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SFTG international collaborative study on in vitro micronucleus test III. Using CHO cells

Marilyn J. Aardema[a], Ronald D. Snyder[b], Carol Spicer[c], Katyayini Divi[d], Takeshi Morita[e], Robert J. Mauthe[f], David P. Gibson[a], Sandra Soelter[g], Patrick T. Curry[h], Veronique Thybaud[i], Giocondo Lorenzon[j], Daniel Marzin[k], Elisabeth Lorge[l,*]

[a] *Procter & Gamble Co., Miami Valley Laboratories, P.O. Box 538707, Cincinnati, OH 45253-8707, USA*
[b] *Schering-Plough, Research Institute, Lafayette, NJ 07848, USA*
[c] *Covance Laboratories Inc., 9200, Leesburg Pike, Vienna, VA 22182, USA*
[d] *Bioreliance, 14920 Broschart Road, Rockville, MD 20850-3349, USA*
[e] *National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku 158-8501, Tokyo, Japan*
[f] *Pfizer Inc., Central Research Division, Eastern Point Road B274 #40, Groton, CT 06340, USA*
[g] *Midwest BioResearch, LLC (formerly Searle) 1801 Maple Avenue, Evanston, IL 60201, USA*
[h] *IIT Research Institute, Chicago, IL 60616, USA*
[i] *Sanofi Aventis, Drug Safety Evaluation, F-94403 Vitry-sur-Seine, France*
[j] *Proskelia Pharmaceuticals, F-93230 Romainville, France*
[k] *Institut Pasteur, Laboratoire de Toxicologie, F-59019 Lille, France*
[l] *Servier Group, Drug Safety Assessment, F-45403 Orleans-Gidy, France*

Abstract

In this report, results are presented from an international study of the in vitro micronucleus assay using Chinese hamster ovary cells. This study was coordinated by an organizing committee supported by the SFTG (the French branch of the European Environmental Mutagen Society). Test chemicals included mannitol, bleomycin, cytosine arabinoside, urethane and diethylstilboestrol. Mitomycin C was used as a positive control. Each chemical was evaluated in at least two laboratories following a variety of different protocols (short and long exposures, varying recovery times, with and without cytochalasin B) in order to help determine a standard protocol for routine testing in Chinese hamster ovary cells. Mannitol and urethane were negative, while bleomycin, cytosine arabinoside and diethylstilboestrol induced a dose dependent increase in micronucleated cells. In the presence of cytochalasin B, increases in micronuclei were observed in binucleated as well as mononucleated cells in cultures treated with bleomycin, cytosine arabinoside or diethylstilboestrol. Importantly, all three of these chemicals were detected in each of the different treatment/recovery regimens. No differences were seen in the sensitivity or accuracy of the responses in the presence of absence of cytochalasin B. Overall, these results demonstrate the suitability of Chinese hamster ovary cells for the in vitro micronucleus assay.

Keywords: In vitro micronucleus assay; CHO cells; Cytochalasin B; Clastogen; Aneugen

* Corresponding author.
E-mail address: elisabeth.lorge@fr.netgrs.com (E. Lorge).

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1. Introduction

Established cell lines, especially those developed from Chinese hamsters (CHO-WBL, CHO-K1, V79, CHL) are commonly used in the standard metaphase chromosome aberration assay (e.g. Ishidate et al. [1]). Chinese hamster cell lines have the advantage of having a relatively small number of chromosomes (20–22 chromosomes), they are readily available, they do not have donor variability like human lymphocytes, and they do not require mitogenic stimulation for growth since most of the cells are dividing. Based on their extensive use in chromosome aberration assays, Chinese hamster cell lines have increasingly been used for the assessment of micronuclei in vitro [2–15]. Not unexpectedly, various protocols have emerged for the in vitro micronucleus assay in Chinese hamster cells and there are few studies that have carefully examined the critical parameters of the protocol. Before an internationally harmonized protocol can be developed for the in vitro micronucleus assay using Chinese hamster cell lines, agreement on the key aspects of the protocol is needed [16,17].

To address this, an international study starting in 1997 was coordinated by an organizing committee supported by the SFTG (the French branch of the European Environmental Mutagen Society) and included laboratories from Europe, America and Japan. As part of this, studies in Chinese hamster ovary cells were conducted with a common protocol. Since the initiation of these studies, Garriott et al. [2] and Phelps et al. [18] reported the results of studies in Chinese hamster ovary cells with a total of 26 chemicals for both, complementary to this present study and that have also a variety of mechanisms of genotoxicity including aneugens, crosslinking agents, and strand breaking agents. They investigated important parameters such as the use of cytochalasin B, length of exposure, number of cells to analyse. The outcome of the present international study, together with these previous papers will contribute to a defined standard protocol for the use of in vitro micronucleus test for routine genotoxicity testing in Chinese hamster ovary cells.

2. Materials and methods

2.1. Cells

CHO-WBL cells were originally obtained from Covance Laboratories (Vienna, VA, USA), Abbott Laboratories (Abbott Park, IL, USA) or BioReliance (Rockville, MD, USA).

2.2. Culture media

Mc Coy's 5A (with bicarbonate buffer and Hepes) supplemented with penicillin–streptomycin (50 UI/ml–50 μg/ml), glutamine (2 mM) and 10% heat-inactivated fetal calf serum.

2.3. Chemicals

The test chemicals were purchased from Sigma Chemicals, coded and dispatched to the participants of the study by the organizing committee (see the general publication in this issue for details [19]).

They were: mannitol (CAS No. 69-65-8), bleomycin (CAS No. 9041-93-4), cytosine arabinoside (CAS No. 147-94-4), urethane (CAS No. 51-79-6) and diethylstilboestrol (CAS No. 56-53-1). Mitomycin C (CAS No. 50-07-7) was used as the positive control.

2.4. Culture conditions

A survey was performed before the study to define the procedures in use in the different participating laboratories and to elaborate a common protocol based on these current practices. Cultures were performed in duplicate. In some studies with cytochalasin B, a separate third culture flask was used for cell counts to provide a complementary evaluation of cytotoxicity to compare to the ratio of binucleated cells. Following the current use validated in each laboratory, different conditions were applied for seeding cells. The day before treatment, the cells were seeded in either 4-well chambers, 8-well chambers, Petriperm chambers, 6-well plates or 35-mm dishes. Cells were cultured at densities of 10,000–14,000 cells/cm^2 in the '3 + 20 h' schedule with cytochalasin B; 2400–5000 cells/cm^2 in the '3 + 45 h' or '24 + 24 h' schedules without cytochalasin B; 2400–7000 cells/cm^2 in the '24 + 20 h' schedule with cytochalasin B or '24 + 0 h' and '3 + 21 h' schedules without cytochalasin B. In these conditions, cells were actively growing upon treatment.

All cultures were incubated at 37 °C in a humidified atmosphere of approximately 5% CO_2 and filtered air.

2.5. Treatment and recovery times

The different treatment and recovery schedules are summarised in Fig. 1.

Cells were treated with the test compound by replacing the culture medium with fresh treatment medium containing various concentrations of the test compound. The cultures were incubated for the required treatment period (3 or 24 h) after which the treatment medium was removed and the cells were rinsed with buffer or culture medium. One set of cultures was harvested immediately after the 24 h treatment with no recovery period ('24 + 0 h'). The cultures that were not harvested immediately after rinsing were then re-fed with either fresh culture medium without cytochalasin B or with culture

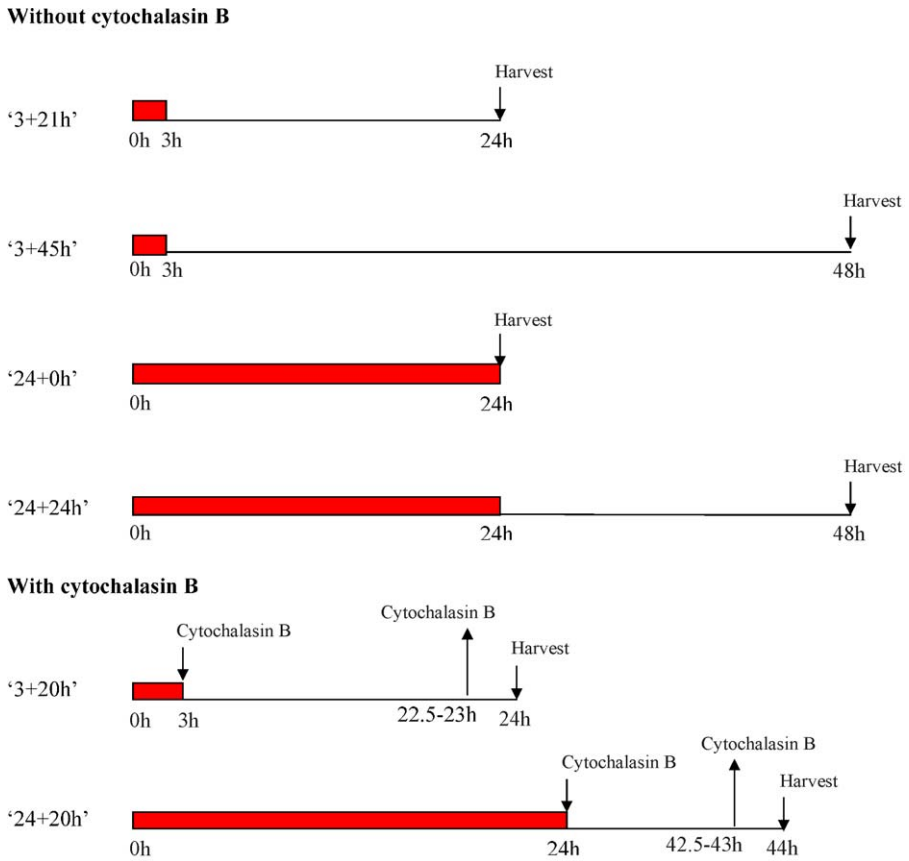

Fig. 1. Treatment and recovery schedules: (■) treatment with a chemical; (—) recovery in the fresh medium.

medium containing cytochalasin B. For recovery in the absence of cytochalasin B, cells were incubated either 21 or 45 h following the 3 h treatment period ('3 + 21' or '3 + 45') or for 24 h following the 24 h treatment ('24 + 24 h') and harvested. For recovery in the presence of cytochalasin B, cells were incubated either 20 h or 45 h following the 3-h treatment period ('3 + 20 h or '3 + 45 h') or incubated for 20 h following the 24 h treatment ('24 + 20 h').

