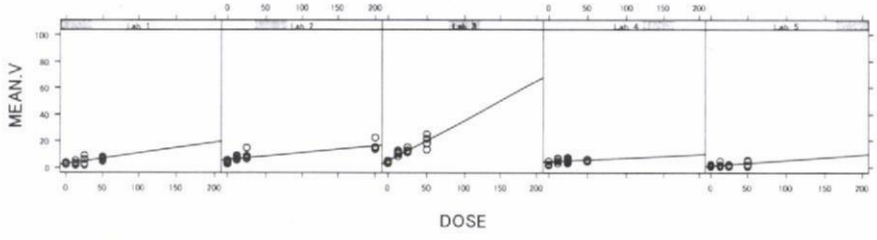


Fig. 5-LB

Table 5-LB Chemical: B
Organ: Liver Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Slope	SE	L95%	U95%	P-value	J_LI
1	id(MEAN_V)	0.0814	0.021	0.0366	0.1262	0.001	1
2	id(MEAN_V)	0.0515	0.007	0.0359	0.0671	1.80E-06	1
3	id(MEAN_V)	0.3008	0.033	0.2312	0.3704	3.90E-08	1
4	id(MEAN_V)	0.0274	0.018	-0.01	0.0647	0.141	0
5	id(MEAN_V)	0.0422	0.018	0.0034	0.081	0.035	1

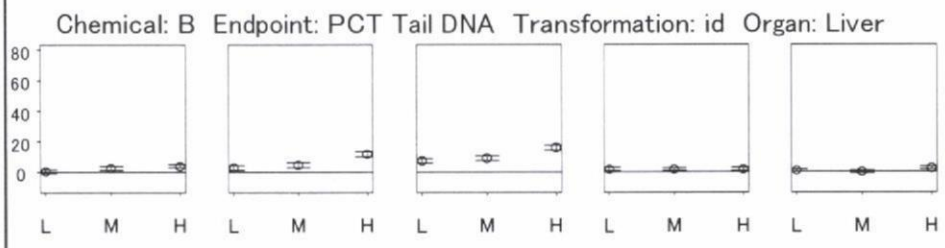


Fig. 6-LB

Table 6-LB

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

		V－L					V－M					V－H						
Lab.	Variable	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	J.D	J.L
1	id(MEAN.V)	0.29	16	1.15	0.651	0.402	2.35	16	1.15	0.07	0.029	3.79	16	1.15	0.006	0.002	1	1
2	id(MEAN.V)	2.61	16	1.61	0.141	0.062	4.65	16	1.61	0.014	0.005	11.78	16	1.61	2.50E−06	8.90E−07	1	1
3	id(MEAN.V)	7.22	16	1.61	5.20E−04	1.90E−04	9.06	16	1.61	5.30E−05	1.90E−05	16.02	16	1.61	4.20E−08	1.40E−08	1	1
4	id(MEAN.V)	1.68	16	0.92	0.102	0.043	1.54	16	0.92	0.129	0.057	1.72	16	0.92	0.094	0.04	0	1
5	id(MEAN.V)	0.76	16	0.92	0.402	0.21	−0.22	16	0.92	0.826	0.592	2.33	16	0.92	0.029	0.011	1	1

