[redacted]

REPORT

[redacted] CD1 Mouse In Vivo Micronucleus Test

[redacted]:	[redacted]
Version ID:	Final report
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Sponsor and Test Facilities Details

Sponsor

[redacted]

Test facility
(Genetic Toxicology)

[redacted]

Test facility
(Statistics)

[redacted]

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[redacted]

Compliance with Good Laboratory Practice

[redacted] **CD1 Mouse In Vivo Micronucleus Test**

The study described in this report was conducted in compliance with the following Good Laboratory Practice and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose formulation.

[redacted]

8 August 2013

Date

[redacted]

Quality Assurance Statement

[redacted]: CD1 Mouse In Vivo Micronucleus Test

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol Audit	09 May 2013	09 May 2013
Protocol Amendment No.1	20 Jun 2013	20 Jun 2013
Report Audit	01 Aug 2013	01 Aug 2013

Process based inspections: At or about the time this study was in progress inspections of procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated below:

Process Based Inspections	Date(s) of Inspection	Date of Reporting to Management
Bodyweights, Dose Administration and post dose observations	05 Feb 2013	05 Feb 2013
Tissue sampling & processing	27 Feb 2013	27 Feb 2013
Slide staining and slide reading	09 Apr 2013-10 Apr 2013	12 Apr 2013
Study management & conduct	11 Jul 2013-12 Jul 2013	12 Jul 2013
Formulation procedures	24 Jul 2013	24 Jul 2013

In addition, process based inspections were conducted of other routine and repetitive procedures employed on this type of study at or about the time this study was in progress. Similarly an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were reported to Company Management.

[redacted]

..... 8 August 2013

Date

Contributing Scientists

[redacted] CD1 Mouse In Vivo Micronucleus Test

Genetic Toxicology Study Management

[redacted]
Study Director

Genetic Toxicology

[redacted]
Senior Animal Technician

[redacted]
Study Scientist

Statistics

[redacted]
Statistician

Summary

This study was designed to assess the potential induction of micronuclei by [redacted] in bone marrow cells of CD1 mice. Animals were treated with [redacted] orally by gavage on two occasions approximately 24 hours apart.

On the basis of results from the preliminary toxicity test, dose levels of 500, 1000 and 2000 mg/kg/day were selected for the micronucleus test. No substantial differences in toxicity were observed between the sexes in the preliminary toxicity test, therefore, in line with current guidelines the micronucleus test was performed using male animals only.

All animals in the vehicle control and test substance dose groups were dosed orally by gavage using a dose volume of 10 mL/kg. The positive control group were dosed orally by gavage using a dose volume of 20 mL/kg.

The vehicle control group received 1% methylcellulose in purified water and the positive control group received Mitomycin at 12 mg/kg.

Bone marrow smears were obtained from animals in the vehicle control and in each of the test substance groups 24 hours after administration of the second dose. In addition, bone marrow smears were also obtained from animals in the positive control group 24 hours after a single dose. One smear from each animal was examined for the presence of micronuclei in 2000 polychromatic erythrocytes. The proportion of polychromatic erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated normochromatic erythrocytes was also kept.

Results

No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes and no statistically significant decreases in the proportion of polychromatic erythrocytes were observed in CD1 mice treated with [redacted] at any treatment level, compared to vehicle control values.

The positive control compound, Mitomycin C, produced a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes ($p<0.01$).

Conclusion

It is concluded that [redacted] did not show any evidence of causing an increase in the induction of micronucleated polychromatic erythrocytes or bone marrow cell toxicity in male CD1 mice when administered orally by gavage in this *in vivo* test procedure.

1. Introduction

1.1 Objective

The purpose of this study was to assess the potential of [redacted] to induce an increase in the induction of micronucleated polychromatic erythrocytes in CD1 mice following oral administration using an *in vivo* cytogenetic system (Boller and Schmid 1970, MacGregor *et al* 1987, Mavournin *et al* 1990).

1.2 Regulatory compliance

The performance of this study was in compliance with the following Guidelines:

OECD Guideline for the Testing of Chemicals. (1997) Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test, Guideline 474.

EC Commission Regulation No. 440/2008. Method B.12: Mutagenicity - In vivo Mammalian Erythrocyte Micronucleus Test. OJ L 142/240.