Cytochalasin B, when used, was added to the cultures at a final concentration of 3 μg/ml in the medium, after the test compound had been removed. Cells were allowed to recover from cytochalasin B for an additional 1–1.5 h prior to harvest to improve cellular morphology and micronucleus scoring. For instance, cells treated with the test chemical for 3 h followed by a 20-h recovery in the presence of cytochalasin B were harvested around 21 h after completion of treatment (24 h from the start of chemical exposure). From the experience of the participants collected during the survey, this step was known to be necessary to obtain nice preparations for these cells.

Mitomycin C was used as the positive control at concentrations of 0.1 μg/ml for all of the 24-h treatments and at 0.2 or 0.5 μg/ml for the 3-h treatments. The concentrations were determined following a preliminary positive control dose range-finding study in all the participating laboratories (see the main publication in this issue [19]).

2.6. Cell sampling and staining

At the appropriate harvest time (see Fig. 1), the cells were rinsed and exposed to a hypotonic shock in situ using either 75 mM KCl or 1% (w/v) sodium citrate. The cells were then fixed with methanol:acetic acid (3:1) and/or 100% (v/v) methanol, and stained with either giemsa or acridine orange.

2.7. Report and evaluation of results

In cultures that were exposed to cytochalasin B, the incidence of micronucleus formation was assessed by counting the number of micronucleated binucleated cells in 1000 binucleated cells per culture (2000 cells per test compound concentration). The incidence of micronucleated mononucleated cells in 1000 mononucleated cells per culture was also analysed, when possible, in the cultures exposed to cytochalasin B. In cultures that were not exposed to cytochalasin B the incidence of micronucleated cells in 1000 cells per culture was analysed (2000 cells per test compound concentration).

Criteria for assay acceptability and interpretation of results are described in the general publication in this issue [19]. Table 1 summarises the assays that were used for this evaluation. The range of tested concentrations is reported for each assay with the highest negative concentration or the lowest positive concentration as well as the corresponding survival percentage and maximal fold increase (MFI), where applicable. The survival percentage was calculated relative to the solvent control and is based on cell counts after treatment when cytochalasin B was not used or on the percentage of binucleated cells when cytochalasin B was used. The fold increase was calculated by dividing the number of micronucleated cells in the treated cultures by the number of micronucleated cells in the solvent control cultures. Data for individual cultures are reported in Appendix A.

The criteria for a positive response were the demonstration of a significant, reproducible, and concentration-dependent increase in the number of micronucleated cells, at one or more test compound concentrations, relative to the number of micronucleated cells in the solvent control. If all of these criteria were not achieved the test compound response was equivocal, and if none of the criteria was achieved the test compound response was negative.

3. Results

3.1. Spontaneous background and mitomycin C-induced responses

The spontaneous background of micronucleated cells among laboratories was around 12 per 1000 cells, whatever the treatment schedule, and ranged from 6 to 27 per 1000 cells. This variability between laboratories was also seen in other cell types in this collaborative study (see the general publication in this issue [19] where the data were detailed). This was also reported for human lymphocytes by the HUMN work [20] where the micronucleus identification was recognised as the main source of variability. However, in most cases, the highest was the negative control, the highest was the induced response. Therefore, this variability was not misleading for the test compounds evaluation but may account for non-reproducible sporadic responses (e.g. for urethane).

The influence of cytochalasin B on spontaneous and mitomycin C-induced numbers of micronucleated cells was also considered. Theoretically, for a given treatment schedule, the incidence of micronucleated cells in binucleates in the presence of cytochalasin B should be divided by two to be compared to the incidence of micronucleated cells in mononucleates in the presence of cytochalasin B. This expected excess of micronucleated cells in the presence of cytochalasin B was actually seen for mitomycin C-induced numbers of micronucleated cells (146 with versus 102 per 1000 without cytochalasin B for the 3 h treatment and 382 with versus 183 per 1000 without cytochalasin B for the 24-h treatment). Nevertheless, the ratio for induced levels was below the theoretical two-fold ratio, and this excess of micronucleated cells in the presence of cytochalasin B was not seen for spontaneous levels. This may be due to a partial inefficiency of cytochalasin B and was probably related to the stage of the cell cycle when the primary lesion leading to a micronucleus occurred. However, this had no consequence on the genotoxic evaluation of the compounds.

3.2. Mannitol

There was no reproducible cytotoxicity, where evaluated, up to 5000 μg/ml. In one laboratory a precipitate was found at 1000 or 5000 μg/ml. In one assay in the presence of cytochalasin B, after the 24 h treatment, the number of micronucleated cells was statistically significantly higher in the treated cultures than in the control. However, this slight increase (1.6 to 2.6-fold the control values), not reproduced in the second assay was considered irrelevant. No statistically significant increases in the number of micronucleated cells were seen in all other assays. Therefore, the compound was concluded negative regardless the treatment schedule used.

3.3. Bleomycin

Bleomycin induced concentration-dependent and reproducible increases in the number of micronucleated cells. These effects were found at concentrations inducing little or no cytotoxicity and were distributed over a wide concentration range (up to a one log difference between lowest and highest positive concentrations).

Surprisingly, in the presence of cytochalasin B, an induction of micronucleated cells was also found in mononucleated cells, in addition to binucleated cells, although to a lesser extent. The incidence of micronucleated cells was generally higher in binucleated cells and was more pronounced after the long treatment. This increase of the number of micronuclei in mononucleated cells, previously reported for aneugens was not expected with bleomycin [21]. Since bleomycin is able to induce DNA damage at every stage of the cell cycle, these micronucleated mononucleated cells may represent the cells that had completed the division before the exposure to cytochalasin B. The difference of micronucleated cell numbers between mononucleated and binucleated cells

Table 1
Summary data with CHO cells

Compound	CAS No.	Schedule	Cyt B[a]	Lab.	Assay	Range of concentrations (μg/ml)[b]	Highest negative concentration (μg/ml)[c]		Lowest positive concentration (μg/ml)[e]		Maximal fold increase of the range[g]	Conclusion for the assay[h]	Judgement for the treatment and recovery schedule[i]
							Result and concentration	Survival (%)[d]	Result and concentration	Survival (%)[f]			
Mannitol	69-65-8	3 + 21	–	1	1	500–5000 p	−5000	nt				–	Negative
				1	2	100–1000 p	−1000	80				–	
				2	1	78–5000	−5000	≥100				–	
				2	2	1250–5000	−5000	≥100				–	
		3 + 45	–	1	1	100–1000 p	−1000	48				–	Negative
				1	2	100–1000 p	−1000	98				–	
				2	1	78–5000	−5000	89				–	
				2	2	1250–5000	−5000	≥100				–	
		24 + 0	–	1	1	100–1000 p	−1000	47				–	Negative
				1	2	100–1000 p	−1000	≥100				–	
				2	1	78–5000	−5000	≥100				–	
				2	2	1250–5000	−5000	87				–	
		24 + 24	–	1	1	500–5000 p	−5000	nt				–	Negative
				1	2	100–1000 p	−1000	≥ 100				–	
				2	1	78–5000	−5000	76				–	
				2	2	1250–5000	−5000	92				–	
		3 + 20	+	1	1	500–5000 p	−5000	83				–	Negative
				1	2	100–1000 p	−1000	≥100				–	
				2	1	78–5000	−5000	≥100				–	
				2	2	1250–5000	−5000	99				–	
		24 + 20	+	1	1	500–5000 p			+500 b	≥100 b	2.6 b	+	Negative
				1	2	100–1000 p	−1000	87				–	
				2	1	78–5000	−5000	≥100				–	
				2	2	1250–5000	−5000	≥100				–	
Bleomycin	9041-93-4	3 + 21	–	1	2	62.5–500			+62.5	69	10.4	+	Positive
				2	1	0.11–500			+0.45	77	15.1	+	
				2	2	15.6–250			+15.6	75	17.7	+	
		3 + 45	–	1	1	0.88–250			+0.88	91	8.0	+	Positive
				1	2	0.88–250			+0.88	93	9.5	+	
				2	1	0.11–500			+0.45	≥100	10.8	+	
		24 + 0	–	1	1	3.5–500			+3.5	77	5.7	+	Positive
				1	2	3.5–500			+3.5	62	6.8	+	
				2	1	0.11–500			+ 0.11	81	26.7	+	
		24 + 24	–	1	2	3.5–125			+3.5	63	11.5	+	Positive
		3 + 20	+	1	1	0.17–14			+0.17 m, b	70 m, b	1.9 m, 8.5 b	+	Positive
				1	2	3.5–500			+3.5 m, b	76 m, b	2.0 m, 6.1 b	+	
				2	1	0.11–62.5			+0.11 b[j]	≥100 b	51.5 b	+	
				2	2	10–100			+10 b[j]	78 b	13.7 b	+	
		24 + 20	+	1	1	0.17–0.875			+0.17 m, b	≥100 m, b	16.3 m, 22.1 b	+	Positive
				1	2	0.29–3.5			+0.29 m, b	93 m, b	11.1 m, 15.5 b	+	

