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Series on Testing and Assessment

No. 114

PERFORMANCE ASSESSMENT OF DIFFERENT CYTOTOXIC AND CYTOSTATIC MEASURES FOR THE IN VITRO MICRONUCLEUS TEST (MNVIT): SUMMARY OF RESULTS IN THE COLLABORATIVE TRIAL

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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides and Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

FOREWORD

This document is the Performance Assessment Report from the collaborative trial of assessing different cytotoxic and cytostatic measurements for the *in vitro* mammalian cell micronucleus test.

The draft Test Guideline for the *in vitro m*ammalian cell micronucleus test was submitted for approval at the 20th meeting of the Working Group of National Coordinators of the Test Guidelines programme (WNT). At the meeting, there were concerns that the Relative Population Doubling (RPD) and Relative Increase in Cell Counts (RICC) methods for estimating cytotoxicity proposed in the draft Test Guideline had not been sufficiently substantiated. The WNT provisionally approved the draft Test Guideline pending the results of the performance assessment of the RICC and RPD methods for assessing cytotoxicity.

The United Kingdom led an EU consortium for the performance assessment of the two methods. Dr. David Kirkland (Covance Laboratories Limited, UK) coordinated the performance assessment, collation of data and the drafting of this performance assessment report. Using the agreed methods, data have been provided for 14 different chemicals in 5 different cell types tested in 12 laboratories, as is presented in the summary tables of this document. Detailed data from individual laboratories can be made available upon request to the Secretariat.

The WNT approved the submission of this report to the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology on 27 November 2009, by written procedure.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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PERFORMANCE ASSESSMENT OF DIFFERENT CYTOTOXIC AND CYTOSTATIC MEASURES FOR THE *IN VITRO* MICRONUCLEUS TEST (MNVIT): SUMMARY OF RESULTS IN THE COLLABORATIVE TRIAL

ABSTRACT

1. To respond to concerns raised by the 20th meeting of the Working Group of National Coordinators of the Test Guidelines programme (WNT20), the performance of the cytotoxicity assays used in the draft TG 487 on "*In Vitro Mammalian Cell Micronucleus Test (MNvit)*" (1) was assessed. This paper summarises the data for 14 different chemicals tested for induction of micronuclei (MN) in 5 different cell types across 12 different laboratories. All 14 chemicals induced biologically and statistically significant increases in MN frequency in the different cell types (L5178Y, TK6, CHO, CHL, V79) in the absence of cytochalasin B at or below target range toxicity (55 ± 5%) irrespective of whether Relative Cell Count (RCC), Relative Increase in Cell Count (RICC) or Relative Population Doubling (RPD) was used as a measure of cytotoxicity/cytostasis to select the top concentration. All measures of cytotoxicity in the absence of cytochalasin B are therefore considered equally acceptable for use, and the responses were comparable to those obtained in the presence of cytochalasin B.

INTRODUCTION

2. Details of the rationale for the trial have been described by Kirkland (2). The objective was to determine whether genotoxic chemicals of different chemical classes and different modes of action would induce significant levels of micronuclei in cultured cells *in vitro* in the absence of cytochalasin B when different measures of cytotoxicity (detecting cytostasis and cell death) were used to select the top concentration. The individual reports from each of the participating laboratories in the trial will be published in a Special Issue of Mutation Research. The detailed data can be reviewed there.

SUMMARY OF FINDINGS

3. It is clear that different cells exhibited different control MN frequencies, and the same cells in different laboratories also exhibited different control MN frequencies. Therefore, for ease of comparison, the key results obtained at or below the target toxicity range (55 ± 5%) are summarised in Tables 1-14 for each chemical. The ranges of control MN responses within this series of experiments are also shown in Table 15, to allow comparison with absolute MN frequencies in treated cultures. However, fold increase in MN response and statistical difference from concurrent control are also given in Tables 1-14 (statistical methods are described for each laboratory in the individual papers). From the data in these tables the following conclusions can be drawn:

4. All chemicals (including the less well defined genotoxins diazepam, phenolphthalein and quinacrine dihydrochloride) were detected as positive in most cell types in the absence of cytochalasin B at levels of toxicity at or below the target range (55 ± 5% toxicity), irrespective of the choice of cytotoxicity measure (RCC, RICC or RPD).