US EPA (1998) Health Effects Test Guidelines; OPPTS 870.5395 Mammalian Erythrocyte Micronucleus Test. EPA 712-C-98-226.

Official notice of MHLW, METI and MOE (31 March 2011)
YAKUSHOKUHATSU 0331 No 7
SEIKYOKU No 5
KANPOKIHATSU No 110331009

Official Notice of J MOL (8 February 1999).

1.3 Test system

The bone marrow micronucleus test is a short-term assay for identification of genotoxic effects associated with mutagens and carcinogens (Mavournin *et al* 1990). Young adult animals are chosen for use because of the high rate of cell division in the bone marrow, the wealth of background data on this species, and their general suitability for toxicological investigations.

In mitotic cells in which chromosomal breakage has been caused by the test substance or its metabolites, acentric fragments of the chromosomes do not separate at the anaphase stage of cell division. After telophase these fragments may not be included in the nuclei of the daughter cells and hence will form single or multiple micronuclei in the cytoplasm of these cells.

A few hours after the last mitosis is completed, erythroblasts expel their nucleus. Young erythrocytes less than 24 hours old are termed polychromatic erythrocytes. More mature erythrocytes are termed normochromatic erythrocytes.

Substances which interfere with the mitotic spindle apparatus will cause non-disjunction or lagging chromosomes at anaphase which may not be incorporated into the daughter nuclei. These lagging chromosomes are not excluded from the erythroblast with the main nucleus and hence also give rise to micronuclei.

Any toxic effects of the test substance on the nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in the number of nucleated cells and polychromatic erythrocytes. If the proportion of polychromatic erythrocytes is found to be significantly less than the control value, this is taken as being indicative of toxicity.

2. Experimental procedure

2.1 Time schedule

Study initiation:	13 May 2013
(Protocol signed by the Study Director)	
Experimental start date:	15 May 2013
Animal arrival date at [redacted]	
Preliminary toxicity test:	15 May 2013
Micronucleus test:	29 May 2013
Study completion:	13 June 2013
(Statistical analysis)	

2.2 Duration of treatment

The test substance was administered on two occasions approximately 24 hours apart.

2.3 Route of administration

The oral route was chosen for this particular study as to maximise exposure to the test system.

2.4 Animal management

2.4.1 Animal supply, acclimatisation and allocation

All animals used on this study were CD1 mice

On the day after arrival from [redacted], animals used on the study weighed:

Preliminary toxicity test:	Males weighed 27.8 g
	Females weighed between 23.6 g to 23.8 g.
Micronucleus test:	Males weighed between 28.6 g to 33.4 g.

Animal age on despatch and on Day 1 of dosing was:

Preliminary toxicity test:	On despatch	Males and females *ca* 35 - 42 days old.
	Day 1	Males and females *ca* 40 - 47 days old.
Micronucleus test:	On despatch	Males *ca* 35 - 42 days old.
	Day 1	Males *ca* 42 - 49 days old.

The day after arrival the weight of the animals was checked and found to be acceptable. The animals were randomly assigned to groups and given a unique tail tattoo. Each group was kept with the sexes separated, in cages and maintained in a controlled environment with the thermostat and relative humidity target ranges set at 19 to 23°C and 40 to 70% respectively. Temperature and humidity were within range throughout the study. The room was illuminated by artificial light for 12 hours per day.

All animals were allowed free access to pelleted expanded rat and mouse No.1 maintenance diet (SQC grade obtained from Special Diets Services Ltd, Witham, Essex, UK) and tap water *ad libitum*.

Food, nestlets and tap water are routinely analysed for quality at source. All animals were given nestlets and a red plastic shelter for environmental enrichment, were acclimatised for a minimum of 5 days, examined daily and weighed prior to dosing.

2.4.2 Clinical observations

Animals were inspected at least twice daily for evidence of ill-health or reaction to treatment. Cages were inspected for evidence of animal ill-health amongst the occupants and any deviations from the normal was recorded at the time in respect of nature and severity, date and time of onset.

2.4.3 Bodyweight

Animal weights were recorded for each animal on the day after arrival, on each day of dosing and on the day of termination.