Table 1 (*Continued*)

Compound	CAS No.	Schedule	Cyt B[a]	Lab.	Assay	Range of concentrations (μg/ml)[b]	Highest negative concentration (μg/ml)[c]		Lowest positive concentration (μg/ml)[e]		Maximal fold increase of the range[g]	Conclusion for the assay[h]	Judgement for the treatment and recovery schedule[i]
							Result and concentration	Survival (%)[d]	Result and concentration	Survival (%)[f]			
				2	1	0.0017–7.8			+0.0068 b[j]	90 b	50.4 b	+	
				2	2	0.0025–0.1			+0.025 b[j]	≥100 b	6.1 b	+	
Cytosine arabinoside	147-94-4	3 + 21	–	1	1	0.03–100			+3	76	7.8	+	Positive
				1	2	1–30			+3	75	5.2	+	
				2	1	0.625–20			+1.25	76	30.7	+	
				2	2	2.5–10			+2.5	≥100	7.3	+	
		3 + 45	–	1	1	0.03–100			+3	76	2.1	+	Positive
				1	2	1–30			+3	67	2.8	+	
				2	1	0.625–20			+5	64	5.4	+	
				2	2	5–30			+5	77	31.6	+	
		24 + 0	–	1	1	0.01–10			+0.1	50	5.2	+	Positive
				1	2	0.03–1			+0.1	72	8.5	+	
				2	1	0.0098–0.3125			+0.156	62	11.4	+	
				2	2	0.078–0.625			+0.078	67	15.3	+	
		24 + 24	–	1	1	0.01–10			+0.1	66	3.2	+	Positive
				1	2	0.03–1			+0.1	69	13.6	+	
				2	1	0.0098–0.3125			+0.0195	≥100	8.5	+	
				2	2	0.0098–0.3125			+0.078	80	15.4	+	
		3 + 20	+	1	1	0.3–100			+3 b	≥100 b	7.8 b	+	Positive
				1	2	1–30			+10 m, + 3 b	67 m, ≥100 b	9.0 m, 8.2 b	+	
				2	1	0.625–20			+20 m, 1.25 b	44 m, ≥100 b	12.0 m, 7.1 b	+	
				2	2	2.5–10			+2.5 m, b	≥100 m, b	7.3 m, 11.8 b	+	
		24 + 20	+	1	1	0.003–1			+0.1 m, b	≥100 m, b	12.5 m, 49.1 b	+	Positive
				1	2	0.03–1			+0.1 b	≥100 b	34.8 b	+	
				2	1	0.0098–0.3125			+0.078 m, b	≥100 m, b	58.3 m, 20.7 b	+	
				2	2	0.039–0.3125			+0.039 m, b	≥100 m, b	422.9 m, 42.7 b	+	
Urethane	51-79-6	3 + 21	–	1	1	1000–5000	−5000	59				–	Negative
				1	2	1000–5000	−5000	71				–	
				2	2	625–5000	−5000	85				–	
				3	1	1250–5000	−5000	83				–	
		3 + 45	–	1	1	1000–5000	−5000	≥100				–	Negative
				1	2	1000–5000	−5000	60				–	
				3	1	1250–5000	−5000	95				–	
		24 + 0	–	1	1	1000-5000	−5000	82				–	Negative
				2	2	625–5000	−5000	63				–	
				3	1	1250–5000	−5000	71				–	
		24 + 24	–	1	1	1000–5000	−5000	53				–	Negative
				1	2	1000–5000	−5000	24				–	
				3	1	1250–5000	−5000	75				–	

		3 + 20	+	1	1	1000–5000			+1000 b	≥100 b	5.8 b	±	Negative
				1	2	1000–5000	−5000	98				−	
				2	2	625–5000			+625 m, b	76 m, b	6.5 m, 1.8 b	±	
				3	1	1250–5000	−5000	54				−	
		24 + 20	+	1	1	500–5000			+3500 b	80 b	3.1 b	±	Negative
				1	2	1000–5000	−5000	62				−	
				2	2	625–5000			+5000 b	60 b	2.1 b	±	
				3	1	1250–5000	−5000	93				−	
Diethylstilboestrol	56-53-1	3 + 21	−	1	2	7.5–40			+40	51	3.0	+	Positive
				2	1	0.5–10			+0.5	87	3.0	+	
		3 + 45	−	2	1	0.5–10			+0.5	≥100	11.8	+	Positive
		24 + 0	−	2	1	0.05–5			+0.05	≥100	11.4	+	Positive
		24 + 24	−	2	1	0.05–5			+0.05	≥100	30.3	+	Positive
		3 + 20	+	2	1	0.5–7			+7 m, + 3 b	49 m, 84 b	5.0 m, 9.0 b	+	Positive
		24 + 20	+	1	2	1.88–5			+5 m, +3.75 b	96 m, 87 b	3.9 m, 4.2 b	+	Positive
				2	1	0.05–1			+0.05 b	73 b	13.0 b	+	

[a] (−) No use of cytochalasin B; (+) use of cytochalasin B.

[b] Range of tested concentrations where genotoxicity was measured; p: precipitate at the highest concentration(s).

[c] (−) Negative, i.e. no significant increase in the number of micronucleated cells over the solvent control.

[d] Relative survival against the solvent control seen at the highest concentration based on cell counts in the absence of cytochalasin B or on percentages of binucleated cells when cytochalasin B was used.

[e] (+) Positive, i.e. significant increase in the number of micronucleated cells over the solvent control at $p < 0.05$ at least one concentration; m: increase in the number of micronucleated mononucleated cells; b: increase in the number of micronucleated binucleated cells.

[f] Relative survival against the solvent control seen at the lowest positive concentration; m: in mononucleated cells, b: in binucleated cells where applicable.

[g] Maximal fold increase in micronucleated cells over the solvent control among the positive concentrations of the tested range, at relevant relative survivals (i.e. >40%); m: in mononucleated cells, b: in binucleated cells where applicable.

[h] (+) Positive: concentration-dependent increase in the number of micronucleated cells over the solvent control; (±) equivocal, i.e. significant increase seen at only one concentration or significant increases not concentration-dependent; (−) negative: no increase in the number of micronucleated cells over the solvent control at any concentration of the range.

[i] (+) Compound judged as positive in the treatment and recovery schedule (all the accepted assays were positive); (−) compound judged as negative in the treatment and recovery schedule (all the accepted assays were negative); (±) equivocal (controversial results between assays); if controversial results were obtained when the negative assay included too low concentrations or a too wide range of concentrations, only the positive assay was taken into account; on the opposite, if an equivocal response was not confirmed in another assay including an adequate range of concentrations, it was concluded as negative. The magnitude of the response was also considered.

[j] Only binucleated cells were evaluated.

seen only after the short treatment is consistent with this hypothesis. However this explanation alone may not account for all the micronucleated cells, as their levels were high in every case. More investigations would be necessary to determine if a part of these micronucleated mononucleated cells escaped from cytokinesis inhibition or passed mitosis without chromosome segregation.

3.4. Cytosine arabinoside

Cytosine arabinoside was found clearly positive in all treatment schedules, with and without cytochalasin B, with reproducible and concentration-dependent increases in the number of micronucleated cells, even at low cytotoxicity. In the presence of cytochalasin B, as seen with bleomycin, increases in micronuclei were observed in both binucleated as well as mononucleated cells.

3.5. Urethane

Urethane, tested up to 5000 μg/ml, produced low or no cytotoxicity and no increase in the number of micronucleated cells in the absence of cytochalasin B. It was clearly negative in all treatment schedules without cytochalasin B. When cytochalasin B was used, sporadic equivocal responses were found; but they were not concentration-related and not reproducible between the assays of a same laboratory and, in some instances, related to especially low spontaneous backgrounds. A third laboratory confirmed the negative results. Thus, urethane was also judged negative after the 3 and 24 h treatments in the presence of cytochalasin B.

3.6. Diethylstilboestrol

Only a few data remained available after evaluating the acceptability of assays, especially as no suitable cytotoxicity was achieved. This reflected the difficulty in finding concentrations sufficiently high to induce genotoxicity with an acceptable cytotoxicity. However, where adequate range of concentrations were obtained, clear concentration-dependent inductions of micronucleated cells were found, in all the treatment schedules, using cytochalasin B or not. In the presence of cytochalasin B, increases in micronuclei were observed in both binucleated as well as mononucleated cells.

4. Discussion

Mannitol was found negative in all treatment schedules, as expected with no cytotoxicity. Bleomycin was detected in all the treatment schedules. With cytochalasin B, an unexpected increase in the number of micronuclei in mononucleated cells would need more investigation to better understand its mechanism. The base analog cytosine arabinoside was also unambiguously found positive both in binucleated and mononucleated cells. Urethane was judged as negative in all the treatment schedules. These results must be considered in the light of published data and of the results obtained in other cell types in this collaborative study, which showed similar results [19]. This confirms previous published results reporting difficulties to detect this compound in vitro without an adequate specific metabolic activation. Indeed, there is some evidence that urethane might not be able to induce clastogenicity in vitro even in the presence of metabolic activation [22].

The aneugen diethylstilboestrol was found positive in all the treatment schedules, despite difficulties in finding a range of concentrations adequate to detect genotoxicity.

Among the different treatment schedules used, the results were comparable. No schedule appeared to give better results on this limited list of compounds. However, on a larger list of compounds, we may assume that the combination of a short and a long treatment would have been shown necessary for a full accurate evaluation of genotoxicity, as it was shown in other cell types in this collaborative study [19]. With or without cytochalasin B, for a given treatment schedule, the induction factors over the controls were of a same magnitude. Lastly, the evaluation of genotoxicity of the tested compounds in this study was concordant with published data [2,9,12,13,18].