5. One chemical (2-aminoanthracene)(2-AA) in one cell type (CHO cells) in one laboratory (Covance) gave a weak but statistically significant MN response when the top concentration was selected by RCC but not when selected by RICC or RPD (Table 9). There was also a positive MN response in the presence of cytochalasin B. However, all responses were weak and not clearly dose-related, and therefore the differences between the different cytotoxicity measures were marginal. When recovery was extended from 21 to 41 hours the MN result was negative by all measures of cytotoxicity, with and without

cytochalasin B. Other cells in other laboratories gave positive responses by all measures of cytotoxicity with 2-AA. Since 2-AA requires CYP1A2 activation followed by acetyltransferase (3) this result may be explained by sub-optimal metabolic activation in these particular experiments at Covance.

6. In addition to 2-AA, 5-FU and cadmium chloride (Tables 7 and 11) also produced quite weak MN responses in some cells and in some laboratories that were not always convincingly positive by any measure of cytotoxicity. In CHO cells at Covance, 5-fluoroucil (5-FU) was only positive by RPD in one experiment using a 24 hr treatment with 24 hr recovery, and it was negative by all cytotoxicity measures in V79 cells. 5-FU can cause severe cell cycle delay, and was not easily detected in the SFTG trial (4).

7. Of the chemicals that were tested in the presence of cytochalasin B, all except quinacrine 2HCl (see below and Table 12) were detected as positive at levels of toxicity at or below the target range. However, the comments below on colchicine in mononucleate and binucleate cells should also be noted.

8. For most chemicals, the concentrations at which target range toxicity was achieved in the presence of cytochalasin B (by Replicative Index, RI) were similar to the concentrations at which target range toxicity was achieved by the 3 measures used in the absence of cytochalasin B. In some cases higher concentrations were needed to achieve target toxicity by RI, and in some cases lower, even within the data set for the same chemical. Thus there was no uniform trend related to the concentration needed to achieve target toxicity in the absence or presence of cytochalasin B.

9. For all chemicals and every cell type either the extent of toxicity according to RCC at a given concentration was less than according to RICC, or the concentration required to achieve a particular level of cytotoxicity was higher in the case of RCC than for RICC. Thus RICC never identified a higher concentration for target range toxicity than RCC.

10. RCC and RPD frequently identified similar concentrations producing toxicity at or near the target range. In some cases RPD identified more toxicity at a given concentration than RCC, and in other cases less toxicity.

11. MN frequencies were often much higher at the same concentration in the presence of cytochalasin B than in its absence. However, control MN frequencies were also generally higher in the presence of cytochalasin B. Obviously in the presence of cytochalasin B the population of cells that has divided is clearly identifiable and therefore the MN frequency is determined only from (binucleate) cells known to have divided. In the absence of cytochalasin B, as cells are mononucleate, the population of cells from which the MN frequency is determined may include some cells that have not divided, and therefore the MN frequency is understandably lower.

12. For colchicine (Table 5) and vinblastine (Table 6) the concentration ranges at which toxicity and MN were induced were very narrow, emphasising the need for close spacing of concentrations, and in many laboratories in this trial it took several attempts before concentrations inducing target range toxicity were identified.

13. Following colchicine treatment in the presence of cytochalasin B, MN frequencies in binucleate cells were low, and on several occasions were not significantly different from controls. This was expected and is due to mitotic slippage (5). When MN were scored in mononucleate cells in these cytokinesis-blocked cultures, significant induction of MN was found in all cases (Table 5).

14. For mitomycin C (Table 2), benzo[a]pyrene (Table 3), diethylstilboestrol (Table 8) and etoposide (Table 10) significant MN induction was seen in most or all cells at levels of toxicity notably <50%.

15. For mitomycin C (Table 2) and etoposide (Table 10), large fold increases in MN frequency over control levels were seen in all cell types. On the other hand, 5-FU (Table 7) and cadmium chloride (Table 11) consistently showed low (2-3-fold) increases in MN frequency even at target range toxicity.

16. For quinacrine 2HCl (Table 12), although it induced significant MN in TK6 and CHO cells in the absence of cytochalasin B, in the one study in CHO cells in the presence of cytochalasin B it did not induce significant MN at concentrations inducing up to 50% cytostasis (reduction in CBPI).

17. In TK6 cells, most chemicals (mitomycin C, benzo[a]pyrene, colchicine, vinblastine, 2-aminoanthracene, etoposide and cadmium chloride) gave lower fold increases in MN response than in the other cell types. This may reflect that TK6 cells are p53 competent, and therefore some of the damaged cells will be lost through apoptosis whereas the p53-defective rodent cell lines are more likely to survive and replicate with the damage, leading to higher MN frequencies.