2.5 Test substance

Identity:	[redacted]
Chemical name:	[redacted]
Batch number:	OF1211
Appearance:	Pale yellow powder
Storage conditions:	Room temperature (ca. 20ºC) in the dark
Purity:	99.9%
Expiry date:	30 April 2014
Date received:	28 January 2013

The above information with regard to the physical characterisation of the test substance is the responsibility of the Sponsor.

2.6 Test substance formulation and administration

Stability and homogeneity of the test substance and of the test substance in the vehicle were not determined in this test and remain the responsibility of the Sponsor. Chemical analysis of dosing formulations for achieved concentration was not performed in this study.

Suspensions of the test substance were prepared in 1% methylcellulose in purified water. The methylcellulose was obtained from Sigma, batch number MKBK1179V

Mitomycin C obtained from Sigma, batch number SLBD1982V was used as the positive control compound. A solution was prepared using purified water at a concentration of 0.6 mg/mL just prior to administration.

All animals in the vehicle control and test substance dose groups were dosed orally by gavage using a dose volume of 10 mL/kg. The positive control group were dosed at 20 mL/kg via oral gavage.

2.7 Preliminary toxicity test

The purpose of this test was to determine suitable dose levels for use in the micronucleus test. The dosages employed were used to give an approximate indication of the maximum tolerated dose, *i.e.* the highest dosage which would be expected to elicit signs of toxicity without producing extreme clinical signs or having a significant effect on survival. The experimental design is shown below:

Group	Treatment	Concentration (mg/mL)	Dosage (mg/kg/day)	Number of animals	
				Males	Females
1	[redacted]	200	2000	2	2

Following dosing, the animals were examined regularly during the working day for a period of 48 hours after the first dose and any mortalities or clinical signs of reaction during the experiment were recorded. At the end of this observation period, surviving animals were killed and discarded.

2.8 Micronucleus test

Following the preliminary toxicity test no substantial differences in toxicity were observed between sexes. Therefore in line with current guidelines, the micronucleus test was performed using males only.

From the results obtained in the preliminary toxicity test (see RESULTS), dose levels of 500, 1000 and 2000 mg/kg/day were used for the micronucleus test. The experimental design is shown below:

Group	Treatment	Concentration (mg/mL)	Dosage (mg/kg/day)	Number of animals Males
1	Vehicle	-	-	6
2	[redacted]	50	500	6
3	[redacted]	100	1000	6
4	[redacted]	200	2000	6
5	Mitomycin C [a]	0.6	12	5

Vehicle 1% methylcellulose in purified water

a Positive control dosed once only approximately 24 hours prior to termination at a dose volume of 20 mL/kg.

Following dosing, the animals were examined regularly and any mortalities or clinical signs of reaction were recorded. Animals from the vehicle control and test substance groups were sacrificed 24 hours after administration of the second dose. In addition animals in the positive control group were sacrificed 24 hours after a single dose.

The animals were killed by exposure to rising levels of carbon dioxide and both femurs dissected out from each animal. The femurs were cleaned of all excess tissue and blood and the proximal epiphysis removed from each bone. The bone marrow of both femurs from each animal was flushed out and pooled in a total volume of 3 mL of filtered foetal calf serum by aspiration.

The resulting cell suspensions were centrifuged at 1000 rpm (150 × *g*) for 5 minutes and the supernatant discarded. The final cell pellet was resuspended in a small volume of foetal calf serum to facilitate smearing in the conventional manner on glass microscope slides (Schmid 1976).

2.9 Fixation and slide staining

1 Fixed for a minimum of 10 minutes in methanol and allowed to air-dry

2 Rinsed in purified water

3 Stained in acridine orange solution (0.0125 mg/mL using purified water) for 4 minutes

4 Washed in purified water for 5 minutes

5 Rinsed in cold tap water for 2 minutes

6 Stored at room temperature until required

7 Immediately prior to scoring, slides are wet mounted with coverslips using purified water

2.10 Microscopic examination

Coded slides were examined by fluorescence microscopy and 2000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. One smear was examined per animal, any remaining smears being held temporarily in reserve in case of technical problems with the first smear.

The proportion of polychromatic erythrocytes was assessed by examination of a total of at least 1000 erythrocytes per animal and the number of micronucleated normochromatic erythrocytes was recorded.