One side tests were performed

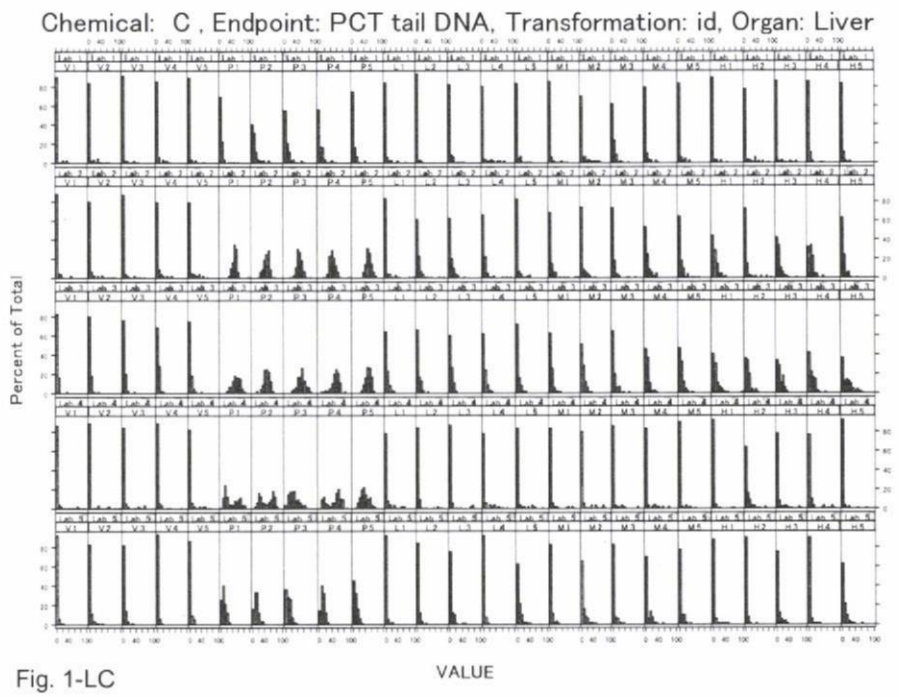


Fig. 1-LC

Table 1-LC

Chemical: C
Organ: Liver Endpoint: PCT tail DNA
Transformation: id

	Group																													
	Vehicle							EMS						Low						Medium						High				
Lab	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max	Dose	N	Mean	Med	SD	Min	Max
1	0	100	3.3	0	8	0	48.2	200	100	8.3	3.1	10.2	0	65.2	125	100	4.2	0.1	10.8	0	87.2	250	100	4.4	0.1	11.2	0	82.1	500	100	3.8	0.1	7.5	0	51.4
	0	100	4.7	0.1	12	0	82.1	200	100	12	7.7	12.9	0.1	66.6	125	100	1.4	0.2	2.6	0	24	250	100	2.1	0.5	16.8	0	66.4	500	100	2.9	0.1	18.7	0	82.8
	0	100	2.6	0	8.8	0	48.6	200	100	9.5	4.4	12.4	0	65.3	125	100	3.2	0.2	8.9	0	49.7	250	100	6	1.5	9	0	54.8	500	100	5.1	0.1	14.1	0	90
	0	100	3.4	0	9	0	45	200	100	9.2	4.1	11.3	0	58.2	125	50	8.6	0.1	15.3	0	69	250	100	4.2	0.2	8.6	0	40.1	500	100	2.8	0.2	7.2	0	50.8
	0	100	3.7	0	9.3	0	59.6	200	100	4.4	1.3	7.3	0	45.9	125	100	4.3	0.2	8.9	0	51.6	250	100	3.9	0.3	8.2	0	37.6	500	100	3.1	0.1	5.5	0	27.9
2	0	100	2.8	0.2	7.8	0	50.2	200	100	50.5	5	9.2	31.2	99.1	125	100	8.2	1.9	15.1	0	94.8	250	100	8.5	2.2	13.8	0	89.8	500	100	8	3.7	9.5	0	42.3
	0	100	7.2	0.9	16.7	0	81.3	200	100	48.9	50.7	9.5	25	72.8	125	100	8.2	4.7	12.8	0	92	250	100	6.1	2	9.1	0	49.2	500	100	8.9	3.2	16.7	0	91.2
	0	100	4.2	0.2	12.2	0	72.7	200	100	48.1	46.9	9.9	15.2	86.1	125	100	8.3	3.2	8.4	0	47.5	250	100	5.8	2.6	7.1	0	35	500	100	9.8	6.5	10.5	0	48
	0	100	5.1	0.8	10.2	0	51.8	200	100	45.7	44.1	11.1	15.4	99.7	125	100	7.4	3.8	13.8	0	88.7	250	100	8.8	3.4	9.2	0	38.9	500	100	10.8	9.1	9.4	0	57.2
	0	100	6.4	1.1	12.3	0	64.9	200	100	57.2	52.9	8.8	32.6	74.4	125	100	4.2	1.3	9.5	0	48.2	250	100	7	2.4	10.7	0	80.3	500	100	6.8	3.3	7.8	0.1	56.3
3	0	100	3.5	2.8	4.8	0	38.6	200	100	54.2	55.3	14.1	22.6	86	125	100	5.4	3.1	6.3	0	31.1	250	100	5.9	4.1	6.8	0	37.6	500	100	11.1	7.6	13.4	0	97