In conclusion, the results of the present study show that CHO cells are suitable for detecting accurately genotoxic compounds of various types in the in vitro micronucleus test. All treatment-recovery schedules are suitable to detect genotoxic compounds. In any case, no genotoxic compound would have been missed with schedules including a short and a long treatment time, whatever the treatment was followed by a recovery period or not and whatever cytochalasin B was used or not. No differences were seen in the sensitivity or accuracy of the responses whether cytochalasin B was used or not.

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Appendix A. Individual data

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
Mannitol														
Lab 1	Assay 1	–	3 + 21	Medium	0	22.0	1.0	na	na	nt	2	g	Y	NEG
					500	24.0	1.1	na	na	nt	2	g		
					1000	14.5	0.7	na	na	nt	2	g		
					1500	15.5	0.7	na	na	nt	2	g		
					2000	9.0	0.4	na	na	nt	2	g		
					2500	9.0	0.4	na	na	nt	2	g		
					3000	8.5	0.4	na	na	nt	2	g		
					3500	15.0	0.7	na	na	nt	2	g		
					4000	17.5	0.8	na	na	nt	2	g		
					4500	14.0	0.6	na	na	nt	2	g		
					5000	11.5	0.5	na	na	nt	2	g, p		
					MMC 0.2	188.0**	8.5	na	na	nt	2	g		
		–	3 + 45	Medium	0	23.5	1.0	na	na	100	2		Y	NEG
					100	13.0	0.6	na	na	70	2			
					200	14.0	0.6	na	na	67	2			
					700	nt	nd	na	na	49	2			
					800	nt	nd	na	na	50	2			
					900	17.0	0.7	na	na	50	2			
					1000	20.0	0.9	na	na	48	2	p		
					MMC 0.2	151.5**	6.4	na	na	90	2			
		–	24 + 0	Medium	0	20.5	1.0	na	na	100	2		Y	NEG
					100	25.0	1.2	na	na	47	2			
					200	18.5	0.9	na	na	48	2			
					700	nt	nt	na	na	48	2			
					800	nt	nt	na	na	51	2			
					900	21.0	1.0	na	na	48	2			
					1000	18.5	0.9	na	na	47	2	p		
					MMC 0.1	187.5**	9.1	na	na	51	2			
		–	24 + 24	Medium	0	32.5	1.0	na	na	nt	2	g	Y	NEG
					500	24.0	0.7	na	na	nt	2	g		
					1000	20.5	0.6	na	na	nt	2	g		
					3500	26.5	0.8	na	na	nt	2	g		
					4000	22.5	0.7	na	na	nt	2	g		
					4500	26.0	0.8	na	na	nt	2	g		
					5000	20.5	0.6	na	na	nt	2	g, p		
					MMC 0.1	111.0**	3.4	na	na	nt	2	g		

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
		+	3 + 20	Medium	0	16.5	1.0	18.5	1.0	100	2		Y	NEG
					500	14.0	0.8	18.5	1.0	100	2			
					1000	nd	nd	11.5	0.6	94	2			
					3500	9.5	0.6	19.5	1.1	93	2			
					4000	10.5	0.6	25.5	1.4	88	2			
					4500	10.0	0.6	22.5	1.2	85	2			
					5000	11.5	0.7	22.5	1.2	83	2	p		
					MMC 0.2	23.0	1.4	120.0**	6.5	80	2			
		+	24 + 20	Medium	0	5.0	1.0	21.0	1.0	100	2		Y	POS
					500	9.0	1.8	40.0**	1.9	117	2			
					4000	8.0	1.6	48.0**	2.3	97	2			
					4500	9.5	1.9	54.5**	2.6	102	2			
					5000	8.0	1.6	34.5*	1.6	88	2	p		
					MMC 0.1	26.5**	5.3	224.5**	10.7	82	2			
Lab 1	Assay 2	–	3 + 21	Medium	0	21.0	1.0	na	na	100	2		Y	NEG
					100	14.5	0.7	na	na	95	2			
					200	16.0	0.8	na	na	86	2			
					700	17.5	0.8	na	na	88	2			
					800	13.5	0.6	na	na	84	2			
					900	22.0	1.0	na	na	77	2			
					1000	9.0	0.4	na	na	80	2	p		
					MMC 0.2	155.5**	7.4	na	na	71	2			
		–	3 + 45	Medium	0	26.5	1.0	na	na	100	2		Y	NEG
					100	16.5	0.6	na	na	153	2			
					200	18.5	0.7	na	na	125	2			
					700	17.0	0.6	na	na	7	2	T		
					800	19.5	0.7	na	na	123	2			
					900	17.5	0.7	na	na	123	2			
					1000	15.5	0.6	na	na	98	2	p		
					MMC 0.2	132.5**	5.0	na	na	97	2			
		–	24 + 0	Medium	0	17.0	1.0	na	na	100	2		Y	NEG
					100	19.0	1.1	na	na	91	2			
					200	18.5	1.1	na	na	97	2			
					700	14.0	0.8	na	na	97	2			
					800	14.5	0.9	na	na	104	2			
					900	16.0	0.9	na	na	101	2			
					1000	15.5	0.9	na	na	101	2	p		
					MMC 0.1	122.5**	7.2	na	na	39	2			

		–	24 + 24	Medium	0	18.0	1.0	na	na	100	2		Y	NEG
					100	14.5	0.8	na	na	105	2			
					200	16.0	0.9	na	na	73	2			
					700	16.5	0.9	na	na	118	2			
					800	15.0	0.8	na	na	108	2			
					900	16.5	0.9	na	na	116	2			
					1000	17.0	0.9	na	na	104	2	p		
					MMC 0.2	169.5**	9.4	na	na	28	2			
		+	3 + 20	Medium	0	14.0	1.0	19.0	1.0	100	2		Y	NEG
					100	14.0	1.0	22.0	1.2	92	2			
					200	20.0	1.4	20.0	1.1	94	2			
					700	16.0	1.1	17.5	0.9	98	2			
					800	19.0	1.4	25.5	1.3	98	2			
					900	15.0	1.1	16.5	0.9	101	2			
					1000	17.0	1.2	25.5	1.3	104	2	p		
					MMC 0.2	40.0**	2.9	114.5**	6.0	89	2			
		+	24 + 20	Medium	0	22.0	1.0	24.5	1.0	100	2		Y	NEG
					100	18.0	0.8	21.0	0.9	100	2			
					200	11.0	0.5	22.0	0.9	100	2			
					700	13.0	0.6	14.5	0.6	95	2			
					800	15.0	0.7	15.5	0.6	96	2			
					900	19.0	0.9	13.5	0.6	90	2			
					1000	13.0	0.6	14.5	0.6	87	2	p		
					MMC 0.1	55.0**	2.5	169.0**	6.9	98	2			
Lab 2	Assay 1	–	3 + 21	Medium	0	12.0	1.0	na	na	100	1		Y	NEG
					78	8.0	0.7	na	na	105	1			
					156	15.0	1.3	na	na	107	1			
					312	8.0	0.7	na	na	123	1			
					625	14.0	1.2	na	na	123	1			
					1250	9.0	0.8	na	na	124	1			
					5000	14.0	1.2	na	na	121	1			
					MMC 0.2	60.0**	5.0	na	na	110	1			
		–	3 + 45	Medium	0	24.0	1.0	na	na	100	1		Y	NEG
					78	21.0	0.9	na	na	111	1			
					156	12.0	0.5	na	na	91	1			
					312	13.0	0.5	na	na	113	1			
					625	19.0	0.8	na	na	78	1			
					1250	14.0	0.6	na	na	82	1			
					5000	13.0	0.5	na	na	89	1			
					MMC 0.2	72.0**	3.0	na	na	117	1			

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
		–	24 + 0	Medium	0	9.0	1.0	na	na	100	1		Y	NEG
					78	14.0	1.6	na	na	95	1			
					156	16.0	1.8	na	na	113	1			
					312	6.0	0.7	na	na	105	1			
					625	8.0	0.9	na	na	116	1			
					1250	7.0	0.8	na	na	117	1			
					5000	16.0	1.8	na	na	111	1			
					MMC 0.1	51.0**	5.7	na	na	74	1			
		–	24 + 24	Medium	0	21.0	1.0	na	na	100	1		Y	NEG
					78	20.0	1.0	na	na	92	1			
					156	11.0	0.5	na	na	109	1			
					312	16.0	0.8	na	na	nt	1			
					625	17.0	0.8	na	na	103	1			
					1250	14.0	0.7	na	na	94	1			
					5000	8.0	0.4	na	na	76	1			
					MMC 0.1	192.0**	9.1	na	na	nt	1			
		+	3 + 20	Medium	0	3.0	1.0	9.0	1.0	100	1		Y	NEG
					78	4.0	1.3	9.0	1.0	96	1			
					156	6.0	2.0	19.0	2.1	96	1			
					312	6.0	2.0	7.0	0.8	105	1			
					625	4.0	1.3	11.0	1.2	104	1			
					1250	3.0	1.0	7.0	0.8	102	1			
					5000	3.0	1.0	6.0	0.7	105	1			
					MMC 0.2	26.0**	8.7	49.0**	5.4	95	1			
		+	24 + 20	Medium	0	7.0	1.0	16.0	1.0	100	1		Y	NEG
					78	6.0	0.9	17.0	1.1	100	1			
					156	5.0	0.7	9.0	0.6	105	1			
					312	7.0	1.0	29.0	1.8	112	1			
					625	9.0	1.3	18.0	1.1	103	1			
					1250	8.0	1.1	24.0	1.5	104	1			
					5000	4.0	0.6	10.0	0.6	101	1			
					MMC 0.1	156.0**	22.3	299.0**	18.7	107	1			
Lab 2	Assay 2	–	3 + 21	Medium	0	9.5	1.0	na	na	100	2		Y	NEG
					1250	12.5	1.3	na	na	98	2			
					2500	8.0	0.8	na	na	108	2			
					5000	11.5	1.2	na	na	100	2			
					MMC 0.2	52.5**	5.5	na	na	94	2			