3. Assessment of results

3.1 Acceptance criteria

The following criteria were applied for assessment of assay acceptability:

1. Each treated and control group should include at least 5 analysable animals.
2. Vehicle control values for micronucleated polychromatic erythrocytes must be consistent with the laboratory historical vehicle control data.
3. Positive controls must show clear unequivocal positive responses.

3.2 Analysis of data

For the proportion of polychromatic erythrocytes at 24 hours, an asymptotic one-tailed Jonckheere's test for trend (Jonckheere 1954) with "step-down" was used on Groups 1 to 4 for a decrease from control. If significant, then the analysis was carried out on Groups 1 to 3, then on Groups 1 and 2. Exact one-tailed Wilcoxon pairwise tests (Wilcoxon 1945), for a decrease from control, were also carried out on Group 1 (control) versus Groups 2, 3, 4 and 5.

For incidences of micronucleated polychromatic erythrocytes at 24 hours, an exact one-tailed Linear-by-Linear association test (Cytel 1995) with "step-down" was used on Groups 1 to 4 for an increase from control. If significant, then the analysis was carried out on Groups 1 to 3. Also, exact one-tailed pairwise Permutation tests (Cytel 1995), for an increase from control, were carried out on Group 1 (control) versus Groups 2, 3, 4 and 5.

Statistical significance was declared at the 5% level for all tests.

The data were received in an Excel document and analysed using SAS 9.1.3 (SAS Institute Inc., 2002) (Jonckheere's and Wilcoxon tests) and StatXact 3 (Cytel 1995) (Linear-by-Linear and Permutation tests).

3.3 Criteria for assessing clastogenic/aneugenic potential

A positive response is normally indicated by a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes for the treatment group compared with the vehicle control group ($p<0.05$); individual and/or group mean values should exceed the laboratory historical control range (Morrison and Ashby 1995).

A negative result is indicated where individual and group mean incidences of micronucleated polychromatic erythrocytes for the group treated with the test substance are not significantly greater than incidences for the concurrent vehicle control group and where these values fall within the historical control range.

An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant decrease in the proportion of polychromatic erythrocytes ($p<0.05$).

4. Archiving

Following completion of this study all raw data, specimens and samples, except those generated or used during any Sponsor's or supplier's analysis, were stored in the archives of [redacted] Types of sample and specimen which are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in [redacted] Standard Operating Procedures.

A copy of the final report will be retained indefinitely and all Quality Assurance inspection records for a period of 20 years. All other appropriate specimens and records will be retained for a minimum period of 1 year from the date of issue of the final report. At the end of this retention period the Sponsor will be contacted and advice sought on future archiving requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

5. Results

The historical control data is summarised in Appendix 2.

5.1 Preliminary toxicity test

No mortalities were observed throughout the duration of the preliminary test. Detailed bodyweights are presented in Appendix 1.

5.1.1 Clinical signs

To determine suitable dose levels for use in the micronucleus test, one group consisting of two male and two female animals were administered [redacted] at 2000 mg/kg/day on two consecutive days approximately 24 hours apart.

At 2000 mg/kg/day the standard limit dose for the micronucleus test, no clinical signs of toxicity were observed. Some small incidences of bodyweight loss were observed during the preliminary toxicity test.

On the basis of this result 2000 mg/kg/day was considered to be the maximum tolerated dose in both male and female animals.

In line with current guidelines and as no substantial differences in toxicity were observed between the sexes, male animals only were used in the micronucleus test, dose levels of 500, 1000 and 2000 mg/kg/day were selected.

5.2 Micronucleus test

The micronucleus test was carried out in male animals only.

Table 1 gives a summary of the results of the micronucleus test and the results of statistical analysis. The results for individual animals are presented in Table 2.

No mortalities were observed throughout the duration of the micronucleus test. Detailed bodyweights are presented in Appendix 1.

5.2.1 Clinical signs

Animals were treated with [redacted] at dose levels of 500, 1000 and 2000 mg/kg/day.
No clinical signs of toxicity were observed for the vehicle control, positive control or animals administered [redacted] over the duration of the test.

Some small incidences of bodyweight loss were observed during the micronucleus test.