	0	100	3.1	1.3	4.1	0	29.3	200	100	49.2	49.1	10.1	18.5	73.5	125	100	5.7	3.5	8.5	0	38.8	250	100	7.1	5.8	7.2	0	36.1	500	100	10.2	9.2	8.8	0	41.4
	0	100	4	2.6	5.2	0	39.4	200	100	53	54.1	12.6	20.1	81.6	125	100	5.7	4	5.6	0	19.8	250	100	6.1	3.8	9	0	59.6	500	100	11.3	8.6	9.7	0	39.3
	0	100	3.9	2.6	3.9	0	19.8	200	100	53.6	55.4	12.5	12.9	81.6	125	100	5.6	2.9	6.4	0	28	250	100	7.6	6.3	7	0	37.4	500	100	10.5	9	9.9	0	53.7
	0	100	4	1.4	6	0	41.3	200	100	51.2	51.8	9.1	28.6	72.9	125	100	5.3	3.2	6.2	0	31.9	250	100	7.2	6.3	7.8	0	42.5	500	100	16.3	12.8	15.9	0	62.3
4	0	100	6	0.3	17.3	0	91	200	100	37.8	37	24.2	5.9	82.3	125	100	7.5	2.3	13.8	0	98	250	100	6.9	1.3	18.8	0	83.9	500	100	2.9	0.6	9.6	0	73.4
	0	100	5.8	0.2	17.5	0	98.7	200	100	46.1	50.8	22	19.2	81.7	125	100	5.7	0.8	13.8	0	85.1	250	100	3.2	1.2	13.8	0	80.4	500	100	6.3	4.1	12	0	71.3
	0	100	5.9	0.2	15.8	0	73.5	200	100	27.2	25.8	15.1	3.5	68.1	125	100	5.5	0.6	16.8	0	77.8	250	100	5.1	0.6	14	0	85.3	500	100	7.5	2	15.8	0	82.1
	0	100	3.2	0.3	10.2	0	82.1	200	100	47.8	56.2	22.8	5.9	68.1	125	100	8	1.4	15.8	0	79.9	250	100	6.9	0.7	15.7	0	78.6	500	100	8.9	1.7	19.2	0	82.8
	0	100	5.6	0.1	13.5	0	68	200	100	38.6	36.8	13.6	11.3	74.6	125	100	6.5	0.5	16.2	0	82.3	250	100	5.1	0.3	8.8	0	52.4	500	100	2.7	0.4	9.2	0	28
5	0	100	1.4	0.1	3	0	21.4	200	100	11.2	10.4	6.2	1.7	29.2	87.5	100	1.9	0.2	4.1	0	25.7	175	100	2.2	0.6	5.5	0	28.9	350	100	2.6	0.5	6	0	49
	0	100	3.4	0.4	8.5	0	44.2	200	100	13.6	12.9	7.5	2.1	38.8	87.5	100	2.3	0.3	4.3	0	27.2	175	100	6.7	0.2	10.4	0	49.3	350	100	1.9	0.2	4.8	0	39.7
	0	100	2.8	0.4	4.8	0	32.2	200	100	10.2	8.2	7.1	0.5	35.8	87.5	100	4.3	1.1	8.2	0	57.7	175	100	2.4	0.4	6.8	0	33.2	350	100	4.9	0.5	7.9	0	33.4
	0	100	1.2	0.2	2.5	0	16.1	200	100	11.2	11.1	6.3	0.3	30.6	87.5	100	1.1	0.4	2.6	0	15.5	175	100	0.9	1	0.8	0	53.8	350	100	2.6	0.4	5.3	0	38.1
	0	100	2.3	0.3	3.9	0	19.2	200	100	8.5	6.7	6.1	1.2	29.8	87.5	100	1.9	1	4	0	40.8	175	100	4.1	1	6.5	0	36.6	350	100	4	1.4	5.3	0	61.5