		–	3 + 45	Medium	0	11.5	1.0	na	na	100	2		Y	NEG
					1250	11.0	1.0	na	na	121	2			
					2500	8.0	0.7	na	na	119	2			
					5000	10.0	0.9	na	na	122	2			
					MMC 0.2	48.5**	4.2	na	na	126	2			
		–	24 + 0	Medium	0	15.5	1.0	na	na	100	2		Y	NEG
					1250	11.0	0.7	na	na	111	2			
					2500	9.0	0.6	na	na	96	2			
					5000	10.5	0.7	na	na	87	2			
					MMC 0.1	100.0**	6.5	na	na	86	2			
		–	24 + 24	Medium	0	17.0	1.0	na	na	100	2		Y	NEG
					1250	15.5	0.9	na	na	101	2			
					2500	8.5	0.5	na	na	93	2			
					5000	12.5	0.7	na	na	92	2			
					MMC 0.1	198.5**	11.7	na	na	72	2			
		+	3 + 20	Medium	0	4.5	1.0	13.0	1.0	100	2		Y	NEG
					1250	1.5	0.3	9.0	0.7	97	2			
					2500	5.5	1.2	17.0	1.3	97	2			
					5000	2.5	0.6	14.0	1.1	99	2			
					MMC 0.2	5.5	1.2	55.5**	4.3	93	2			
		+	24 + 20	Medium	0	3.0	1.0	10.5	1.0	100	2		Y	NEG
					1250	2.5	0.8	18.5*	1.8	95	2			
					2500	3.0	1.0	12.5	1.2	102	2			
					5000	3.0	1.0	15.0	1.4	100	2			
					MMC 0.1	119.0**	39.7	276.0**	26.3	105	2			
Bleomycin														
Lab 1	Assay 1	–	3 + 45	Medium	0	23.5	1.0	na	na	100	2		Y	POS
					0.88	185.0**	7.9	na	na	91	2			
					62.5	187.5**	8.0	na	na	49	2			
					125	174.0**	7.4	na	na	43	2			
					250	144.0**	6.1	na	na	14	2	T		
					MMC 0.2	160.0**	6.8	na	na	79	2			
		–	24 + 0	Medium	0	25.0	1.0	na	na	100	2		Y	POS
					3.5	143.0**	5.7	na	na	77	2			
					15	123.0**	4.9	na	na	62	2			
					62.5	101.0**	4.0	na	na	46	2			
					250	44.0**	1.8	na	na	30	2	T		
					500	29.5	1.2	na	na	21	2	T		
					MMC 0.1	119.0**	4.8	na	na	72	2			
		+	3 + 20	Medium	0	9.5	1.0	20.0	1.0	100	2		Y	POS
					0.17	18.0*	1.9	150.5**	7.5	70	2			
					0.39	18.5*	1.9	133.0**	6.7	86	2			
					0.875	13.5	1.4	113.5**	5.7	82	2			

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
					1.75	15.0	1.6	169.5**	8.5	67	2			
					14	13.5	1.4	tox	tox	39	2	T		
					MMC 0.2	19.0*	2.0	199.0**	10.0	83	2			
		+	24 + 20	Medium	0	7.5	1.0	15.5	1.0	100	2		Y	POS
					0.17	43.0**	5.7	155.0**	10.0	104	2			
					0.26	56.5**	7.5	211.5**	13.6	101	2			
					0.39	74.0**	9.9	231.0**	14.9	73	2			
					0.587	102.5**	13.7	342.5**	22.1	66	2			
					0.875	122.5**	16.3	319.0**	20.6	54	2			
					MMC 0.1	22.0**	2.9	275.5**	17.8	92	2			
Lab 1	Assay 2	–	3 + 21	Medium	0	17.0	1.0	na	na	100	2		Y	POS
					62.5	176.0**	10.4	na	na	69	2			
					125	138.0**	8.1	na	na	53	2			
					250	123.5**	7.3	na	na	46	2			
					500	74.0**	4.4	na	na	37	2	T		
					MMC 0.2	171.0**	10.1	na	na	79	2			
		–	3 + 45	Medium	0	19.5	1.0	na	na	100	2		Y	POS
					0.88	184.5**	9.5	na	na	93	2			
					62.5	241.5**	12.4	na	na	18	2	T		
					125	189.0**	9.7	na	na	20	2	T		
					250	150.5**	7.7	na	na	13	2	T		
					MMC 0.2	190.0**	9.7	na	na	91	2			
		–	24 + 0	Medium	0	18.0	1.0	na	na	100	2		Y	POS
					3.5	121.5**	6.8	na	na	62	2			
					15	113.5**	6.3	na	na	38	2	T		
					62.5	90.5**	5.0	na	na	40	2			
					250	29.5*	1.6	na	na	25	2	T		
					500	24.5	1.4	na	na	27	2	T		
					MMC 0.1	87.0**	4.8	na	na	49	2			
		–	24 + 24	Medium	0	25.0	1.0	na	na	100	2		Y	POS
					3.5	282.0**	11.3	na	na	63	2			
					15	288.0**	11.5	na	na	43	2			
					62.5	290.5**	11.6	na	na	32	2	T		
					125	278.0**	11.1	na	na	31	2	T		
					MMC 0.1	173.5**	6.9	na	na	59	2			
		+	3 + 20	Medium	0	13.0	1.0	24.0	1.0	100	2		Y	POS
					3.5	26.0**	2.0	147.5**	6.1	76	2			
					30	30.0**	2.3	233.5**	9.7	38	2	T		

					62.5	38.5**	3.0	208.0**	8.7	23	2	T		
					250	34.0**	2.6	tox	tox	74	1			
					500	21.0	1.6	tox	tox	20	1	T		
					MMC 0.2	32.0**	2.5	119.0**	5.0	88	2			
		+	24 + 20	Medium	0	11.0	1.0	19.5	1.0	100	2		Y	POS
					0.29	65.0**	5.9	208.5**	10.7	93	2			
					0.88	87.0**	7.9	272.5**	14.0	95	2			
					3.5	122.0**	11.1	302.5**	15.5	92	2			
					MMC 0.1	23.5**	2.1	165.5**	8.5	94	2			
Lab 2	Assay 1	–	3 + 21	Medium	0	12.0	1.0	na	na	100	2		Y	POS
					0.11	20.0	1.7	na	na	84	2			
					0.45	29.0**	2.4	na	na	77	2			
					0.9	41.0**	3.4	na	na	80	2			
					1.8	41.5**	3.5	na	na	86	2			
					3.6	54.5**	4.6	na	na	93	2			
					15.6	86.0**	7.2	na	na	79	2			
					31.2	104.0**	8.7	na	na	86	2			
					62.5	148.0**	12.3	na	na	94	2			
					250	181.0**	15.1	na	na	46	2			
					500	249.0**	20.8	na	na	17	2	T		
					MMC 0.2	43.0*	3.6	na	na	nt	2			
		–	3 + 45	Medium	0	13.0	1.0	na	na	100	2		Y	POS
					0.11	15.0	1.2	na	na	69	2			
					0.23	19.0	1.5	na	na	100	2			
					0.45	22.5*	1.8	na	na	100	2			
					0.9	17.0	1.3	na	na	94	2			
					1.8	41.5**	3.2	na	na	66	2			
					3.6	51.0**	3.9	na	na	93	2			
					7.8	47.5**	3.7	na	na	60	2			
					15.6	86.5**	6.7	na	na	55	2			
					31.2	97.5**	7.5	na	na	46	2			
					62.5	139.5**	10.8	na	na	46	2			
					250	214.5**	16.5	na	na	12	2	T		
					500	203.0**	15.6	na	na	7	2	T		
					MMC 0.2	43.0**	3.3	na	na	nt	1			
		–	24 + 0	Medium	0	7.5	1.0	na	na	100	2		Y	POS
					0.11	114.5**	15.3	na	na	81	2			
					0.23	154.0**	20.5	na	na	80	2			
					0.45	158.0**	21.1	na	na	63	2			
					0.9	148.5**	19.8	na	na	73	2			
					1.8	200.5**	26.7	na	na	79	2			
					3.9	128.5**	17.1	na	na	33	2	T		
					7.8	115.5**	15.4	na	na	36	2	T		
					15.6	84.5**	11.3	na	na	35	2	T		
					31.2	90.5**	12.1	na	na	31	2	T		

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
					62.5	35.0**	4.7	na	na	17	2	T		
					125	18.0**	2.4	na	na	15	2	T		
					250	7.0	0.9	na	na	18	1	T		
					500	7.0	0.9	na	na	10	1	T		
					MMC 0.1	190.0**	25.3	na	na	58	1			
		+	3 + 20	Medium	0	nt	nt	6.0	1.0	100	1		Y	POS
					0.11	nt	nt	54.0**	9.0	103	1			
					0.23	nt	nt	54.0**	9.0	105	1			
					0.45	nt	nt	62.0**	10.3	101	1			
					0.9	nt	nt	53.0**	8.8	99	1			
					1.8	nt	nt	83.0**	13.8	101	1			
					3.9	nt	nt	124.0**	20.7	93	1			
					7.8	nt	nt	175.0**	29.2	91	1			
					15.6	nt	nt	185.0**	30.8	80	1			
					31.2	nt	nt	227.0**	37.8	63	1			
					62.5	nt	nt	309.0**	51.5	65	1			
					MMC 0.2	nt	nt	105.0**	17.5	95	1			
		+	24 + 20	Medium	0	nt	nt	10.0	1.0	100	2		Y	POS
					0.0017	nt	nt	15.0	1.5	93	1			
					0.0034	nt	nt	16.0	1.6	92	1			
					0.0068	nt	nt	25.0**	2.5	90	1			
					0.0137	nt	nt	27.0**	2.7	95	1			
					0.0275	nt	nt	47.0**	4.7	86	1			
					0.055	nt	nt	80.0**	8.0	90	1			
					0.11	nt	nt	163.5**	16.4	98	2			
					0.23	nt	nt	215.0**	21.5	92	2			
					0.45	nt	nt	236.5**	23.7	94	2			
					0.9	nt	nt	301.5**	30.2	82	2			
					1.8	nt	nt	309.5**	31.0	78	2			
					3.9	nt	nt	428.0**	42.8	65	1			
					7.8	nt	nt	504.0	50.4	57	1			
					MMC 0.1	nt	nt	321.0**	32.1	99	1			
Lab 2	Assay 2	–	3 + 21	Medium	0	9.8	1.0	na	na	100	2		Y	POS
					15.6	120.0**	12.3	na	na	75	2			
					31.2	172.5**	17.7	na	na	54	2			
					62.5	162.0**	16.6	na	na	51	2			
					125	157.5**	16.2	na	na	50	2			
					250	136.0**	13.9	na	na	30	2	T		
					MMC 0.2	109.5**	11.2	na	na	59	2			

		+	3 + 20	Medium	0	nt	nt	16.5	1.0	100	2		Y	POS
					10	nt	nt	132.0**	8.0	78	2			
					15	nt	nt	114.5**	6.9	72	2			
					20	nt	nt	148.5**	9.0	70	2			
					25	nt	nt	207.0**	12.5	64	2			
					30	nt	nt	175.5**	10.6	59	2			
					35	nt	nt	190.0**	11.5	60	2			
					40	nt	nt	211.5**	12.8	56	2			
					50	nt	nt	226.5**	13.7	51	2			
					100	nt	nt	240.5**	14.6	39	2	T		
					MMC 0.2	nt	nt	97.5**	5.9	95	2			
		+	24 + 20	Medium	0	nt	nt	10.5	1.0	100	2		Y	POS
					0.0025	nt	nt	14.0	1.3	97	2			
					0.005	nt	nt	12.0	1.1	106	2			
					0.0075	nt	nt	11.5	1.1	101	2			
					0.01	nt	nt	12.0	1.1	105	2			
					0.025	nt	nt	24.5**	2.3	105	2			
					0.05	nt	nt	26.0**	2.5	106	2			
					0.075	nt	nt	55.5**	5.3	108	2			
					0.1	nt	nt	64.5**	6.1	99	2			
					MMC 0.1	nt	nt	448.5**	42.7	109	2			
Cytosine arabinoside														
Lab 1	Assay 1	–	3 + 21	Medium	0	5.0	1.0	na	na	100	1		Y	POS
					0.03	nt	nt	na	na	132	1			
					0.1	nt	nt	na	na	111	1			
					0.3	4.0	0.8	na	na	120	1			
					1	13.0	2.6	na	na	105	1			
					3	39.0**	7.8	na	na	76	1			
					10	41.0**	8.2	na	na	31	1	T		
					30	30.0**	6.0	na	na	32	1	T		
					100	38.0**	7.6	na	na	29	1	T		
					MMC 0.2	91.0**	18.2	na	na	57	1			
		–	3 + 45	Medium	0	14.0	1.0	na	na	100	1		Y	POS
					0.03	17.0	1.2	na	na	94	1			
					0.1	9.0	0.6	na	na	103	1			
					0.3	8.0	0.6	na	na	104	1			
					1	12.0	0.9	na	na	91	1			
					3	29.0*	2.1	na	na	76	1			
					10	17.0	1.2	na	na	43	1			
					30	46.0**	3.3	na	na	39	1	T		
					100	185.0**	13.2	na	na	27	1	T		
					MMC 0.2	100.0**	7.1	na	na	78	1			

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
		–	24 + 0	Medium	0	11.0	1.0	na	na	100	1		Y	POS
					0.01	6.0	0.5	na	na	78	1			
					0.03	8.0	0.7	na	na	60	1			
					0.1	24.0*	2.2	na	na	50	1			
					0.3	57.0**	5.2	na	na	43	1			
					1	17.0	1.5	na	na	36	1	T		
					3	10.0	0.9	na	na	33	1	T		
					10	15.0	1.4	na	na	24	1	T		
					MMC 0.1	106.0**	9.6	na	na	40	1			
		–	24 + 24	Medium	0	11.0	1.0	na	na	100	1		Y	POS
					0.01	8.0	0.7	na	na	79	1			
					0.03	13.0	1.2	na	na	73	1			
					0.1	35.0**	3.2	na	na	66	1			
					0.3	55.0**	5.0	na	na	33	1	T		
					1	156.0**	14.2	na	na	18	1	T		
					3	12.0	1.1	na	na	12	1	T		
					10	17.0	1.5	na	na	6	1	T		
					MMC 0.1	213.0**	19.4	na	na	40	1			
		+	3 + 20	Medium	0	12.0	1.0	13.0	1.0	100	1		Y	POS
					0.3	12.0	1.0	12.0	0.9	120	1			
					1	24.0	2.0	18.0	1.4	118	1			
					3	4.0	0.3	34.0**	2.6	112	1			
					10	8.0	0.7	72.0**	5.5	79	1			
					30	20.0	1.7	102.0**	7.8	55	1			
					100	24.0	2.0	252.0**	19.4	26	1	T		
					MMC 0.2	24.0	2.0	321.0**	24.7	107	1			
		+	24 + 20	Medium	0	4.0	1.0	11.0	1.0	100	1		Y	POS
					0.003	12.0	3.0	10.0	0.9	104	1			
					0.01	12.0	3.0	14.0	1.3	101	1			
					0.03	8.0	2.0	14.0	1.3	106	1			
					0.1	50.0*	12.5	54.0**	4.9	115	1			
					0.3	tox	tox	213.0**	19.4	116	1			
					1	tox	tox	540.0**	49.1	116	1			
					MMC 0.1	256.0**	64.0	800.0**	72.7	91	1			
Lab 1	Assay 2	–	3 + 21	Medium	0	7.5	1.0	na	na	100	2		Y	POS
					1	10.5	1.4	na	na	97	2			
					3	26.0**	3.5	na	na	75	2			
					10	39.0**	5.2	na	na	47	2			
					30	31.5**	4.2	na	na	29	2	T		
					MMC 0.2	142.0**	18.9	na	na	30	2	T		

		–	3 + 45	Medium	0	8.0	1.0	na	na	100	2		Y	POS
					1	9.5	1.2	na	na	80	2			
					3	22.0**	2.8	na	na	67	2			
					10	22.0**	2.8	na	na	49	2			
					30	27.0**	3.4	na	na	27	2	T		
					MMC 0.2	73.5**	9.2	na	na	47	2			
		–	24 + 0	Medium	0	11.0	1.0	na	na	100	2		Y	POS
					0.03	8.0	0.7	na	na	81	2			
					0.1	40.0**	3.6	na	na	72	2			
					0.3	94.0**	8.5	na	na	49	2			
					1	28.5**	2.6	na	na	51	2			
					MMC 0.1	166.5**	15.1	na	na	87	2			
		–	24 + 24	Medium	0	7.0	1.0	na	na	100	2		Y	POS
					0.03	9.5	1.4	na	na	83	2			
					0.1	23.0**	3.3	na	na	69	2			
					0.3	95.5**	13.6	na	na	48	2			
					1	187.5**	26.8	na	na	23	2	T		
					MMC 0.1	450.5**	64.4	na	na	36	2	T		
		+	3 + 20	Medium	0	2.0	1.0	11.0	1.0	100	2		Y	POS
					1	14.0	7.0	12.0	1.1	105	2			
					3	10.0	5.0	41.0**	3.7	101	2			
					10	18.0	9.0	90.0**	8.2	67	2			
					30	16.0*	8.0	84.5**	7.7	55	2			
					MMC 0.2	4.0*	2.0	328.5**	29.9	97	2			
		+	24 + 20	Medium	0	8.0	1.0	15.5	1.0	100	2		Y	POS
					0.03	2.0	0.3	8.5	0.5	108	2			
					0.1	4.0	0.5	26.0*	1.7	115	2			
					0.3	6.0	0.8	193.5**	12.5	119	2			
					1	0.0	0.0	540.0**	34.8	122	2			
					MMC 0.1	190.0**	23.8	832.0**	53.7	109	2			
Lab 2	Assay 1	–	3 + 21	Medium	0	3.0	1.0	na	na	100	1		Y	POS
					0.625	5.0	1.7	na	na	109	1			
					1.25	13.0*	4.3	na	na	76	1			
					2.5	54.0**	18.0	na	na	84	1			
					5	74.0**	24.7	na	na	50	1			
					10	92.0**	30.7	na	na	43	1			
					20	115.0**	38.3	na	na	37	1	T		
					MMC 0.2	70.0**	23.3	na	na	69	1			
		–	3 + 45	Medium	0	8.0	1.0	na	na	100	1		Y	POS
					0.625	11.0	1.4	na	na	89	1			
					1.25	5.0	0.6	na	na	109	1			
					2.5	19.0	2.4	na	na	106	1			
					5	23.0*	2.9	na	na	64	1			
					10	43.0**	5.4	na	na	59	1			
					20	38.0**	4.8	na	na	55	1			
					MMC 0.1	32.0**	4.0	na	na	112	1			

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
		–	24 + 0	Medium	0	9.0	1.0	na	na	100	1		Y	POS
					0.00975	11.0	1.2	na	na	89	1			
					0.0195	13.0	1.4	na	na	100	1			
					0.039	16.0	1.8	na	na	114	1			
					0.078	14.0	1.6	na	na	65	1			
					0.156	27.0**	3.0	na	na	62	1			
					0.3125	103.0**	11.4	na	na	69	1			
					MMC 0.1	107.0**	11.9	na	na	64	1			
		–	24 + 24	Medium	0	13.0	1.0	na	na	100	1		Y	POS
					0.00975	6.0	0.5	na	na	111	1			
					0.0195	36.0**	2.8	na	na	102	1			
					0.039	20.0	1.5	na	na	111	1			
					0.078	35.0**	2.7	na	na	57	1			
					0.156	110.0**	8.5	na	na	45	1			
					0.3125	143.0**	11.0	na	na	35	1	T		
					MMC 0.05	137.0**	10.5	na	na	99	1			
		+	3 + 20	Medium	0	0.0	0.0	15.0	1.0	100	1		Y	POS
					0.625	0.0	0.0	18.0	1.2	105	1			
					1.25	8.0	8.0	35.0**	2.3	104	1			
					2.5	4.0	4.0	58.0**	3.9	106	1			
					5	2.0	2.0	106.0**	7.1	83	1			
					10	4.0	4.0	92.0**	6.1	58	1			
					20	12.0*	12.0	67.0**	4.5	44	1			
					MMC 0.2	20.0**	20.0	156.0**	10.4	103	1			
		+	24 + 20	Medium	0	4.0	1.0	22.0	1.0	100	1		Y	POS
					0.00975	6.0	1.5	5.0	0.2	108	1			
					0.0195	2.0	0.5	21.0	1.0	105	1			
					0.039	14.0	3.5	32.0	1.5	111	1			
					0.078	26.0**	6.5	50.0**	2.3	109	1			
					0.156	161.3**	58.3	184.0**	8.4	115	1			
					0.3125	233.3**	50.0	456.0**	20.7	124	1			
					MMC 0.05	40.0**	10.0	123.0**	5.6	106	1			
Lab 2	Assay 2	–	3 + 21	Medium	0	7.5	1.0	na	na	100	2		Y	POS
					2.5	33.5**	4.5	na	na	101	2			
					5	54.5**	7.3	na	na	68	2			
					10	52.5**	7.0	na	na	53	2			
					MMC 0.2	75.0**	10.0	na	na	60	2			

		–	3 + 45	Medium	0	7.5	1.0	na	na	100	2	Y	POS
					5	57.5**	7.7	na	na	77	2		
					10	91.5**	12.2	na	na	93	2		
					20	91.0**	12.1	na	na	65	2		
					30	237.0**	31.6	na	na	52	2		
					MMC 0.2	102.5**	13.7	na	na	105	2		
		–	24 + 0	Medium	0	9.5	1.0	na	na	100	2	Y	POS
					0.078	51.0**	5.4	na	na	67	2		
					0.156	145.5**	15.3	na	na	84	2		
					0.3125	92.0**	9.7	na	na	90	2		
					0.625	68.0**	7.2	na	na	60	2		
					MMC 0.1	149.0**	15.7	na	na	40	2		
		–	24 + 24	Medium	0	10.0	1.0	na	na	100	2	Y	POS
					0.00975	13.5	1.4	na	na	108	2		
					0.0195	14.5	1.5	na	na	99	2		
					0.039	10.0	1.0	na	na	106	2		
					0.078	23.0**	2.3	na	na	80	2		
					0.156	67.0**	6.7	na	na	97	2		
					0.3125	153.5**	15.4	na	na	45	2		
					MMC 0.1	176.5**	17.7	na	na	92	2		
		+	3 + 20	Medium	0	3.0	1.0	12.0	1.0	100	2	Y	POS
					2.5	12.0*	4.0	54.5**	4.5	102	2		
					5	13.0*	4.3	107.0**	8.9	85	2		
					10	22.0**	7.3	141.5**	11.8	53	2		
					MMC 0.2	25.4**	8.5	230.0**	19.2	96	2		
		+	24 + 20	Medium	0	1.0	1.0	14.5	1.0	100	2	Y	POS
					0.039	14.0**	14.0	32.5**	2.2	105	2		
					0.078	16.0**	16.0	120.5**	8.3	105	2		
					0.156	422.9**	422.9	338.5**	23.3	104	2		
					0.3125	nt	nt	619.5**	42.7	105	2		
					MMC 0.1	157.0**	157.0	643.0**	44.3	97	2		
Urethane													
Lab 1	Assay 1	–	3 + 21	Medium	0	0.5	1.0	na	na	100	2	Y	NEG
					1000	0.0	0.0	na	na	104	2		
					2000	1.0	2.0	na	na	111	2		
					3500	0.0	0.0	na	na	72	2		
					5000	1.5	3.0	na	na	59	2		
					MMC 0.2	12.0**	24.0	na	na	nt	2		
		–	3 + 45	Medium	0	1.0	1.0	na	na	100	2	Y	NEG
					1000	2.0	2.0	na	na	119	2		
					2000	2.0	2.0	na	na	130	2		
					3500	1.0	1.0	na	na	116	2		
					5000	1.5	1.5	na	na	123	2		
					MMC 0.2	9.5**	9.5	na	na	nt	2		

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
		–	24 + 0	Medium	0	1.5	1.0	na	na	100	2		Y	NEG
					1000	1.5	1.0	na	na	120	2			
					2000	0.5	0.3	na	na	125	2			
					3500	2.0	1.3	na	na	71	2			
					5000	2.5	1.7	na	na	82	2			
					MMC 0.1	7.5**	5.0	na	na	nt	2			
		–	24 + 24	Medium	0	0.0	0.0	na	na	100	2		Y	NEG
					1000	1.5	1.5	na	na	63	2			
					2000	0.5	0.5	na	na	73	2			
					3500	2.0	2.0	na	na	48	2			
					5000	2.5	2.5	na	na	53	2			
					MMC 0.1	32.0**	32.0	na	na	nt	2			
		+	3 + 20	Medium	0	5.5	1.0	2.5	1.0	100	2		Y	EQ
					1000	2.4	0.4	14.5**	5.8	101	2			
					2000	10.6	1.9	8.5*	3.4	98	2			
					3500	6.0	1.1	9.0*	3.6	97	2			
					5000	4.0	0.7	9.5**	3.8	100	2			
					MMC 0.2	5.0	0.9	60.0**	24.0	nt	2			
		+	24 + 20	Medium	0	6.5	1.0	3.5	1.0	100	2		Y	POS
					1000	3.0	0.5	7.5	2.1	93	2			
					2000	7.0	1.1	8.5	2.4	93	2			
					3500	5.0	0.8	11.0**	3.1	80	2			
					5000	3.5	0.5	7.5	2.1	75	2			
					MMC 0.1	23.0**	6.5	285.5**	161.0	nt	2			
Lab 1	Assay 2	–	3 + 21	Medium	0	1.5	1.0	na	na	100	2		Y	NEG
					1000	0.5	0.3	na	na	125	2			
					2000	0.5	0.3	na	na	108	2			
					3500	0.5	0.3	na	na	183	2			
					5000	1.5	1.0	na	na	71	2			
					MMC 0.2	12.5**	8.3	na	na	nt	2			
		–	3 + 45	Medium	0	1.5	1.0	na	na	100	2		Y	NEG
					1000	0.0	0.0	na	na	97	2			
					2000	2.0	1.3	na	na	69	2			
					3500	0.5	0.3	na	na	101	2			
					5000	2.0	1.3	na	na	60	2			
					MMC 0.2	11.5**	7.7	na	na	nt	2			

		–	24 + 24	Medium	0	1.0	1.0	na	na	100	2		Y	NEG
					1000	0.5	0.5	na	na	69	2			
					2000	0.5	0.5	na	na	66	2			
					3500	1.5	1.5	na	na	43	2			
					5000	2.0	2.0	na	na	24	2	T		
					MMC 0.1	88.5**	88.5	na	na	nt	2			
		+	3 + 20	Medium	0	4.5	1.0	4.5	1.0	100	2		Y	NEG
					1000	0.5	0.1	4.5	1.0	94	2			
					2000	3.5	0.8	4.5	1.0	97	2			
					3500	2.5	0.6	5.0	1.1	99	2			
					5000	4.5	1.0	3.0	0.7	98	2			
					MMC 0.2	8.5	1.9	65.0**	14.4	nt	2			
		+	24 + 20	Medium	0	6.0	1.0	7.0	1.0	100	2		Y	NEG
					1000	0.0	0.0	0.5	0.1	83	2			
					2000	0.5	0.1	1.0	0.1	85	2			
					3500	2.0	0.3	5.0	0.7	73	2			
					5000	1.0	0.2	9.5	1.4	62	2			
					MMC 0.1	72.5**	12.1	403.5**	57.6	nt	2			
Lab 2	Assay 2	–	3 + 21	Medium	0	19.5	1.0	na	na	100	2		Y	NEG
					625	22.5	1.2	na	na	101	2			
					1250	13.0	0.7	na	na	130	2			
					2500	18.0	0.9	na	na	93	2			
					5000	16.5	0.8	na	na	85	2			
					MMC 0.2	167.5**	8.6	na	na	52	2			
		–	24 + 0	Medium	0	17.5	1.0	na	na	100	2		Y	NEG
					625	23.5	1.3	na	na	123	2			
					1250	19.0	1.1	na	na	134	2			
					2500	14.0	0.8	na	na	79	2			
					5000	12.0	0.7	na	na	63	2			
					MMC 0.1	88.0**	5.0	na	na	40	2			
		–	24 + 24	Medium	0	29.0	1.0	na	na	100	2		N	na
					625	19.5	0.7	na	na	95	2			
					1250	17.0	0.6	na	na	86	2			
					2500	24.5	0.8	na	na	66	2			
					5000	64.5**	2.2	na	na	38	2	T		
					MMC 0.1	81.0**	2.8	na	na	17	2	T		
		+	3 + 20	Medium	0	9.0	1.0	22.0	1.0	100	2		Y	EQ
					625	58.5**	6.5	38.5**	1.8	76	2			
					1250	37.0**	4.1	29.5	1.3	102	2			
					2500	41.5**	4.6	34.5*	1.6	101	2			
					5000	32.5**	3.6	33.5*	1.5	101	2			
					MMC 0.2	28.5**	3.2	252.0**	11.5	25	2	T		
		+	24 + 20	Medium	0	6.0	1.0	9.5	1.0	100	2		Y	EQ
					625	5.5	0.9	16.5	1.7	91	2			
					1250	8.5	1.4	14.0	1.5	87	2			

