

SFTG international collaborative study on in vitro micronucleus test IV. Using CHL cells

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Abstract

In this report, are presented the results of an international collaborative study on the in vitro micronucleus assay, using CHL cells. Fourteen laboratories participated in this study which was coordinated by an organizing committee supported by the SFTG (the French branch of the European Environmental Mutagen Society). Nine coded substances, having different modes of action and at different levels were assessed in the in vitro micronucleus test, using a common protocol. Mitomycin C was used as a positive control. In order to help to define a standard protocol on CHL cells, short and long treatment periods followed by various recovery times, with or without cytochalasin B, were compared. After an evaluation of the acceptability of the assays, the tested chemicals were classified as negative, positive or equivocal. Mannitol and clofibrate were judged as negative in all treatment schedules. Bleomycin was positive in all the treatment schedules, with an increase in the number of micronucleated cells in both mononucleate and binucleate cells when using cytochalasin B. This was also shown for the aneugens colchicine, diethylstilboestrol and griseofulvin,

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as expected. Urethane was judged as equivocal only after long treatment with cytochalasin B, and negative in all other treatment schedules. In any case, no genotoxic compound would have been missed with schedules including a short and a long treatment time, whether the treatment was followed by a recovery period or not and whether cytochalasin B was used or not. Thus, these results show that CHL cells were suitable for accurately detecting clastogenic and aneugenic compounds of various types in the *in vitro* micronucleus test.

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1. Introduction

The Chinese hamster lung established cell line (CHL cell line) has been used for years in cytogenetic studies in Japan. The CHL cell line was shown to be suitable in regulatory genotoxicity assessment for the detection of clastogenic activity in the *in vitro* chromosome aberration test. The need to detect aneugens led to an evaluation of the *in vitro* micronucleus test, originally in cultured human lymphocytes [1], but then applied to cell lines, including the CHL cell line [2]. Recently, many *in vitro* micronucleus studies using CHL cells were reported [3–7]. An interlaboratory validation study [8], showed a good concordance between the *in vitro* chromosome aberration test and the *in vitro* micronucleus test using CHL cells. Moreover, all the aneugens tested in this study, polyploidy inducers in the *in vitro* chromosome aberration test, were also detected by the *in vitro* micronucleus test in CHL cells. The SFTG collaborative study was set up to evaluate the performances of the *in vitro* micronucleus test using different cell systems, including the CHL cell line, with a common protocol. A large number of substances were previously tested in CHL cells for induction of chromosome aberrations [9] or for induction of micronuclei [8]. Most of these compounds were included in the present study. Regarding the compounds selected as non-genotoxic chemicals, mannitol was previously found negative and clofibrate was reported as positive but marginal in the *in vitro* chromosome aberration test on CHL cells [9]. In the same study, cytosine arabinoside and mitomycin C, which were chosen as clastogens, showed positive results and urethane, chosen as an *in vivo* specific genotoxic compound, revealed clastogenicity only at the extremely high concentrations (>10 mM) [9]. Bleomycin and 5-fluorouracil were known to induce chromosome aberrations in CHL cells [9]. Colchicine [8], diethylstilboestrol [10] and griseofulvin [11], known as aneugens, induced polyploidy but no structural aberrations. The present study, set up to evaluate the performance of the *in vitro* micronucleus test included these chemicals and was conducted with a common protocol and com-

mon treatment schedules to help to define a standard protocol.

2. Materials and methods

The general conditions of the study are described in a previous paper in this issue [12].

2.1. Cells

CHL cells were chosen for their ability to grow actively and for their chromosome stability. CHL/IU cells (JCRB0030, Lot 070197) were obtained fresh from Health Science Research Resources Bank (Osaka, Japan) for the study and distributed to all the participants.

2.2. Culture media

MEM (GIBCO, 61100-061) was supplemented with 50 IU/ml penicillin–50 µg/ml streptomycin (GIBCO, 15140-122), and 10% (v/v) heat-inactivated calf serum (Moregate Lot 596072 and Cansera International Inc. Lot 28110754).

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 for details). They were: mannitol (CAS No. 69-65-8), clofibrate (CAS No. 637-07-0), bleomycin (CAS No. 9041-93-4), 5-fluorouracil (CAS No. 51-21-8), cytosine arabinoside (CAS No. 147-94-4), urethane (CAS No. 51-79-6), colchicine (CAS No. 64-86-8), diethylstilboestrol (CAS No. 56-53-1) and griseofulvin (CAS No. 126-07-8). Mitomycin C (CAS No. 50-07-7) was used as the positive control. Cytochalasin B was purchased from Sigma Chemicals.