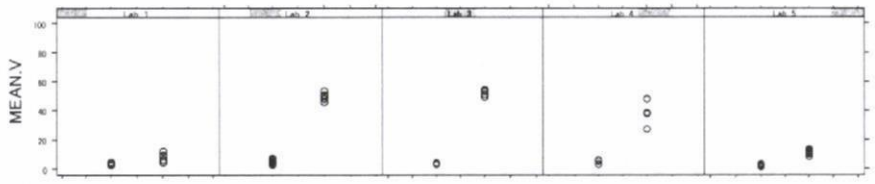


Fig. 2-LC

Table 2-LC

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

		Vehicle					EMS				
Lab.	Variable	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
1	id(MEAN_V)	5	3.3	0.8	2.6	4.7	5	8.3	3	4.4	12
2	id(MEAN_V)	5	5.1	1.7	2.8	7.2	5	49.5	2.9	45.7	53.4
3	id(MEAN_V)	5	3.7	0.4	3.1	4	5	52.2	2	49.2	54.2
4	id(MEAN_V)	5	5.3	1.2	3.2	6	5	39.9	8.6	27.2	48.1
5	id(MEAN_V)	5	2.2	0.9	1.2	3.4	5	11.2	2	8.5	13.6

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

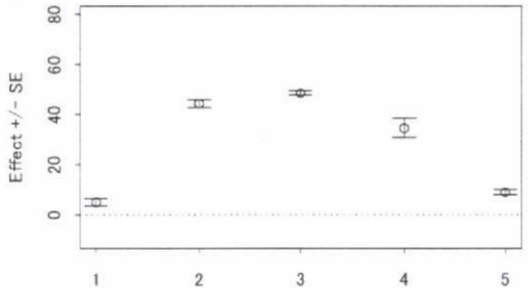


Fig. 3-LC

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

Table 3-LC

						Method					
						Pooled			Satterthwaite		
Lab.	Variable	Effect	SE	L95%	U95%	df	P-value	J T	df	P-value	J T
1	id(MEAN V)	4.97	1.39	1.77	8.17	8	0.007	1	4.58	0.018	1
2	id(MEAN V)	44.41	1.49	40.97	47.86	8	2.00E-09	1	6.58	2.90E-08	1
3	id(MEAN V)	48.54	0.93	46.4	50.68	8	2.00E-11	1	4.3	3.40E-07	1
4	id(MEAN V)	34.53	3.9	25.55	43.52	8	2.10E-05	1	4.15	7.60E-04	1
5	id(MEAN V)	8.99	0.97	6.75	11.24	8	1.50E-05	1	5.63	1.30E-04	1

For the calculation for CIs the pooled method were used.

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

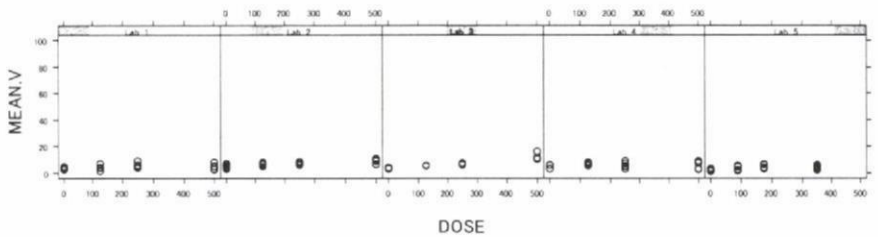


Fig. 4-LC

Table 4-LC

Chemical: C
Organ: Liver 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	3.3	0.8	2.6	4.7	125	5	4.1	1.9	1.4	6.8	250	5	5.5	2.2	3.9	9.1	500	5	4.3	2.2	2.6	7.9
2	id(MEAN V)	0	5	5.1	1.7	2.8	7.2	125	5	6.7	1.3	4.7	8.2	250	5	7.2	1.3	5.8	8.6	500	5	8.9	1.6	6.3	10.8
3	id(MEAN V)	0	5	3.7	0.4	3.1	4	125	5	5.5	0.2	5.3	5.7	250	5	6.8	0.7	5.9	7.6	500	5	11.9	2.5	10.2	16.3
4	id(MEAN V)	0	5	5.3	1.2	3.2	6	125	5	6.6	1	5.5	8	250	5	6.2	2.3	3.1	9.2	500	5	6.1	3	2.7	8.9
5	id(MEAN V)	0	5	2.2	0.9	1.2	3.4	87.5	5	3.2	1.8	1.7	5.6	175	5	4.7	1.6	3.2	6.7	350	5	3.8	1.6	1.9	6