5.2.2 Micronucleated polychromatic erythrocyte counts (MPCE)

[redacted] did not cause any statistically significant increases in the number of micronucleated polychromatic erythrocytes in male CD1 mice.

Mitomycin C caused a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes ($p<0.01$) in male CD1 mice.

5.2.3 Micronucleated normochromatic erythrocytes (MNCE)

[redacted] did not cause any significant increases in the incidence of micronucleated normochromatic erythrocytes in male CD1 mice.

5.2.4 Proportion of polychromatic erythrocytes (%PCE)

[redacted] did not cause any statistically significant decreases in the proportion of polychromatic erythrocytes in male CD1 mice.

Mitomycin C did not cause a statistically significant decrease in the proportion of polychromatic erythrocytes in male CD1 mice.

6. Conclusion

It is concluded that [redacted] did not show any evidence of causing an increase in the induction of micronucleated polychromatic erythrocytes or bone marrow cell toxicity in male CD1 mice when administered orally by gavage in this *in vivo* test procedure.

7. References

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SCHMID, W. (1976) The micronucleus test for cytogenetic analysis. In: HOLLANDER, A. (ed.) *Chemical Mutagens, Principles and Methods for their Detection*, **4**, 31. Published by Plenum Press, New York.

WILCOXON, F. (1945). Individual comparisons by ranking methods. *Biometrics Bulletin*, **1**, 80-83.

Table 1 Summary of results and statistical analysis

Sampling time after 2nd dose	Treatment	Dose (mg/kg/day)	Proportion of PCE (Group mean %) #	Incidence MPCE (Group mean) #
24 Hours	Vehicle	-	49.6	1.7
	[redacted]	500	50.0	1.5
	[redacted]	1000	47.5	1.0
	[redacted]	2000	46.5	1.2
	Mitomycin C [a]	12	52.8	93.6**

Vehicle 1% methylcellulose in purified water

PCE Polychromatic erythrocytes

MPCE Number of micronucleated polychromatic erythrocytes observed per 2000 polychromatic erythrocytes examined

[a] Positive control dosed once only 24 hours prior to termination

Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

** $p < 0.01$ (significant)

otherwise $p > 0.01$ (not significant)

Table 2 Individual animal data

Results for individual animals - 24 hour sampling time

Treatment (mg/kg/day)	Animal number	Proportion PCE (%)	MPCE	PCE	NCE	MNCE
Vehicle (-)	701	58.1	1	584	422	0
	702	45.5	0	455	545	0
	703	49.0	4	493	514	0
	704	43.8	1	461	591	0
	705	50.0	2	503	502	0
	706	51.5	2	542	511	0
[redacted] (500)	711	48.1	3	485	524	0
	712	48.5	0	489	520	0
	713	54.0	3	558	476	0
	714	56.7	0	584	446	0
	715	45.9	2	460	543	0
	716	46.9	1	483	546	0
[redacted] (1000)	721	44.3	1	484	609	1
	722	42.4	0	436	593	1
	723	53.3	0	559	490	0
	724	47.9	0	488	530	0
	725	48.0	5	481	522	0
	726	49.4	0	506	518	0
[redacted] (2000)	731	31.5	3	323	701	0
	732	41.3	0	451	640	0
	733	43.5	0	440	571	0
	734	52.4	2	676	615	0
	735	51.5	2	519	489	0
	736	59.0	0	599	417	0
Mitomycin C [a] (12)	741	51.3	118	515	488	0
	742	47.3	89	523	582	0
	743	51.2	92	528	503	0
	744	60.3	53	644	424	0
	745	53.6	116	546	472	0

Vehicle 1% methylcellulose in purified water
PCE Number of polychromatic erythrocytes per 1000 erythrocytes
MPCE Number of micronucleated cells observed per 2000 polychromatic erythrocytes examined
NCE Total number of normochromatic erythrocytes examined for micronuclei
MNCE Number of micronucleated normochromatic erythrocytes observed
[a] Positive control dosed once only 24 hours prior to termination