2.4. Culture conditions

Cells were incubated at 37 °C with 5% CO₂.

For the genotoxicity evaluation, cultures were performed in duplicate in at least one assay.

The day before treatment, cells were seeded at 4 × 10⁵ cells/plate (3-h treatments with cytochalasin B), 2 × 10⁵ cells/plate (24-h treatments with cytochalasin B) or 1 × 10⁵ cells/plate

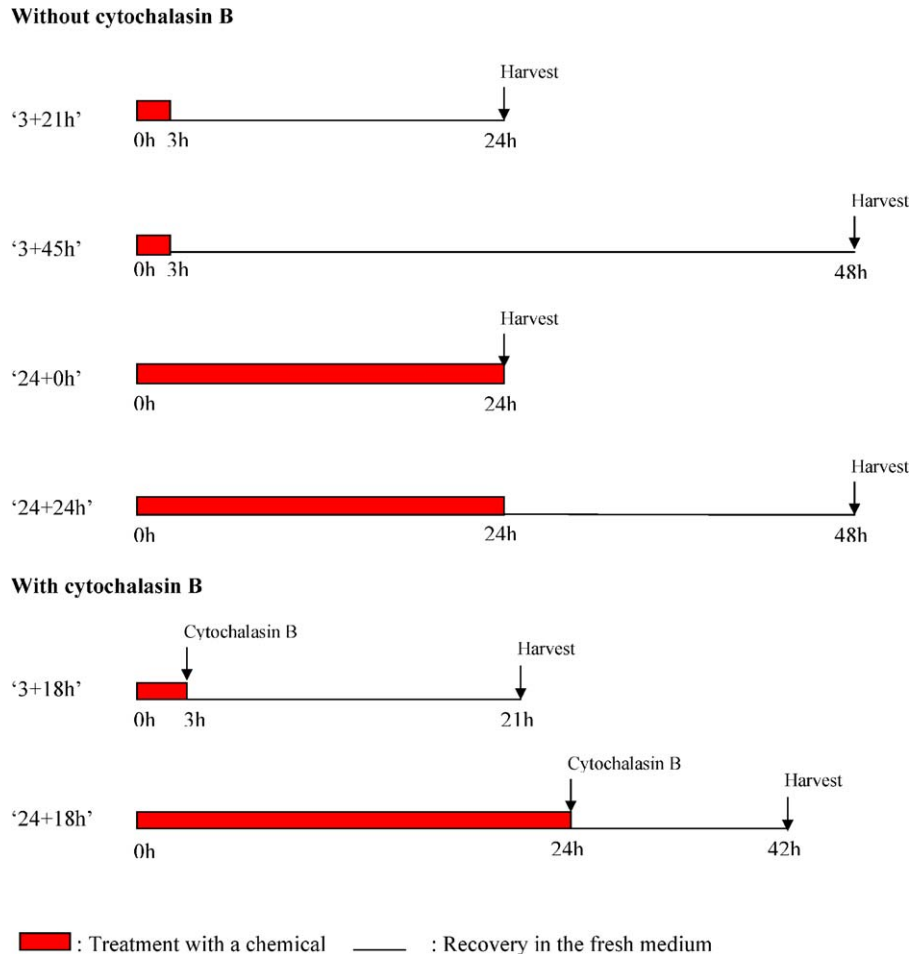


Fig. 1. Treatment and recovery schedules.

(treatments without cytochalasin B) in 6-cm plates containing 5 ml of medium. In these conditions, cells were actively growing during the treatment.

2.5. Treatment and recovery times

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

When cytochalasin B was not used, cells were treated with the compound for 3 h and harvested after a 21-h or 45-h recovery ('3 + 21 h' schedule or '3 + 45 h' schedule), or treated for 24-h and harvested immediately or after a 24-h recovery ('24 + 0 h' schedule or '24 + 24 h' schedule).

When using cytochalasin B, after the treatment period (3 h or 24 h) (schedule), cells were incubated for 18 h with cytochalasin B (3 $\mu\text{g}/\text{ml}$). These schedules were therefore designated '3 + 18 h' or '24 + 18 h' schedule. The treatment period with cytochalasin B was determined in a preliminary study in order to select the most appropriate treatment time to obtain an optimal frequency of binucleate cells.