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

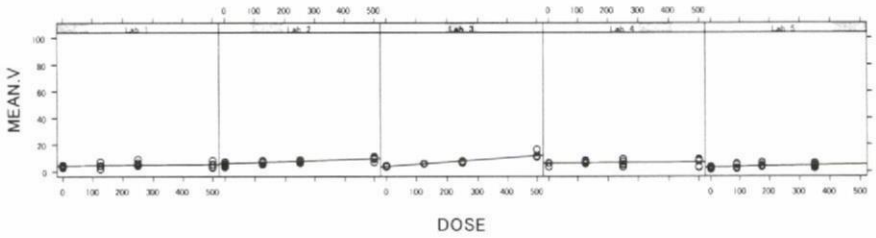


Fig. 5-LC

Table 5-LC Chemical: C
Organ: Liver Endpoint: PCT tail DNA
Transformation: id Link: id

Lab.	Variable	Slope	SE	L95%	U95%	P-value	J LI
1	id(MEAN_V)	0.0021	0.002	-0.003	0.0069	0.384	0
2	id(MEAN_V)	0.0072	0.002	0.0035	0.0108	6.10E-04	1
3	id(MEAN_V)	0.0164	0.002	0.013	0.0197	6.00E-09	1
4	id(MEAN_V)	0.0009	0.002	-0.004	0.006	0.7	0
5	id(MEAN_V)	0.0045	0.003	-0.001	0.0103	0.129	0

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

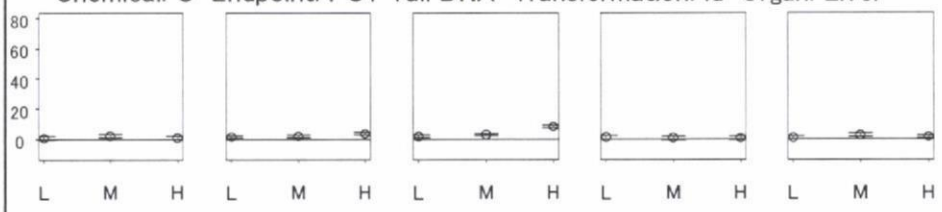


Fig. 6-LC

Table 6-LC

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

		V - L					V - M					V - H						
Lab.	Variable	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	Effect	df	SE	Dunnett	LSD	J D	J L
1	id(MEAN_V)	0.79	16	1.18	0.47	0.257	2.21	16	1.18	0.094	0.04	1.02	16	1.18	0.387	0.201	0	1
2	id(MEAN_V)	1.59	16	0.95	0.128	0.056	2.05	16	0.95	0.055	0.023	3.8	16	0.95	0.001	5.00E-04	1	1
3	id(MEAN_V)	1.83	16	0.83	0.052	0.021	3.08	16	0.83	0.003	9.40E-04	8.23	16	0.83	4.40E-08	1.50E-08	1	1
4	id(MEAN_V)	1.26	16	1.3	0.344	0.174	0.91	16	1.3	0.458	0.248	0.78	16	1.3	0.501	0.279	0	0
5	id(MEAN_V)	0.99	16	0.96	0.32	0.159	2.52	16	0.96	0.024	0.009	1.57	16	0.96	0.141	0.062	1	1