CONCLUSIONS

18. All 14 chemicals induced biologically and statistically significant increases in MN frequency in different cell types (L5178Y, TK6, CHO, CHL, V79) in the absence of cytochalasin B at or below target range toxicity (55 ± 5%) irrespective of whether relative cell count (RCC), relative increase in cell count (RICC) or relative population doubling (RPD) was used. There was one exception (2-AA in CHO cells tested at Covance) where RCC gave a weak positive response yet RICC and RPD did not, but all responses were weak and not clearly dose-related (even in the presence of cytochalasin B), which suggests the batch of S9 used may not have been optimal. All measures of cytotoxicity in the absence of cytochalasin B are therefore considered equally acceptable for use. The responses were comparable to those obtained in the presence of cytochalasin B, and therefore there should be a similar level of confidence in results obtained in the absence of cytochalasin B. Therefore if scientists have a preference for one measure of cytotoxicity (*e.g.* perhaps to use RPD to reduce the risk of misleading positives, (6)) over another, then the data obtained in this trial indicate that is acceptable.

REFERENCES

1. OECD (*draft*), *In Vitro* Mammalian Cell Micronucleus Test (MNvit). OECD Guideline for Testing of Chemicals No. 487, OECD, Paris. Available at: [http://www.oecd.org/env/testguidelines]

2. Kirkland, D. (*In preparation*), Evaluation of different cytotoxic and cytostatic measures for the *in vitro* micronucleus test (MNVit): Introduction to the collaborative trial. To be submitted to *Mutation Res*.

3. Rodrigues, A.S., Silva, I.D., Caria, M.H., Laires, A., Chaveca, T., Glatt, H.R. and Rueff, J. (1994), Genotoxicity assessment of aromatic amines and amides in genetically engineered V79 cells. *Mutation Res.* 341, 93-100.

4. Lorge, E., Thybaud, V., Aardema, M.J., Oliver, J., Wakata, A., Lorenzon, G. and Marzin, D. (2006), SFTG international collaborative study on *in vitro* micronucleus test. I. General conditions and overall conclusions of the study. *Mutation Res.* 607, 13-36.

5. Elhajouji, A., Cunha, M., and Kirsch-Volders, M. (1998), Spindle poisons can induce polyploidy by mitotic slippage and micronucleate mononucleates in the cytokinesis-block assay. *Mutagenesis* 13, 193-198.

6. Greenwood, S.K., Hill, R.B., Sun, J.T., Armstrong, M.J., Johnson, T.E., Gara, J.P. and Galloway, S.M. (2004), Population doubling: A simple and more accurate estimation of cell growth suppression in the *in vitro* assay for chromosomal aberrations that reduces irrelevant positive results. *Environ. Mol. Mutagen.* 43 36-44.

APPENDIX 1

TABLES 1-15: SUMMARY RESULTS OF THE *IN VITRO* MN TEST CYTOTOXICITY ASSESSMENT

Abbreviations and symbols:

RCC = relative cell count
RICC = relative increase in cell count
RPD = relative population doubling
RI = replication index
%MN = % micronucleated cells
Monunucs = mononucleaed cells
Binucs = binucleated cells
FI = fold increase over concurrent control (control ranges for each lab and cell type are given in the table below)
* the 2 Sanofi-Aventis labs use different sources of S9, and therefore a full set of tests was performed in each facility
** = statistically different from concurrent control
NS = not significant
# = replication index **increased** at all doses scored
ND = not done

Table 1: Cytosine arabinoside

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Sanofi-Aventis Lab 1*	24 + 0 [-S9]	51	0.25	**6.59**** **[65.9x]**	51	0.05	**1.4**** **[14.0x]**	46	0.1	**4.2**** **[42.0x]**	ND	ND	ND
	Sanofi-Aventis Lab 2*	24 + 0 [-S9]	51	0.15	**7.5**** **[16.7x]**	53	0.075	**2.45**** **[5.44x]**	54	0.15	**7.5**** **[16.7x]**	ND	ND	ND
L5178Y	HLS	3 + 21 [-S9]	53	1.5	**5.8**** **[19.3x]**	49	1.0	**2.25**** **[7.5x]**	41	1.5	**5.8**** **[19.3x]**	ND	ND	ND
L5178Y	HLS	3 + 21 [+S9]	46	1.5	**4.4**** **[9.78x]**	55	1.5	**4.4**** **[9.78x]**	34	1.5	**4.4**** **[9.78x]**	ND	ND	ND
TK6	Institut Pasteur	27 + 27 [-S9]	58	0.05	**5.15**** **[20.6x]**	53	0.012	**2.25**** **[9.0x]**	43	0.012	**2.25**** **[9.0x]**	ND	ND	ND
CHO	Covance	24 + 0 [-S9]	53	0.35	**7.2**** **[12.0x]**	51	0.175	**2.7**** **[4.5x]**	48	0.3	**3.8**** **[6.33x]**	64	0.2	**2.7**** **[3.18x]**
CHO	Covance	24 + 24 [-S9]	50	0.4	**5.6**** **[11.2x]**	58	0.4	**5.6**** **[11.2x]**	57	1.5	**13.1**** **[29.1x]**	<0#	0.4	**14.1**** **[15.7x]**
CHO	Pfizer	24 + 0 [-S9]	46	3.69	**3.9**** **[13.0x]**	54	0.461	**7.1**** **[23.7x]**	42	3.69	**3.9**** **[13.0x]**	51	0.461	**5.4**** **[4.91x]**
V79	Covance	24 + 0	52	0.0125	**10.2****	53	0.01	**6.5****	55	0.0125	**10.2****	54	0.015	**6.3****

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
		[-S9]			**[10.2x]**			**[6.5x]**			**[10.2x]**			**[3.94x]**

Table 2: Mitomycin C

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Servier Group	3 + 21 [-S9]	50	0.36	**28.0** [93.3x]**	46	0.26	**20.1** [67.0x]**	59	0.36	**28.0** [93.3x]**	ND	ND	ND
L5178Y	Roche	3 + 21 [-S9]	8	0.12	**2.7** [13.5x]**	13	0.12	**2.7** [13.5x]**	9	0.12	**2.7** [13.5x]**	ND	ND	ND
L5178Y	Roche	24 + 0 [-S9]	25	0.06	**9.7% [48.5x]**	40	0.06	**9.7% [48.5x]**	30	0.06	**9.7% [48.5x]**	ND	ND	ND
TK6	Novartis	3 + 27 [-S9]	26	2.0	**6.55** [7.28x]**	35	2.0	**6.55** [7.28x]**	22	2.0	**6.55** [7.28x]**	25	2.0	**4.55** [2.76x]**
TK6	Servier Group	3 + 21 [-S9]	49	0.133	**4.40** [8.80x]**	41	0.068	**3.55** [7.10x]**	46	0.095	**5.10** [10.2x]**	ND	ND	ND
CHO	Pfizer	24 + 0 [-S9]	52	1.26	**10.8** [18.0x]**	44	0.95	**12.2** [20.3x]**	46	1.26	**10.8** [18.0x]**	40	1.26	**8.6** [10.8x]**
CHL	Covance	3 + 21 [-S9]	28	0.25	**15** [12.5x]**	36	0.25	**15** [12.5x]**	27	0.25	**15** [12.5x]**	8	0.25	**27.4** [54.8x]**

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Table 3: Benzo(a)pyrene

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Astra Zeneca	3 + 24 [+S9]	57	1.5	**1.99**** **[18.1x]**	42	0.75	**0.77**** **[7.0x]**	28	0.75	**0.77**** **[7.0x]**	35	1.5	**2.46**** **[4.17x]**
L5178Y	HLS	3 + 21 [+S9]	34	2.0	**5.3**** **[13.3x]**	41	2.0	**5.3**** **[13.3x]**	25	2.0	**5.3**** **[13.3x]**	ND	ND	ND
TK6	Covance	3 + 21 [+S9]	32	33	**1.65**** **[3.67x]**	48	3	**1.25**** **[2.78x]**	60	9	**1.6**** **[3.56x]**	57	24	**1.95**** **[4.33x]**
CHO	Covance	3 + 21 [+S9]	30	18	**6.35**** **[11.8x]**	54	16	**8.3**** **[15.5x]**	47	16	**8.3**** **[15.5x]**	15	5	**6.5**** **[8.13x]**
V79	Covance	3 + 21 [+S9]	32	11	**6.55**** **[4.52x]**	57	5	**4.95**** **[3.41x]**	53	5	**4.95**** **[3.41x]**	50	5	**3.88**** **[4.31x]**

Table 4: Cyclophosphamide

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Astra Zeneca	3 + 24 [+S9]	51	6	**7.52**** **[68.4x]**	54	3	**5.49**** **[49.9x]**	56	6	**7.52**** **[68.4x]**	4	3	**10.86**** **[18.4x]**
L5178Y	Roche	3 + 21 {+S9]	50	8	**18.2**** **[72.8x]**	51	5	**11.9**** **[47.6x]**	42	5	**11.9**** **[47.6x]**	ND	ND	ND
TK6	Covance	3 + 21 [+S9]	21	8	**1.85**** **[1.95x]**	59	8	**1.85**** **[1.95x]**	53	8	**1.85**** **[1.95x]**	47	10	**1.45**** **[2.42x]**
CHL	Covance	3 + 21 [+S9]	34	15	**10.8**** **[10.8x]**	34	12	**12.45**** **[12.45x]**	27	12	**12.45**** **[12.45x]**	52	18	**11.18**** **[23.5x]**

Table 5: Colchicine

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Astra Zeneca	3 + 24 [-S9]	57	0.007	**0.71**** **[6.45x]**	55	0.005	**0.73**** **[6.64x]**	39	0.005	**0.73**** **[6.64x]**	7	0.005	**0.92 NS in binucs [1.70x]** **(5.04** in mononucs [7.52x])**
L5178Y	Roche	24 + 0 [-S9]	27	0.025	**6.3**** **[63.0x]**	43	0.025	**6.3**** **[63.0x]**	32	0.025	**6.3**** **[63.0x]**	ND	ND	ND
TK6	Novartis	3 + 27 [-S9]	49	0.028	**3.65**** **[2.15x]**	23	0.016	**4.35**** **2.56x]**	14	0.016	**4.35**** **[2.56x]**	1	0.005	**5.4**** **[2.4x]**
CHO	Covance	3 + 21 [-S9]	55	2	**2.7**** **[13.5x]**	0	1.25	**1.9**** **[9.5x]**	56	1.5	**1.5**** **[7.5x]**	17	5	**2.3** in binucs [3.29x]** **(14.1** in mononucs [14.1x])**
CHO	Covance	24 + 0 [-S9]	55	0.3	**21.0**** **[210.0x]**	61	0.2	**10.7**** **[107.0x]**	45	0.2	**10.7**** **[107.0x]**	65	0.2	**1.3 in binucs [1.0x]** **4.7** in mononucs [4.27x]**
CHL	Covance	3 + 21 [-S9]	54	1	**6.7****	50	0.5	**2.0****	53	0.75	**3.7****	52	1.5	**2.1** in binucs [4.2x]**

					[6.7x]			**[2.0x]**			**[3.7x]**			**(16.1** in mononucs [26.8x])**
V79	Covance	3 + 21 [-S9]	52	0.35	**5.2** **[6.50x]**	53	0.25	**5.2** **[6.50x]**	53	0.35	**5.2** **[6.50x]**	27	0.25	**4.1** in binucs [2.93x] **(16.5** in mononucs [27.5x])**

Table 6: Vinblastine

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Sanofi-Aventis Lab 1	3 + 21 [-S9]	52	0.0175	**3.01**** [37.6x]	56	0.0125	**1.00**** [12.5x]	55	0.015	**1.66**** [20.8x]	ND	ND	ND
	Sanofi-Aventis Lab 2	3 + 21 [-S9]	43	0.01	**6.05**** [10.1x]	41	0.0075	**2.5**** [4.17x]	56	0.01	**6.05**** [10.1x]	ND	ND	ND
L5178Y	Servier Group	3 + 21 [-S9]	57	0.0157	**0.9**** [18.0x]	53	0.0146	**0.4**** [8.0x]	38	0.0146	**0.4**** [8.0x]	ND	ND	ND
TK6	Servier Group	3 + 21 [-S9]	58	0.012	**12.3**** [6.83x]	48	0.004	**7.75**** [4.31x]	52	0.006	**11.55**** [6.42x]	ND	ND	ND
TK6	Institut Pasteur	3 + 27 [-S9]	45	0.00359	**2.3**** [6.13x]	52	0.00272	**1.125**** [3.00x]	59	0.003125	**1.7**** [4.53x]	ND	ND	ND
CHO	Swansea	3 + 21 [-S9]	48	2.0	**10.3**** [7.63x]	35	1.0	**9.96**** [7.38x]	49	2.0	**10.3**** [7.63x]	64	0.8	**9.84**** [4.90x]
CHO	Pfizer	24 + 0 [-S9]	58	0.122	**17.2**** [24.6x]	38	0.051	**8.0**** [11.4x]	49	0.079	**14.2**** [20.3x]	46	0.033	**37.8**** [19.9x]
V79	BAT Expt 1	3 + 21 [-S9]	50	0.8	**29.6**** [29.6x]	55	0.4	**24.7**** [24.7x]	52	0.8	**29.6**** [29.6x]	41	0.4	**34.7**** [26.7x]
V79	BAT Expt 2	3 + 21 [-S9]	49	0.2	**11.45**** [15.3x]	56	0.15	**8.85**** [11.8x]	53	0.2	**11.45**** [15.3x]	11	0.3	**41.3**** [37.5x]

Table 7: 5-Fluorouracil

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Sanofi-Aventis Lab 1	24 + 24 [-S9]	55	0.125	**3.74**** **[12.5x]**	38	0.1	**1.43**** **[4.77x]**	46	0.15	**4.55**** **[15.2x]**	ND	ND	ND
	Sanofi-Aventis lab 2	24 + 24 [-S9]	45	0.15	**1.45**** **[2.9x]**	49	0.15	**1.45**** **[2.9x]**	41	0.2	**1.85**** **[3.7x]**	ND	ND	ND
L5178Y	Servier Group	24 + 24 [-S9]	51	0.13	**0.75** **[2.50x]**	54	0.105	**0.6** **[2.0x]**	41	0.105	**0.6** **[2.0x]**	ND	ND	ND
TK6	Novartis	24 + 24 [-S9]	51	0.9	**4.75**** **[3.39x]**	44	0.7	**5.05**** **[3.61x]**	41	0.9	**4.75**** **[3.39x]**	0	0.9	**3.25**** **[2.95x]**
CHO	Covance Expt 1	24 + 24 [-S9]	56	1.0	**0.9** **[3.0x]**	62	1.0	**0.9** **[3.0x]**	36	1.0	**0.9** **[3.0x]**	52	3.0	**0.8** **[1.14x]**
	Covance Expt 2	24 + 24 [-S9]	51	1.5	**0.8** **[1.6x]**	55	1.5	**0.8** **[1.6x]**	49	7.5	**1.35**** **[2.7x]**	0	7.5	**1.75** **[1.46x]**
V79	Covance	24 + 0 [-S9]	47	5.0	**0.9** **[1.29x]**	51	2.5	**0.9** **[1.29x]**	45	5.0	**0.9** **[1.29x]**	16	5.0	**2.0** **[1.05x]**
	Covance	24 + 24 [-S9]	56	1.0	**0.7** **[1.4x]**	60	1.0	**0.7** **[1.4x]**	29	1.0	**0.7** **[1.4x]**	42	5.0	**1.0** **[0.625x]**

Table 8: Diethylstilboestrol

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	HLS	27+0 [-S9]	60	9	**2.15**** **[2.68x]**	29	7.5	**1.50**** **[1.89x]**	52	10	**2.5**** **[3.13x]**	ND	ND	ND
L5178Y	Roche	24 + 24 [-S9]	42	10.4	**4.70**** **[5.88x]**	58	10.4	**4.70**** **[5.88x]**	42	10.4	**4.70**** **[5.88x]**	ND	ND	ND
TK6	Institut Pasteur	27 + 0 [-S9]	51	15	**3.75**** **[4.69x]**	50	7.5	**3.25**** **[4.06x]**	41	7.5	**3.25**** **[4.06x]**	ND	ND	ND
CHO	Swansea	24 + 0 [-S9]	23	4.0	**2.87**** **[2.52x]**	47	4.0	**2.87**** **[2.52x]**	39	4.0	**2.87**** **[2.52x]**	32	4.0	**9.79**** **[5.20x]**
V79	BAT Expt 1	24 + 0 [-S9]	47	4.0	**8.3**** **[9.22x]**	56	4.0	**8.3**** **[9.22x]**	33	4.0	**8.3**** **[9.22x]**	45	4.0	**10.5**** **[6.36x]**
V79	BAT Expt 2	24 + 0 [-S9]	38	3.0	**7.1**** **[6.45x]**	58	3.0	**7.1**** **[6.45x]**	46	3.0	**7.1**** **[6.45x]**	61	4.5	**8.3**** **[7.55x]**

Table 9: 2-Aminoanthracene

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Sanofi-Aventis Lab 1	3 + 21 [+S9]	52	1.25	**0.73**** **[9.13x]**	53	1.0	**0.63**** **[7.88x]**	47	1.25	**0.73**** **[9.13x]**	ND	ND	ND
	Sanofi-Aventis Lab 2	3 + 21 [+S9]	43	0.75	**4.80**** **[13.7x]**	45	0.6	**2.0**** **[5.70x]**	49	0.75	**4.8**** **[13.7x]**	ND	ND	ND
L5178Y	Servier Group	3 + 21 [+S9]	49	0.75	**2.00**** **[8.00x]**	57	0.62	**0.90**** **[3.60x]**	59	068	**1.25**** **[5.00x]**	ND	ND	ND
TK6	Astra Zeneca	3 + 24 [+S9]	31	1.0	**1.95**** **[2.6x]**	60	1.0	**1.95**** **[2.6x]**	50	1.0	**1.95**** **[2.6x]**	ND	ND	ND
	Astra Zeneca	3 + 42 [+S9]	36	1.0	**2.6**** **[3.13x]**	47	1.0	**2.6**** **[3.13x]**	31	1.0	**2.6**** **[3.13x]**	ND	ND	ND
TK6	Servier Group	3 + 21 [+S9]	52	1.0	**4.3**** **[3.91x]**	51	0.62	**2.20**** **[2.00x]**	58	0.683	**2.65**** **[2.41x]**	ND	ND	ND
TK6	Institut Pasteur	3 + 27 [+S9]	34	1.43	**1.3**** **[2.89x]**	50	1.43	**1.3**** **[2.89x]**	37	1.43	**1.3**** **[2.89x]**	ND	ND	ND
CHO	Covance	3 + 21 [+S9]	50	4	**1.3**** **[2.89x]**	60	3.5	**0.75** **[1.67x]**	33	2	**0.7** **[1.56x]**	52	3.5	**1.9**** **[1.73x]**
	Covance	3 + 41 [+S9]	57	4.5	**0.85** **[1.89x]**	41	3.5	**0.75** **[1.67x]**	51	4	**0.75** **[1.67x]**	52	3.5	**1.2** **[1.0x]**
V79	BAT Expt 1	3 + 21 [+S9]	32	8.0	**2.3**** **[4.18x]**	50	8.0	**2.3**** **[4.18x]**	44	8.0	**2.3**** **[4.18x]**	34	16.0	**3.4**** **[3.09x]**
V79	BAT Expt 2	3 + 21 [+S9]	50	4.0	**4.0**** **[3.64x]**	58	3.0	**3.4**** **[3.09x]**	48	3.0	**3.4**** **[3.09x]**	38	3.0	**5.3**** **[3.12x]**

Table 10: Etoposide

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Astra Zeneca	3 + 24 [-S9]	26	0.4	**9.8**** **[89.1x]**	37	0.4	**9.8**** **[89.1x]**	25	0.4	**9.8**** **[89.1x]**	2	0.1	**6.7**** **[27.9x]**
L5178Y	HLS	3 + 21 [-S9]	56	0.31	**7.15**** **[47.7x]**	50	0.16	**6.65**** **[44.3x]**	49	0.31	**7.15**** **[47.7x]**	ND	ND	ND
L5178Y	HLS	3 + 21 [+S9]	43	0.31	**7.85**** **[19.6x]**	55	0.31	**7.85**** **[19.6x]**	36	0.31	**7.85**** **[19.6x]**	ND	ND	ND
TK6	Novartis	3 + 27 [-S9]	22	0.2	**5.25**** **[4.38x]**	30	0.2	**5.25**** **[4.38x]**	19	0.2	**5.25**** **[4.38x]**	0	0.2	**5.45**** **[2.66x]**
CHL	Covance	3 + 21 [-S9]	52	5.5	**12.1**** **[30.3x]**	36	3.0	**14.0**** **[35.0x]**	51	5.0	**13.0**** **[32.5x]**	50	5.0	**36.0**** **[45.0x]**

Table 11: Cadmium chloride

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN mononucs [FI]	% tox	Conc. (μg/ml)	% MN binucs [FI]
L5178Y	Astra Zeneca	3 + 24 [-S9]	42	0.34	**0.85**** **[7.73x]**	51	0.21	**0.51**** **[4.64x]**	50	0.27	**0.83**** **[7.73x]**	30	0.27	**2.05**** **[3.80x]**
L5178Y	Servier Group	3 + 45 [-S9]	47	0.48	**0.65**** **[6.50x]**	55	0.48	**0.65**** **[6.50x]**	33	0.48	**0.65**** **[6.50x]**	ND	ND	ND
TK6	Covance	3 + 21 [-S9]	45	6	**1.25**** **[1.92x]**	30	4	**1.35**** **[2.08x]**	55	6	**1.25**** **[1.92x]**	53	4	**2.7**** **[3.0x]**
CHO	Covance	3 + 21 [-S9]	42	1.0	**2.9**** **[3.63x]**	40	0.8	**1.8**** **[2.25x]**	47	1.0	**2.9**** **[3.63x]**	24	0.26	**1.45**** **[1.93x]**

Table 12: Quinacrine dihydrochloride

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN binucs [FI]
TK6	BioReliance	24 + 0 [-S9]	59	2	**2.35** [2.76x]**	50	1	**1.40** [1.65x]**	61	1	**1.40** [1.65x]**	ND	ND	ND
CHO	BioReliance	24 + 0 [-S9]	56	3.5	**4.20** [3.82x]**	45	2	**2.60** [2.36x]**	56	2.5	**3.50** [3.18x]**	ND	ND	ND
CHO	Pfizer	24 + 0 [-S9]	52	3.07	**3.5** [5.00x]**	54	1.36	**2.8** [4.00x]**	54	3.07	**3.5** [5.00x]**	56	2.61	**1.0 NS [1.43x]**

Table 13: Phenolphthalein

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN binucs [FI]
TK6	BioReliance	24 + 0 [-S9]	6	20	**2.60**** **[2.36x]**	8	20	**2.60**** **[2.36x]**	5	20	**2.60**** **[2.36x]**	ND	ND	ND
CHO	BioReliance	24 + 0 [-S9]	57	25	**4.10**** **[3.28x]**	46	15	**3.10**** **[2.48x]**	35	15	**3.10**** **[2.48x]**	ND	ND	ND
CHO	Pfizer	24 + 0 [-S9]	47	31.2	**3.0**** **[3.33x]**	57	31.2	**3.0**** **[3.33x]**	36	31.2	**3.0**** **[3.33x]**	15	31.2	**4.5**** **[3.21x]**

Table 14: Diazepam

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target range toxicity (55 ± 5%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN mononucs [FI]	% tox	Conc. (µg/ml)	% MN binucs [FI]
TK6	BioReliance	24 + 0 [-S9]	51	55	**3.10**** **[3.10x]**	54	50	**2.65**** **[2.65x]**	57	55	**3.10**** **[3.10x]**	ND	ND	ND
CHO	BioReliance	24 + 0 [-S9]	48	100	**4.30**** **[3.58x]**	56	55	**3.30**** **[2.75x]**	54	65	**3.85**** **[3.21x]**	ND	ND	ND
CHO	Pfizer	24 + 0 [-S9]	55	85	**1.3**** **[4.33x]**	53	52.2	**0.8**** **[2.67x]**	50	85	**1.3**** **[4.33x]**	52	52.2	**0.9**** **[4.5x]**

Table 15: Control ranges of micronucleated cells for each laboratory during this series of experiments

Laboratory	Cell type	Range of % MN in controls:	
		Mononucs	Binucs (+ cytoB)
Sanofi-Aventis Lab 1	L5178Y	0.08-0.3	Not done
Sanofi-Aventis lab 2	L51`78Y	0.35-0.6	Not done
Astra Zeneca	L5178Y	0.11	0.24-0.59
HLS	L5178Y	0.15-0.8	Not done
Servier	L5178Y	0-0.50	Not done
Roche	L5178Y	0.1-0.8	
Novartis	TK6	0.9-1.7	1.1-2.25
Servier	TK6	0.5-1.8	Not done
Institut Pasteur	TK6	0.15-0.8	Not done
Astra Zeneca	TK6	0.75-0.83	Not done
Covance	TK6	0.45-0.95	0.45-0.9
Swansea	CHO	0.96-1.90	1.48-2.87
Covance	CHO	0.2-0.8	0.7-1.2
Covance	CHL	0.4-1.2	0.475-0.8
BAT	V79	0.55-1.1	1.0-1.7
Covance	V79	0.5-1.9	0.9-1.9
BioReliance	TK6	0.85-1.1	Not done
BioReliance	CHO	1.1-1.25	Not done
Pfizer	CHO	0.3-0.9	0.2-1.9