At the end of the treatment period, cells were rinsed with buffer and cultured in fresh medium for the recovery period.

Mitomycin C was used as a positive control at 0.1 $\mu\text{g}/\text{ml}$ for all the 3-h treatments and at 0.05 $\mu\text{g}/\text{ml}$ for all the 24-h treatments. The concentrations were determined on the basis of a preliminary study performed in all the participating laboratories (see the main publication in this issue [12]).

2.6. Cell sampling and staining

Cells were generally collected by trypsinization, exposed to 75 mM KCl to induce a hypotonic shock, and then fixed with methanol:acetic acid (3:1). The fixed cell suspension was dropped onto the slides and the slides were air-dried. The cells were stained by being mounted in 40 $\mu\text{g}/\text{ml}$ acridine orange in phosphate buffered saline and immediately observed using a fluorescence microscope.

2.7. Report and evaluation of results

The conditions for cytotoxicity and genotoxicity assessment are described in the general publication in this issue [12]. Table 1 summarizes all the assays which were retained for genotoxicity evaluation. The range of concentrations tested is

Table 1
Summary data with CHL 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) ^c		Maximal fold increase of the range ^e (MFI)	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	313–5000	–5000	≥100				–	Negative			
						625–5000	–5000	≥100								
		3+45	–	1	1	313–5000	–5000	91		+2500	≥100	2.8	±	Negative		
									625–5000	–5000	93					
		24+0	–	1	1	313–5000	–5000	93					–	Negative		
									625–5000	–5000	≥100					
		24+24	–	1	1	313–5000	–5000	≥100					–	Negative		
									625–5000	–5000	≥100					
		3+18	+	1	1	625–5000	–5000	≥100					–	Negative		
									313–5000	–5000	83					
		24+18	+	1	1	625–5000	–5000	78		+1250 b ^j	≥100 b	1.8 b	±	Negative		
									313–5000	–5000	83	+625 b ^j	96 b		1.8 b	
Clofibrate	637-07-0	3+21	–	1	1	15.6–125	–125	25				–	Negative			
						15.6–125	–125	31								
						5–70	–70	48								
		3+45	–	1	1	15.6–125	–125	21					–	Negative		
									15.6–125	–125	21	+62.5 ^k	98		2.1	±
									5–70	–70	36					
		24+0	–	1	1	15.6–125	–125	16					–	Negative		
									15.6–125	–125	20					
									25–75	–75	11					
		24+24	–	1	1	15.6–125	–125	12					–	Negative		
									15.6–125	–125	12					
									5–70	–70	36					
		3+18	+	1	1	31.3–250	–250	43					–	Negative		
									31.3–250	–250	43					
									25–125	–125	27					
		24+18	+	1	1	15.6–125	–125	22					–	Negative		
									15.6–125	–125	22					
									25–100	–100	46					
Bleomycin	9041-93-4	3+21	–	1	2	1–8			+1	75	23.6	+	Positive			
						2–500			+2	99	27.5	+				
						12.8–200			+12.8	≥100	35.7	+				
		3+45	–	1	2	0.25–2	–	21.5			+0.25	≥100	21.5	+	Positive	
									2–500			+2	84	18.3		+
									12.8–200			+12.8	95	11.4		+
		24+0	–	1	2	0.5–4	–	15.7			+0.5	85	15.7	+	Positive	
									0.81–200			+0.81	95	22.6		+
									2–32			+2	96	39.4		+
		24+24	–	1	2	0.5–4	–	50.5			+0.5	83	50.5	+	Positive	
									0.81–200			+0.81	94	25.2		+
									2–32			+2	77	27.1		+