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
					2500	7.0	1.2	17.0	1.8	73	2			
					5000	5.0	0.8	19.5*	2.1	60	2			
					MMC 0.1	55.5**	9.3	209.0**	22.0	29	2	T		
Lab 3	Assay 1	–	3 + 21	Medium	0	8.0	1.0	na	na	100	2		Y	NEG
					1250	4.5	0.6	na	na	102	2			
					2500	9.0	1.1	na	na	94	2			
					5000	9.5	1.2	na	na	83	2			
					MMC 0.05	138.0**	17.3	na	na	94	2			
		–	3 + 45	Medium	0	10.5	1.0	na	na	100	2		Y	NEG
					1250	7.0	0.7	na	na	93	2			
					2500	17.5	1.7	na	na	95	2			
					5000	13.0	1.2	na	na	95	2			
					MMC 0.05	161.0**	15.3	na	na	85	2			
		–	24 + 0	Medium	0	10.0	1.0	na	na	100	2		Y	NEG
					1250	11.0	1.1	na	na	98	2			
					2500	10.0	1.0	na	na	94	2			
					5000	7.5	0.8	na	na	71	2			
					MMC 0.05	136.5**	13.7	na	na	82	2			
		–	24 + 24	Medium	0	13.0	1.0	na	na	100	2		Y	NEG
					1250	15.0	1.2	na	na	106	2			
					2500	16.0	1.2	na	na	97	2			
					5000	8.0	0.6	na	na	75	2			
					MMC 0.05	166.5**	12.8	na	na	89	2			
		+	3 + 20	Medium	0	3.0	1.0	10.0	1.0	100	2		Y	NEG
					1250	4.5	1.5	7.0	0.7	98	2			
					2500	1.0	0.3	10.0	1.0	89	2			
					5000	3.5	1.2	13.5	1.4	54	2			
					MMC 0.25	70.5**	23.5	287.5**	28.8	73	2			
		+	24 + 20	Medium	0	1.5	1.0	9.0	1.0	100	2		Y	NEG
					1250	2.5	1.7	8.5	0.9	99	2			
					2500	3.5	2.3	7.0	0.8	95	2			
					5000	1.0	0.7	14.0	1.6	93	2			
					MMC 0.05	23.5**	15.7	137.5**	15.3	93	2			
Diethylstilboestrol														
Lab 1	Assay 2	–	3 + 21	Ethanol	0	16.5	1.0	na	na	100	2		Y	POS
					7.5	12.0	0.7	na	na	96	2			

					15	8.0	0.5	na	na	104	2			
					30	8.0	0.5	na	na	91	2			
					40	49.5**	3.0	na	na	51	2			
					MMC 0.2	168.5**	10.2	na	na	51	2			
		–	3 + 45	Ethanol	0	5.5	1.0	na	na	100	2		N	na
					15	7.5	1.4	na	na	98	2			
					30	12.0*	2.2	na	na	93	2			
					40	20.5**	3.7	na	na	48	2			
					MMC 0.2	nt	nt	na	na	10	2	T		
		–	24 + 0	Ethanol	0	19.0	1.0	na	na	100	2		N	na
					7.5	242.0**	12.7	na	na	18	2	T		
					15	166.5**	8.8	na	na	13	2	T		
					30	18.0	0.9	na	na	18	2	T		
					40	17.5	0.9	na	na	25	2	T		
					MMC 0.1	184.0**	9.7	na	na	44	2			
		–	24 + 24	Ethanol	0	9.5	1.0	na	na	100	2		N	na
					1.88	13.0	1.4	na	na	82	2			
					3.75	21.5**	2.3	na	na	31	2	T		
					5	28.5**	3.0	na	na	37	2	T		
					MMC 0.1	164.0**	17.3	na	na	15	2	T		
		+	3 + 20	Ethanol	0	43.5	1.0	34.0	1.0	100	2		N	na
					7.5	21.0	0.5	21.5	0.6	96	2			
					15	29.5	0.7	26.0	0.8	97	2			
					22.5	22.0	0.5	19.0	0.6	97	2			
					30	16.0	0.4	15.0	0.4	86	2			
					40	18.0	0.4	nt	nt	10	2	T		
					MMC 0.2	31.5	0.7	241.5**	7.1	28	2	T		
		+	24 + 20	Ethanol	0	12.0	1.0	10.5	1.0	100	2		Y	POS
					1.88	18.0	1.5	15.5	1.5	103	2			
					3.75	20.0	1.7	28.0**	2.7	87	2			
					5	46.5**	3.9	44.0**	4.2	96	2			
					MMC 0.1	156.0**	13.0	nt	nt	nt	2			
Lab 2	Assay 1	–	3 + 21	Ethanol	0	1.0	1.0	na	na	100	2		Y	POS
					0.5	25.5**	25.5	na	na	87	2			
					1	19.5**	19.5	na	na	89	2			
					5	23.0**	23.0	na	na	82	2			
					10	17.5**	17.5	na	na	53	2			
					MMC 0.2	115.5**	115.5	na	na	61	2			
		–	3 + 45	Ethanol	0	2.0	1.0	na	na	100	2		Y	POS
					0.5	18.0**	9.0	na	na	101	2			
					1	23.5**	11.8	na	na	81	2			
					5	22.5**	11.3	na	na	79	2			
					10	17.0**	8.5	na	na	53	2			
					MMC 0.2	220.0**	110.0	na	na	43	2			

Appendix A (*Continued*)

Lab number	Assay number	CytB[a]	Schedule: treatment + recovery (h)	Solvent	Concentration (μg/ml)[b]	Micronucleated mononucleated cells[c]		Micronucleated binucleated cells[c]		Survival (%)	Number of cultures analysed	Others[d]	Acc.[e]	Pos.[f]
						Number per 1000 cells	Induction factor	Number per 1000 cells	Induction factor					
		−	24 + 0	Ethanol	0	3.5	1.0	na	na	100	2		Y	POS
					0.05	32.5**	9.3	na	na	107	2			
					0.25	30.5**	8.7	na	na	91	2			
					0.5	40.0**	11.4	na	na	85	2			
					5	33.0**	9.4	na	na	28	2	T		
					MMC 0.1	165.5**	47.3	na	na	50	2			
		−	24 + 24	Ethanol	0	3.0	1.0	na	na	100	2		Y	POS
					0.05	91.0**	30.3	na	na	104	2			
					0.125	91.0**	30.3	na	na	110	2			
					1	64.0**	21.3	na	na	72	2			
					5	75.0**	25.0	na	na	40	2			
					MMC 0.1	198.0**	66.0	na	na	60	2			
		+	3 + 20	Ethanol	0	0.0	0.0	0.0	0.0	100	2		Y	POS
					0.5	0.5	0.5	0.0	0.0	91	2			
					1	1.0	1.0	0.5	0.5	91	2			
					3	2.0	2.0	4.0*	4.0	84	2			
					5	0.5	0.5	4.5**	4.5	90	2			
					7	5.0**	5.0	9.0**	9.0	49	2			
					MMC 0.2	6.0**	6.0	60.0**	60.0	89	2			
		+	24 + 20	Ethanol	0	3.0	1.0	1.5	1.0	100	2		Y	POS
					0.05	5.0	1.7	11.0**	7.3	73	2			
					0.125	1.0	0.3	3.0	2.0	80	2			
					0.25	0.5	0.2	19.5**	13.0	80	2			
					0.5	4.0	1.3	13.5**	9.0	69	2			
					0.75	3.5	1.2	18.5**	12.3	61	2			
					1	0.0	0.0	17.0**	11.3	59	2			
					MMC 0.2	1.5	0.5	106.0**	70.7	73	2			

[a] (+) With cytochalasin B; (−) without cytochalasin B.

[b] MMC: positive control, mitomycin C.

[c] Number of micronucleated cells given for 1000 cells; induction factor: when the number of micronucleated cells was 0 in the control, it was set to 1 to calculate the induction factor for treated cultures (see text for the formula). NS: not statistically higher than controls (Yates Chi-square test); nt: not tested; nd: not determined; na: not applicable; tox: not evaluated due to cytotoxicity. *Statistically higher than controls at $p < 0.05$. **Statistically higher than controls at $p < 0.01$; (–) lower than the control: statistical analysis not done.

[d] p: precipitate; low cells: low number of cells; mmn: multi-micronucleated cells; T: genotoxicity data obtained at survivals clearly below 40% were taken into account for genotoxicity evaluation positive results obtained only at such concentrations were discarded; negative results obtained at such concentrations when lower concentrations gave positive results were not considered; g: no evaluation of survival but as the highest concentration was 5000 μg/ml and as mannitol showed no cytotoxicity, the assay was used for genotoxicity evaluation.

[e] Y: assay accepted; N: assay not accepted (see text).

[f] POS: assay concluded as positive; NEG: assay concluded as negative; EQ: only one positive concentration in the range or no concentration–effect relationship; na: not appropriate.

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