Appendix 1 Bodyweights

Preliminary toxicity test

Treatment (mg/kg/day)	Animal Number		Bodyweights (g) At Treatment Day after arrival Individual	Mean	Day 1 Individual	Mean	Day 2 Individual	Mean	At Termination Day 3 Individual	Mean
[redacted] (2000)	M	81	27.8	27.8	29.8	30.1	30.6	31.0	31.2	31.0
	M	82	27.8		30.4		31.4		30.8#	
	F	83	23.6	23.7	25.1	25.3	24.1#	25.3	24.2	25.5
	F	84	23.8		25.4		26.4		26.8	

#	Denotes weight loss from previous weighing
M	Male
F	Female

Appendix 1 Bodyweights - continued

Micronucleus test

Treatment (mg/kg/day)	Animal Number	Bodyweights (g) Day after arrival		At Treatment Day 1		Day 2		At Termination Day 3	
		Individual	Mean	Individual	Mean	Individual	Mean	Individual	Mean
Vehicle (-)	701	30.4	30.3	33.1	33.0	32.8#	33.3	33.8	33.5
	702	30.3		32.6		32.7		33.0	
	703	28.8		30.6		31.0		30.9#	
	704	31.0		33.6		34.6		34.6	
	705	31.2		34.8		34.8		35.2	
	706	29.9		33.2		33.6		33.4#	
[redacted] (500)	711	30.8	30.6	34.0	33.7	34.1	33.8	34.8	34.3
	712	33.4		36.7		36.8		37.2	
	713	30.0		32.8		32.6#		32.8	
	714	29.8		31.8		32.2		32.6	
	715	29.9		33.1		33.6		34.2	
	716	29.4		33.6		33.5#		34.2	
[redacted] (1000)	721	31.8	30.3	33.6	33.6	34.4	33.8	34.4	34.1
	722	29.7		33.4		33.5		33.9	
	723	30.4		34.0		34.0		34.1	
	724	29.6		34.3		34.0#		34.8	
	725	30.8		34.7		34.9		35.5	
	726	29.4		31.8		32.1		32.0#	
[redacted] (2000)	731	28.6	30.2	31.7	33.7	31.2#	33.8	32.3	34.3
	732	29.1		32.3		32.4		31.9#	
	733	33.1		35.4		35.0#		36.1	
	734	30.1		32.8		33.9		34.2	
	735	29.0		33.6		34.0		34.2	
	736	31.2		36.2		36.3		36.9	
Mitomycin C [a] (12)	741	32.6	31.1	-	-	36.2	34.8	35.4#	34.3
	742	31.0		-		34.4		34.3#	
	743	29.5		-		32.6		32.2#	
	744	30.4		-		34.3		33.5#	
	745	31.8		-		36.7		36.2#	

Vehicle 1% methylcellulose in purified water
# Denotes weight loss from previous weighing
a Positive control dosed once only 24 hours prior to termination

Appendix 2 Historical control data

CD1 Mice

Vehicle Control Values –April 2011– March 2013 (11 studies)

Proportion of Polychromatic Erythrocytes (% PCE)

	Individual	Group
Minimum	32.0	43.2
Maximum	71.0	54.8
Mean	49.1	49.4
Standard Deviation	6.4	3.6

Micronucleated Polychromatic Erythrocytes (MPCE)
(per 2000 PCE cells)

	Individual	Group
Minimum	0	0.4
Maximum	5	2.6
Mean	1.3	1.3
Standard Deviation	1.2	0.6

Positive Control Values – April 2011 – March 2013 (11 studies)

Proportion of Polychromatic Erythrocytes (% PCE)

	Individual	Group
Minimum	33.0	44.0
Maximum	67.0	53.2
Mean	47.7	47.7
Standard Deviation	6.1	2.8

Micronucleated Polychromatic Erythrocytes (MPCE)
(per 2000 PCE cells)

	Individual	Group
Minimum	24	27.5
Maximum	163	94.0
Mean	68.2	66.7
Standard Deviation	28.7	20.0

Annex 1 GLP compliance statements

DH Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

[redacted]

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Ecosystems
Phys.Chem. Testing
Residue studies
Mutagenicity
Toxicology

DATE OF INSPECTION
18th – 20th June 2012

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999)

14/9/12

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

Annex 1 GLP compliance statements - continued

Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

[redacted]

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Ecosystems
Toxicology
Residue Studies

DATE OF INSPECTION **20 to 22 November 2012**

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

22/2/13

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA