

OECD Environment, Health and Safety Publications

Series on Testing and Assessment

No. xx

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

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNEP, UNIDO, UNITAR, WHO and OECD**

**Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris 2009**

Also published in the Series on Testing and Assessment:

- No. 1, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals (1993; reformatted 1995, revised 2006)*
- No. 2, *Detailed Review Paper on Biodegradability Testing (1995)*
- No. 3, *Guidance Document for Aquatic Effects Assessment (1995)*
- No. 4, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment (1995)*
- No. 5, *Report of the SETAC/OECD Workshop on Avian Toxicity Testing (1996)*
- No. 6, *Report of the Final Ring-test of the Daphnia magna Reproduction Test (1997)*
- No. 7, *Guidance Document on Direct Phototransformation of Chemicals in Water (1997)*
- No. 8, *Report of the OECD Workshop on Sharing Information about New Industrial Chemicals Assessment (1997)*
- No. 9, *Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application (1997)*
- No. 10, *Report of the OECD Workshop on Statistical Analysis of Aquatic Toxicity Data (1998)*
- No. 11, *Detailed Review Paper on Aquatic Testing Methods for Pesticides and industrial Chemicals (1998)*
- No. 12, *Detailed Review Document on Classification Systems for Germ Cell Mutagenicity in OECD Member Countries (1998)*
- No. 13, *Detailed Review Document on Classification Systems for Sensitising Substances in OECD Member Countries (1998)*
- No. 14, *Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (1998)*
- No. 15, *Detailed Review Document on Classification Systems for Reproductive Toxicity in OECD Member Countries (1998)*
- No. 16, *Detailed Review Document on Classification Systems for Skin Irritation/Corrosion in OECD Member Countries (1998)*
- No. 17, *Environmental Exposure Assessment Strategies for Existing Industrial Chemicals in OECD Member Countries (1999)*
- No. 18, *Report of the OECD Workshop on Improving the Use of Monitoring Data in the Exposure Assessment of Industrial Chemicals (2000)*

- No. 19, *Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation* (1999)
- No. 20, *Revised Draft Guidance Document for Neurotoxicity Testing* (2004)
- No. 21, *Detailed Review Paper: Appraisal of Test Methods for Sex Hormone Disrupting Chemicals* (2000)
- No. 22, *Guidance Document for the Performance of Out-door Monolith Lysimeter Studies* (2000)
- No. 23, *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures* (2000)
- No. 24, *Guidance Document on Acute Oral Toxicity Testing* (2001)
- No. 25, *Detailed Review Document on Hazard Classification Systems for Specifics Target Organ Systemic Toxicity Repeated Exposure in OECD Member Countries* (2001)
- No. 26, *Revised Analysis of Responses Received from Member Countries to the Questionnaire on Regulatory Acute Toxicity Data Needs* (2001)
- No. 27, *Guidance Document on the Use of the Harmonised System for the Classification of Chemicals which are Hazardous for the Aquatic Environment* (2001)
- No. 28, *Guidance Document for the Conduct of Skin Absorption Studies* (2004)
- No. 29, *Guidance Document on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media* (2001)
- No. 30, *Detailed Review Document on Hazard Classification Systems for Mixtures* (2001)
- No. 31, *Detailed Review Paper on Non-Genotoxic Carcinogens Detection: The Performance of In-Vitro Cell Transformation Assays* (2007)
- No. 32, *Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies* (2000)
- No. 33, *Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures* (2001)
- No. 34, *Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment* (2005)
- No. 35, *Guidance notes for analysis and evaluation of chronic toxicity and carcinogenicity studies* (2002)

No. 36, *Report of the OECD/UNEP Workshop on the use of Multimedia Models for estimating overall Environmental Persistence and long range Transport in the context of PBTS/POPS Assessment (2002)*

No. 37, *Detailed Review Document on Classification Systems for Substances Which Pose an Aspiration Hazard (2002)*

No. 38, *Detailed Background Review of the Uterotrophic Assay Summary of the Available Literature in Support of the Project of the OECD Task Force on Endocrine Disrupters Testing and Assessment (EDTA) to Standardise and Validate the Uterotrophic Assay (2003)*

No. 39, *Guidance Document on Acute Inhalation Toxicity Testing (2009)*

No. 40, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures Which Cause Respiratory Tract Irritation and Corrosion (2003)*

No. 41, *Detailed Review Document on Classification in OECD Member Countries of Substances and Mixtures which in Contact with Water Release Toxic Gases (2003)*

No. 42, *Guidance Document on Reporting Summary Information on Environmental, Occupational and Consumer Exposure (2003)*

No. 43, *Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (2008)*

No. 44, *Description of Selected Key Generic Terms Used in Chemical Hazard/Risk Assessment (2003)*

No. 45, *Guidance Document on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long-range Transport (2004)*

No. 46, *Detailed Review Paper on Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances (2004)*

No. 47, *Detailed Review Paper on Fish Screening Assays for the Detection of Endocrine Active Substances (2004)*

No. 48, *New Chemical Assessment Comparisons and Implications for Work Sharing (2004)*

No. 49, *Report from the Expert Group on (Quantitative) Structure-Activity Relationships [(Q)SARs] on the Principles for the Validation of (Q)SARs (2004)*

No. 50, *Report of the OECD/IPCS Workshop on Toxicogenomics (2005)*

No. 51, *Approaches to Exposure Assessment in OECD Member Countries: Report from the Policy Dialogue on Exposure Assessment in June 2005 (2006)*

No. 52, *Comparison of emission estimation methods used in Pollutant Release and Transfer Registers (PRTRs) and Emission*

Scenario Documents (ESDs): Case study of pulp and paper and textile sectors (2006)

No. 53, *Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms) (2006)*

No. 54, *Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application (2006)*

No. 55, *Detailed Review Paper on Aquatic Arthropods in Life Cycle Toxicity Tests with an Emphasis on Developmental, Reproductive and Endocrine Disruptive Effects (2006)*

No. 56, *Guidance Document on the Breakdown of Organic Matter in Litter Bags (2006)*

No. 57, *Detailed Review Paper on Thyroid Hormone Disruption Assays (2006)*

No. 58, *Report on the Regulatory Uses and Applications in OECD Member Countries of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models in the Assessment of New and Existing Chemicals (2006)*

No. 59, *Report of the Validation of the Updated Test Guideline 407: Repeat Dose 28-Day Oral Toxicity Study in Laboratory Rats (2006)*

No. 60, *Report of the Initial Work Towards the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1A) (2006)*

No. 61, *Report of the Validation of the 21-Day Fish Screening Assay for the Detection of Endocrine Active Substances (Phase 1B) (2006)*

No. 62, *Final OECD Report of the Initial Work Towards the Validation of the Rat Hershberger Assay: Phase-1, Androgenic Response to Testosterone Propionate, and Anti-Androgenic Effects of Flutamide (2006)*

No. 63, *Guidance Document on the Definition of Residue (2006)*

No. 64, *Guidance Document on Overview of Residue Chemistry Studies (2006)*

No. 65, *OECD Report of the Initial Work Towards the Validation of the Rodent Uterotrophic Assay - Phase 1 (2006)*

No. 66, *OECD Report of the Validation of the Rodent Uterotrophic Bioassay: Phase 2. Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories (2006)*

No. 67, *Additional data supporting the Test Guideline on the Uterotrophic Bioassay in rodents (2007)*

No. 68, *Summary Report of the Uterotrophic Bioassay Peer Review Panel, including Agreement of the Working Group of the*

National Coordinators of the Test Guidelines Programme on the follow up of this report (2006)

No. 69, Guidance Document on the Validation of (Quantitative) Structure-Activity Relationship [(Q)SAR] Models (2007)

No. 70, Report on the Preparation of GHS Implementation by the OECD Countries (2007)

No. 71, Guidance Document on the Uterotrophic Bioassay - Procedure to Test for Antioestrogenicity (2007)

No. 72, Guidance Document on Pesticide Residue Analytical Methods (2007)

No. 73, Report of the Validation of the Rat Hershberger Assay: Phase 3: Coded Testing of Androgen Agonists, Androgen Antagonists and Negative Reference Chemicals by Multiple Laboratories. Surgical Castrate Model Protocol (2007)

No. 74, Detailed Review Paper for Avian Two-generation Toxicity Testing (2007)

*No. 75, Guidance Document on the Honey Bee (*Apis Mellifera* L.) Brood test Under Semi-field Conditions (2007)*

No. 76, Final Report of the Validation of the Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances: Phase 1 - Optimisation of the Test Protocol (2007)

No. 77, Final Report of the Validation of the Amphibian Metamorphosis Assay: Phase 2 - Multi-chemical Interlaboratory Study (2007)

No. 78, Final Report of the Validation of the 21-day Fish Screening Assay for the Detection of Endocrine Active Substances. Phase 2: Testing Negative Substances (2007)

*No. 79, Validation Report of the Full Life-cycle Test with the Harpacticoid Copepods *Nitocra Spinipes* and *Amphiascus Tenuiremis* and the Calanoid Copepod *Acartia Tonsa* - Phase 1 (2007)*

No. 80, Guidance on Grouping of Chemicals (2007)

No. 81, Summary Report of the Validation Peer Review for the Updated Test Guideline 407, and Agreement of the Working Group of National Coordinators of the Test Guidelines Programme on the follow-up of this report (2007)

No. 82, Guidance Document on Amphibian Thyroid Histology (2007)

No. 83, Summary Report of the Peer Review Panel on the Stably Transfected Transcriptional Activation Assay for Detecting Estrogenic Activity of Chemicals, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)

No. 84, *Report on the Workshop on the Application of the GHS Classification Criteria to HPV Chemicals, 5-6 July Bern Switzerland (2007)*

No. 85, *Report of the Validation Peer Review for the Hershberger Bioassay, and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2007)*

No. 86, *Report of the OECD Validation of the Rodent Hershberger Bioassay: Phase 2: Testing of Androgen Agonists, Androgen Antagonists and a 5 α -Reductase Inhibitor in Dose Response Studies by Multiple Laboratories (2008)*

No. 87, *Report of the Ring Test and Statistical Analysis of Performance of the Guidance on Transformation/Dissolution of Metals and Metal Compounds in Aqueous Media (Transformation/Dissolution Protocol) (2008)*

No.88, *Workshop on Integrated Approaches to Testing and Assessment (2008)*

No.89, *Retrospective Performance Assessment of the Test Guideline 426 on Developmental Neurotoxicity (2008)*

No.90, *Background Review Document on the Rodent Hershberger Bioassay (2008)*

No.91, *Report of the Validation of the Amphibian Metamorphosis Assay (Phase 3) (2008)*

No.92, *Report of the Validation Peer Review for the Amphibian Metamorphosis Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-Up of this Report (2008)*

No.93, *Report of the Validation of an Enhancement of OECD TG 211: Daphnia Magna Reproduction Test (2008)*

No.94, *Report of the Validation Peer Review for the 21-Day Fish Endocrine Screening Assay and Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow-up of this Report (2008)*

No.95, *Detailed Review Paper on Fish Life-Cycle Tests (2008)*

No.96, *Guidance Document on Magnitude of Pesticide Residues in Processed Commodities (2008)*

No.97, *Detailed Review Paper on the use of Metabolising Systems for In Vitro Testing of Endocrine Disruptors (2008)*

No. 98, *Considerations Regarding Applicability of the Guidance on Transformation/Dissolution of Metals Compounds in Aqueous Media (Transformation/Dissolution Protocol) (2008)*

No. 99, *Comparison between OECD Test Guidelines and ISO Standards in the Areas of Ecotoxicology and Health Effects (2008)*

No.100, *Report of the Second Survey on Available Omics Tools (2009)*

No.101, *Report on the Workshop on Structural Alerts for the OECD (Q)SAR Application Toolbox (2009)*

No.102, *Guidance Document for using the OECD (Q)SAR Application Toolbox to Develop Chemical Categories According to the OECD Guidance on Grouping of Chemicals (2009)*

No.103, *Detailed Review Paper on Transgenic Rodent Mutation Assays (2009)*

No.104, *Performance Assessment: Comparison of 403 and CxT Protocols via Simulation and for Selected Real Data Sets (2009)*

No. 105, *Report on Biostatistical Performance Assessment of the draft TG 436 Acute Toxic Class Testing Method for Acute Inhalation Toxicity (2009)*

No.106, *Guidance Document for Histologic Evaluation of Endocrine and Reproductive Test in Rodents (2009)*

No.107, *Preservative treated wood to the environment for wood held in storage after treatment and for wooden commodities that are not cover and are not in contact with ground (2009)*

No.108, *Intact, Stimulated, Weanling Male Rat Version of the Hershberger Bioassay (2009)*

No.109, *Literature Review on the 21-Day Fish Assay and the Fish Short-Term Reproduction Assay (2009)*

No.110, *Report of the Validation Peer Review for the Weanling Hershberger Bioassay and Agreement of the Working of national Coordinators of the Test Guidelines Programme on the Follow-Up of this Report (2009)*

No.111, *Report of the Expert Consultation to Evaluate and Estrogen Receptor Binding Affinity Model for Hazard Identification (2009)*

© OECD 2009

Applications for permission to reproduce or translate all or part of this material should be made to: Head of Publications Service, OECD, 2 rue André-Pascal, 75775 Paris Cedex 16, France

ABOUT THE OECD

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.

This publication is available electronically, at no charge.

**For this and many other Environment,
Health and Safety publications, consult the OECD's
World Wide Web site (www.oecd.org/ehs/)**

or contact:

**OECD Environment Directorate,
Environment, Health and Safety Division**

**2 rue André-Pascal
75775 Paris Cedex 16
France**

**Fax: (33-1) 44 30 61 80
E-mail: ehscont@oecd.org**

FOREWORD

(To be added)

This document is published on the responsibility of the Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals of the OECD.

Contact for further details:
Environment, Health and Safety Division
Environment Directorate
Organisation for Economic Co-Operation and Development
2, rue André Pascal
75775 Paris Cedex 16, France

Tel : 33-1-45-24-16-74
E.mail : env.edcontact@oecd.org

TABLE OF CONTENTS

ABOUT THE OECD	9
FOREWORD	11
PREAMBLE	13
ABSTRACT	14
INTRODUCTION	14
SUMMARY OF FINDINGS	14
CONCLUSIONS	16
REFERENCES	17
APPENDIX 1: Summary results of the in vitro MN test cytotoxicity assessment	18
<u>Table 1:</u> Cytosine arabinoside	19
<u>Table 2:</u> Mitomycin C	20
<u>Table 3:</u> Benzo(a)pyrene	21
<u>Table 4:</u> Cyclophosamide	22
<u>Table 5:</u> Colchicine	23
<u>Table 6:</u> Vinblastine	25
<u>Table 7:</u> 5-Fluorouracil	26
<u>Table 8:</u> Diethylstilbestrol	27
<u>Table 9:</u> 2-Aminoanthracene	28
<u>Table 10:</u> Etoposide	29
<u>Table 11:</u> Cadmium chloride	30
<u>Table 12:</u> Quinacrine dihydrochloride	31
<u>Table 13:</u> Phenolphthalein	32
<u>Table 14:</u> Diazepam	33
<u>Table 15:</u> Control ranges of micronucleated cells for each laboratory during this series of experiments.	33

PREAMBLE

1. At WNT20, the United States raised some concerns that the Relative Population Doubling (RPD) and Relative Increase in Cell Counts (RICC) methods for estimating cytotoxicity proposed in the MNvit TG 487 had not been adequately substantiated. This concern rendered paragraph 26 and Annex 2 of the draft Test Guideline unacceptable to the US at that time. The UK agreed to lead an EU consortium for the performance assessment of the RICC and RPD protocols for assessing cytotoxicity and the United States agreed to accept the draft Test Guideline if they considered that the additional data substantiated their use. WNT20 provisionally adopted the TG 487 pending the resolution of the issue.
2. After extensive negotiations, agreement between the EU consortium and the United States on the scope and acceptance criteria of the performance assessment of RPD/RICC was achieved. Using the agreed protocols, data has now 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.
3. Dr. David Kirkland (Covance Laboratories Limited, UK) coordinated the performance assessment, collation of data and the drafting of the performance assessment report and is gratefully acknowledged for his contributions to the final adoption of the "*In Vitro Mammalian Cell Micronucleus Test Guideline 487*".

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

ABSTRACT

1. 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 ($55 \pm 5\%$) 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 (1). 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 ($55 \pm 5\%$) 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 ($55 \pm 5\%$ 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 (2) 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-fluorouracil (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 (3).

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 [4]. 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 ($55 \pm 5\%$) 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, (5)) over another, then the data obtained in this trial indicate that is acceptable.

REFERENCES

1. 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.*
2. 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.
3. 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.
4. 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.
5. 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 = mononucleated 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 toxicity (50-60%) 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 [-S9]	52	0.0125	10.2** [10.2x]	53	0.01	6.5** [6.5x]	55	0.0125	10.2** [10.2x]	54	0.015	6.3** [3.94x]

Table 2: Mitomycin C

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target toxicity (50-60%) 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]

Table 3: Benzo(a)pyrene

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target toxicity (50-60%) 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 toxicity (50-60%) 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 + recover y (hr) [+/- S9]	Key findings at or near target toxicity (50-60%) for different toxicity measures:											
			RCC			RICC			RPD			RI (with cytoB)		
			% to x	Conc. (µg/ml)	% MN mononuc s [FI]	% to x	Conc. (µg/ml)	% MN mononuc s [FI]	% to x	Conc. (µg/ml)	% MN mononuc s [FI]	% tox	Conc. (µg/ml)	% MN binuc s [FI]
L5178 Y	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 binuc s [1.70x] (5.04** in mononuc s [7.52x])
L5178 Y	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]	N D	N D	N D
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 binuc s [3.29x] (14.1** in mononuc s [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 binuc s [1.0x]

														4.7** in mononuc s [4.27x]
CHL	Covanc e	3 + 21 [-S9]	54	1	6.7** [6.7x]	50	0.5	2.0** [2.0x]	53	0.75	3.7** [3.7x]	52	1.5	2.1** in binucs [4.2x] (16.1** in mononuc s [26.8x])
V79	Covanc e	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 mononuc s [27.5x])

Table 6: Vinblastine

Cell type	Lab	Treat + recovery (hr) [+/- S9]	Key findings at or near target toxicity (50-60%) 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 toxicity (50-60%) 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 toxicity (50-60%) 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 toxicity (50-60%) 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	0.68	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 toxicity (50-60%) 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 toxicity (50-60%) 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 toxicity (50-60%) 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 toxicity (50-60%) 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 toxicity (50-60%) 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	L5178Y	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