One side tests were performed

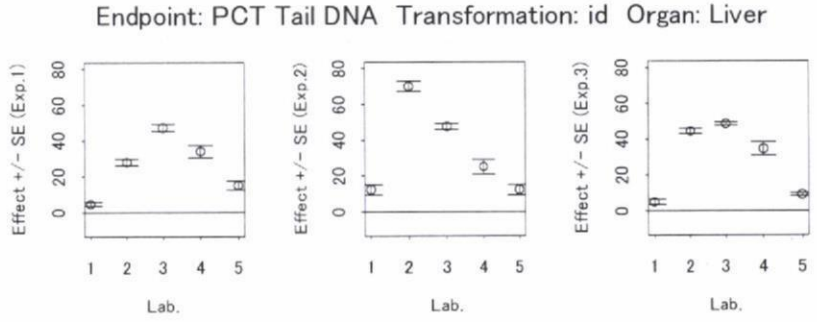


Fig. 7-L

Table 7-L

Organ: Liver 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				10.021		443.655		0		11.1734		6.96485
EXP	A	id(MEAN_V)	4.5554	1.36697	27.912	2.92431	47.274	3.96385	33.984	11.871	15.033	6.5359
	B	id(MEAN_V)	12.219	7.90982	70.048	7.95682	47.567	2.47403	24.937	17.5753	12.182	8.16573
	C	id(MEAN_V)	4.9697	1.92639	44.413	2.22959	48.542	0.86008	34.532	15.1863	8.9931	0.9457

Table 8-L

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

Variable	Component		Variance
id(MEAN_V)	Chem.(Lab.)		94.872
	Lab.		330.7
	Residual	1 A	1.367
		1 B	7.9098
		1 C	1.9264
		2 A	2.9243
		2 B	7.9568
		2 C	2.2296
		3 A	3.9638
		3 B	2.474
		3 C	0.8601
		4 A	11.871
		4 B	17.575
		4 C	15.186
		5 A	6.5359
		5 B	8.1657
		5 C	0.9457

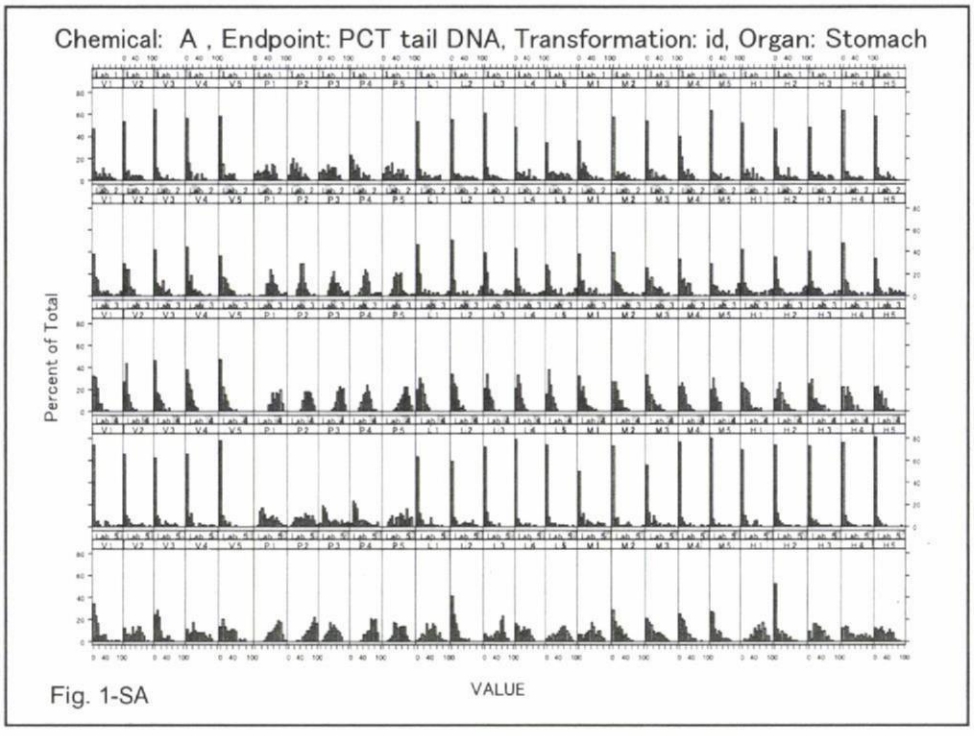


Fig. 1-SA

Table 1-SA