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 (MFI)	Conclusion for the assay ^h	Judgement for the treatment and recovery schedule ⁱ	
							Result and concentration	Survival % ^d	Result and concentration	Survival % ^f				
5-Fluorouracil	51-21-8	3+18	+	1	1	2–32			+2 m, b	83 m, b	26.3 m, 73.2 b	+	Positive	
						1–8			+1 m, b	98 m, b	36.2 m, 91.4 b	+		
						2–500			+5.12 m, +2 b	94 m, 96 b	4.2 m, 14.4 b	+		
		24+18	+	2	2	32–500			+32 m,b	≥100 m,b	3.6 m, 19.1 b	+		
						0.125–1			+0.125 m,b	76 m,b	78.9 m, 80.4 b	+		
						2–200			+2 m,b	98 m,b	20.7 m, 26.8 b	+		
	51-21-8	3+21	–	1	1	5.12–80			+5.12 m,b	94 m,b	25.5 m, 25.1 b	+		
						150–5000			+500	62	11.1	+		
						150–5000			+500	76	7.8	+		
						125–1000			+500	56	4.4	+		
						125–1000			+125	74	9.5	+		
						3–100			+30	51	2.7	±		
51-21-8	3+45	–	1	1	3–100	–100	44					–	Equivocal	
					3–100									
					1.25–10			+2.5	66	3.6	+			
					1.25–10			+10	43	2.0	+			
					150–5000			+500	63	6.2	+			
					150–5000			+150	72	23.6	+			
51-21-8	24+0	–	1	1	1–30			+10	40	2.5	+	Equivocal		
					0.25–2	–2	43							
					0.25–2			+1	43	2.2	+			
					12.5–100	–100	44							
					625–5000			+1250 m, 625 b	83 m, 88 b	2.9 m, 13.1 b	+			
					625–5000			+625 b ^j	52 b	11.2 b	+			
51-21-8	24+18	+	1	2	25–75			+25 m,b	87 m,b	3.1 m, 12.5 b	+	Positive		
					10–80			+10 b ^j	59 b	16.6 b	+			
					0.0625–1			+0.0625	74	5.3	+			
					0.0625–1			+0.0625	73	12.9	+			
					0.0625–4			+0.0625	65	5.6	+			
					0.0125–0.2			+0.05	90	2.9	+			
51-21-8	3+45	–	1	1	0.0625–1			+0.0625	75	11.8	+	Positive		
					0.0625–1			+0.125	77	22.4	+			
					0.0625–4			+0.0625	62	3.4	+			
					0.0125–0.2			+0.2 ^k	86	1.8	±			
					0.0078–0.125			+0.0156	55	7.8	+			
					0.00625–0.1			+0.00625	73	18.6	+			
51-21-8	24+0	–	1	2	0.00025–0.004			+0.002	73	2.2	+	Positive		
					0.0078–0.125			+0.0156	76	16.7	+			
					0.00625–0.1			+0.00625	87	11.3	+			
					0.00025–0.004			+0.004	74	1.9	±			
					0.0625–1			+0.5 b ^j	62 b	3.4 b	+			
					0.0625–1			+0.0625 b ^j	99 b	4.4 b	+			
51-21-8	24+24	–	1	1	0.0125–0.2			+0.1 b ^j	98 b	1.8 b	+	Positive		
					0.0125–0.2			+0.1 b ^j	98 b	1.8 b	+			
					0.0078–0.125			+0.0078 b ^j	≥100 b	17.4 b	+			
					0.00625–1.6			+0.00625 b ^j	≥100 b	41.3 b	+			
					0.00025–0.004			+0.004 b ^j	≥100 b	1.4 b	±			
					0.00025–0.004			+0.004 b ^j	≥100 b	1.4 b	±			
Cytosine arabinoside	147-94-4	3+21	–	1	1	0.0625–1			+0.0625	74	5.3	+	Positive	
						0.0625–1			+0.0625	73	12.9	+		
						0.0625–4			+0.0625	65	5.6	+		
		3+45	–	2	2	0.0125–0.2			+0.05	90	2.9	+		
						0.0625–1			+0.0625	75	11.8	+		
						0.0625–1			+0.125	77	22.4	+		
	147-94-4	24+0	–	1	1	0.0625–4			+0.0625	62	3.4	+		
						0.0125–0.2			+0.2 ^k	86	1.8	±		
						0.0078–0.125			+0.0156	55	7.8	+		
						0.00625–0.1			+0.00625	73	18.6	+		
						0.00025–0.004			+0.002	73	2.2	+		
						0.0078–0.125			+0.0156	76	16.7	+		
147-94-4	24+24	–	1	2	0.00625–0.1			+0.00625	87	11.3	+	Positive		
					0.00625–0.1			+0.00625	87	11.3	+			
					0.00025–0.004			+0.004	74	1.9	±			
					0.0625–1			+0.5 b ^j	62 b	3.4 b	+			
					0.0625–1			+0.0625 b ^j	99 b	4.4 b	+			
					0.0125–0.2			+0.1 b ^j	98 b	1.8 b	+			
147-94-4	3+18	+	1	1	0.0078–0.125			+0.0078 b ^j	≥100 b	17.4 b	+	Positive		
					0.00625–1.6			+0.00625 b ^j	≥100 b	41.3 b	+			
					0.00025–0.004			+0.004 b ^j	≥100 b	1.4 b	±			
					0.00025–0.004			+0.004 b ^j	≥100 b	1.4 b	±			
					0.00025–0.004			+0.004 b ^j	≥100 b	1.4 b	±			
					0.00025–0.004			+0.004 b ^j	≥100 b	1.4 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 (MFI)	Conclusion for the assay ^h	Judgement for the treatment and recovery schedule ⁱ		
							Result and concentration	Survival % ^d	Result and concentration	Survival % ^f					
Diethylstilboestrol	56-53-1	3 + 21	–	1	2	10–40	–20	45	+40	51	8.8	±	Equivocal		
				2	1	2.5–20						–			
		24 + 0	–	1	1	3.125–25			+6.25	61	11.3	+	Positive		
				1	2	3.125–25			+6.25	85	32.8	+			
				2	1	2.5–10			+2.5 ^k	81	39.1	+			
				2	2	1.25–10			+2.5 ^k	≥100	7.3	+			
		24 + 24	–	1	1	3.125–25			+6.25	40	3.0	+	Positive		
				1	2	3.125–25			+6.25	46	2.9	+			
		3 + 18	+	2	2	2.5–30			+30 b ^j	81 b	1.7 b	±	Equivocal		
		24 + 18	+	1	1	1	1	3.125–25			+6.25 m, b	≥100 m, b	89.3 m, 20.8 b	+	Positive
1	2					10–20			+10 m, b	81 m, b	31.6 m, 8.3 b	+			
2	1					2.5–10			+5 m, b	71 m, b	18.2 m, 7.7 b	+			
2	2					10–30			+10 m, b	72 m, b	25.8 m, 9.7 b	+			
Griseofulvin	126-07-8	3 + 21	–	1	1	1.4–1000 p			+111 ^k	≥100	18.7	+	Positive		
				1	2	25–400 p			+200 ^k	78	27.5	+			
		3 + 45	–	1	1	1	1	1.4–1000 p			+111 ^k	≥100	23.8	+	Positive
						1	2	25–400 p			+100 ^k	55	3.0	+	
		24 + 0	–	1	1	1	1	1.4–1000 p			+12.3 ^k	64	35.4	+	Positive
						1	2	3.1–50			+6.3 ^k	82	34.8	+	
		24 + 24	–	1	1	1	1	1.4–1000 p			+12.3 ^k	64	18.6	+	Positive
						1	2	25–400 p			+25 ^k	54	13.9	+	
		3 + 18	+	1	2	25–400 p			+25 m, +200 b	≥100 m, 77 b	2.8 m, 4.8 b	+	Positive		
		24 + 18	+	1	1	1	1	1.4–1000 p			+4.1 b ^j	≥100 m, b	12.3 m, b	+	Positive
1	2					25–400 p			+25 m, b	≥100 m, b	59.1 m, 28.2 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 binucleate 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 at least one concentration; m: increase in the number of micronucleated mononucleate cells; b: increase in the number of micronucleated binucleate cells.

^f Relative survival against the solvent control seen at the lowest positive concentration; m: in mononucleate cells, b: in binucleate 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 mononucleate cells, b: in binucleate 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 binucleate cells were evaluated.

^k Increase in the number of multinucleate cells.

reported for each assay with the highest negative concentration or the lowest positive concentration with the corresponding survival percentage and maximal fold increase (MFI), where applicable. The survival percentage indicates the relative survival versus the solvent control and was calculated on the basis of the cell counts for the assays without cytochalasin B or on the basis of the percentage of multinucleate cells for the assays with cytochalasin B. The evaluation of micronuclei induction was based on mononucleate cells when cytochalasin B was not used and on binucleate cells when cytochalasin B was used. In the assays with cytochalasin B, mononucleate cells were also considered. The fold increase was calculated by dividing the number of micronucleated cells in the treated cultures by that in the solvent cultures. The conclusion for each assay and the judgement for the compound in each treatment schedule are given in Table 1. The corresponding individual data are reported in Appendix A. The criteria for the acceptability of the assays and for the interpretation of results are described in the general publication in this issue [12].

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 only some of these criteria were achieved the test compound response was classified as equivocal, and if none of the criteria were achieved the test compound response was classified as negative.

3. Results

3.1. Mannitol

No cytotoxicity was observed whatever the concentration and the treatment schedule up to 5000 $\mu\text{g}/\text{ml}$. No statistically significant increases in the number of micronucleated cells were seen, except in three sporadic instance where a slightly significant ($p < 0.05$) increase appeared. This marginal increase was not concentration-dependent, not reproduced in the other assay and MFI did not exceed 2.8. Thus, these marginal increases in the number of micronucleated cells were concluded irrelevant. Therefore, mannitol was judged to be negative in all the treatment schedules.

3.2. Clofibrate

Clofibrate steeply reduced the percentage of survival at concentrations of more than approximately 70 $\mu\text{g}/\text{ml}$, regardless of treatment schedule or presence of cytochalasin B. Statistically significant increases in the number of micronucleated cells were sporadically found, although none exceeded a MFI of 2.1. Additionally, these responses were not concentration-related and not

reproducible between assays with the same schedule. Therefore, they were considered biologically irrelevant. Consequently, clofibrate was judged to be negative in all the treatment schedules.

3.3. Bleomycin

Bleomycin showed clear positive responses with reproducible and concentration-dependent increases in the number of micronucleated cells and with high MFIs. Bleomycin showed low or no cytotoxicity at the lowest positive concentrations and the positive responses covered a wide range of concentrations. When cytochalasin B was used, it was remarkable that micronuclei were seen not only in binucleate cells, but also in mononucleate cells, although corresponding incidences were lower. The incidence of micronucleated cells was generally higher in binucleate cells.

3.4. 5-Fluorouracil

In the absence of cytochalasin B, 5-fluorouracil showed clear positive responses in both '3+21' and '24+0' treatment schedules. However, in the treatment times followed by long recovery periods without cytochalasin B ('3+45' and '24+24'), contradictory results were found between assays at the same levels of concentration. Since the MFIs were low, we classified the responses as equivocal in these treatment schedules. For the same treatment times without cytochalasin B, the cytotoxicity in the long recovery time was more pronounced than that the one in the short recovery time. This reflected the difficulty of cells to recover after the division-block induced by 5-fluorouracil during the recovery periods. This resulted in lower analysable concentrations in all the schedules that included a long recovery period, in the presence of cytochalasin B. However, 5-fluorouracil was clearly positive when a short or no recovery time was applied. Clear positive responses were also found in binucleate cells in both short and long treatment schedules and the lowest positive concentration was much lower in the long treatment schedule when cytochalasin B was used. Finally, 5-fluorouracil was considered positive in the study.

3.5. Cytosine arabinoside

Cytosine arabinoside showed a clear positive response with reproducible and concentration-dependent increases in the number of micronucleated cells in all treatment schedules with and without cytochalasin B, even at low or no cytotoxicity. Cytosine arabinoside

showed relatively strong cytotoxicity compared to the other compounds tested and the cytotoxicity was related to the treatment times, not to the recovery times. Cytosine arabinoside was judged positive.

3.6. Urethane

Urethane was classified as negative in all treatment schedules without cytochalasin B up to 5000 µg/ml at which urethane produced low or no cytotoxicity. Only sporadic increases in the number of micronucleated cells were found, but were not reproducible or concentration-dependent and they were considered to be biologically irrelevant. When cytochalasin B was used, low MFI (i.e. 2.9), but reproducible increases in the number of micronucleated binucleate cells were observed at the highest concentrations in long treatment in three out of four tests, leading us to classify urethane as equivocal.

3.7. Colchicine

Colchicine induced concentration-dependent increases in the number of micronucleated cells in all the treatment schedules with and without cytochalasin B. With cytochalasin B, micronucleated binucleate cells were induced with high MFIs. The MFI of micronucleated mononucleate cells was similar to or higher than that of the micronucleated binucleate cells. This high induction of micronucleated mononucleate cells is in accordance with previous data [13]. The lowest positive concentrations were lower in the long treatment schedules and in the '3 + 45' schedule, probably related to an accumulation of cytokinesis blocked cells or abnormal divisions in these conditions. In the absence of cytochalasin B, the number of multinucleate cells was increased. Colchicine was judged positive.

3.8. Diethylstilboestrol

In the 3-h treatment schedules with and without cytochalasin B, low increases in micronucleated cells were observed but were neither reproducible nor concentration-dependent. These results led us to classify diethylstilboestrol as equivocal. However, a number of assays were rejected because suitable cytotoxicity was not achieved. It seemed that cytotoxicity occurred steeply at concentrations close to those inducing genotoxic effects, precluding obtaining meaningful results. On the contrary, the 24-h treatments, performed at lower concentrations with and without cytochalasin B, gave rise to clear and concentration-dependent results, the

accumulation of proliferation-blocked cells over a long treatment time resulting, in this case, in delayed cytotoxicity. With cytochalasin B, MFIs in 24 h treatment cultures were higher in mononucleate cells than in binucleate cells. Without cytochalasin B, multinucleate cells were found after the 24-h treatment. Diethylstilboestrol was concluded positive after long treatment both with and without cytochalasin B, irrespective of the recovery period.

3.9. Griseofulvin

Griseofulvin induced unambiguously concentration-related increases in the number of micronucleated cells in all treatment schedules. These were seen in both mononucleate and binucleate cells when cytochalasin B was used. Multinucleate cells were observed without cytochalasin B. Griseofulvin was judged positive.

4. Discussion

Expected results were obtained with all compounds tested in this in vitro micronucleus assay using CHL cells.

Mannitol and clofibrate were judged as negative in all treatment schedules. Bleomycin was positive in all the treatment schedules and the number of micronucleated cells increased in both mononucleate and binucleate cells when using cytochalasin B. The increase in the number of micronucleated mononucleate cells, previously reported for aneugens, was not expected with clastogens [13]. Since bleomycin is a clastogen and able to induce DNA damage at every stage of the cell cycle, these micronucleated mononucleate cells may represent the cells which had completed cell division before the exposure to cytochalasin B. The difference in the level of micronucleus induction between mononucleate and binucleate cells seen only after the 3-h treatment with cytochalasin B is consistent with this hypothesis. However this explanation alone may not account for all the micronucleated cells, as their numbers were high in every case including when mononucleate cells were analysed in the assays with cytochalasin B. More investigations, i.e. the development of a FISH technique [14] on CHL cells, would be necessary to determine if some of these micronucleated mononucleate cells escaped from cytokinesis inhibition or passed mitosis without chromosome segregation. Base analogs, 5-fluorouracil and cytosine arabinoside, were also unambiguously found positive in the in vitro micronucleus test using CHL cells. However, 5-fluorouracil was difficult to detect when treatments were followed

by a long recovery period without cytochalasin B, probably because damaged cells were lost during the long recovery time due to cytotoxicity. In this case, the sensitivity of the test was not improved by a long recovery period after treatment. Urethane was judged as equivocal only after the long treatment with cytochalasin B, and negative in all other treatment schedules. This confirms previous published results reporting difficulties to detect this compound in vitro without adequate specific metabolic activation [15]. Moreover, a previous validation study using the same CHL cell line reported urethane as negative even in the presence of the usual Aroclor-induced rat liver S9 [8]. Our results are considered to be consistent with those published data on urethane [15,8]. The aneugens, colchicine, diethylstilboestrol and griseofulvin, were characterized by induction of micronuclei in both mononucleate and binucleate cells, and by the presence of multinucleate cells. However, with diethylstilboestrol in the short treatments, cytotoxicity interfered with genotoxicity so that genotoxicity was difficult to demonstrate. In this case, the long treatment at lower concentrations allowed micronucleated cells to recover from cytotoxicity. This was in accordance with previous validation studies on CHL cells where colchicine and diethylstilboestrol were also detected after a 24-h treatment [3,8]. The results of

this study were concordant with already available data obtained from the in vitro micronucleus test or from the in vitro chromosome aberration test [3,8–10]. These results with diethylstilboestrol suggest that the long treatment might be more efficient to detect aneugens.

In conclusion, the results of the present study showed that CHL cells were suitable for detecting accurately genotoxic compounds of various types in the in vitro micronucleus test. A long treatment was needed for aneugens, as shown here with diethylstilboestrol. For the other compounds, all treatment-recovery schedules were suitable to detect the genotoxic effects. No differences were seen in the sensitivity or accuracy of the responses whether cytochalasin B was used or not. In any case, no genotoxic compound would have been missed with schedules including a short and a long treatment time, whether the treatment was followed by a recovery period or not and whether cytochalasin B was used or not.

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Appendix A

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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	9.5	1.0	na	na	100	2		Y	NEG	
					313	10.5	1.1	na	na	129	2				
					625	6.5	0.7	na	na	142	2				
					1250	6.0	0.6	na	na	158	2				
					2500	8.0	0.8	na	na	161	2				
					5000	7.0	0.7	na	na	120	2				
			MMC 0.1	58.0**	6.1	na	na	113	2						
			–	3 + 45	Medium	0	4.0	1.0	na	na	100	2		Y	EQ
						313	6.5	1.6	na	na	107	2			
						625	5.0	1.3	na	na	98	2			
						1250	6.5	1.6	na	na	97	2			
						2500	11.0*	2.8	na	na	107	2			
		5000				6.5	1.6	na	na	94	2				
		MMC 0.1	64.0**	16.0	na	na	102	2							
		–	24 + 0	Medium	0	10.5	1.0	na	na	100	2		Y	NEG	
					313	9.0	0.9	na	na	108	2				
					625	12.0	1.1	na	na	105	2				
					1250	10.0	1.0	na	na	102	2				
					2500	11.0	1.0	na	na	91	2				
					5000	8.0	0.8	na	na	93	2				
		MMC 0.05	111.5**	10.6	na	na	82	2							
		–	24 + 24	Medium	0	11.5	1.0	na	na	100	2		Y	NEG	
					313	9.0	0.8	na	na	125	2				
					625	7.0	0.6	na	na	116	2				
					1250	6.5	0.6	na	na	135	2				
					2500	7.0	0.6	na	na	120	2				
					5000	16.5	1.4	na	na	115	2				
		MMC 0.05	306.5**	26.7	na	na	74	2							
+	3 + 18	Medium	0	3.0	1.0	15.5	1.0	100	2		Y	NEG			
			625	nt	nt	15.5	1.0	101	2						
			1250	nt	nt	16.0	1.0	100	2						
			2500	nt	nt	13.0	0.8	99	2						
			5000	5.0	1.7	17.0	1.1	100	2						
			MMC 0.1	8.0	2.7	75.5**	4.9	97	2						
+	24 + 18	Medium	0	4.5	1.0	11.0	1.0	100	2		Y	NEG			
			625	nt	nt	4.5	0.4	103	2						
			1250	nt	nt	8.5	0.8	103	2						
			2500	nt	nt	6.5	0.6	99	2						
			5000	6.0	1.3	6.5	0.6	78	2						
			MMC 0.05	132.5**	29.4	281.5**	25.6	77	2						
Lab 1	Assay 2	–	3 + 21	Medium	0	9.5	1.0	na	na	100	2		Y	NEG	
					625	10.5	1.1	na	na	112	2				
					1250	11.0	1.2	na	na	118	2				
					2500	9.5	1.0	na	na	95	2				
					5000	4.0	0.4	na	na	109	1				
					MMC 0.1	56.0**	5.9	na	na	108	2				

		–	3+45	Medium	0	8.5	1.0	na	na	100	2		Y	NEG
					625	12.5	1.5	na	na	105	2			
					1250	8.5	1.0	na	na	103	2			
					2500	11.5	1.4	na	na	113	2			
					5000	9.5	1.1	na	na	91	2			
					MMC 0.1	60.5**	7.1	na	na	88	2			
		–	24+0	Medium	0	16.0	1.0	na	na	100	2		Y	NEG
					625	13.0	0.8	na	na	132	2			
					1250	9.5	0.6	na	na	94	2			
					2500	10.0	0.6	na	na	151	2			
					5000	13.0	0.8	na	na	127	2			
					MMC 0.05	143.5**	9.0	na	na	134	2			
		–	24+24	Medium	0	9.5	1.0	na	na	100	2		Y	NEG
					625	9.5	1.0	na	na	91	2			
					1250	11.0	1.2	na	na	103	2			
					2500	10.0	1.1	na	na	89	2			
					5000	14.0	1.5	na	na	83	2			
					MMC 0.05	276.5**	29.1	na	na	65	2			
		+	3+18	Medium	0	nt	nt	10.5	1.0	100	2		Y	EQ
					313	nt	nt	7.5	0.7	98	2			
					625	nt	nt	14.0	1.3	99	2			
					1250	nt	nt	18.5*	1.8	100	2			
					2500	nt	nt	16.5	1.6	100	2			
					5000	nt	nt	12.0	1.1	98	2			
					MMC 0.1	nt	nt	89.0**	8.5	97	2			
		+	24+18	Medium	0	nt	nt	12.0	1.0	100	2		Y	EQ
					313	nt	nt	12.5	1.0	99	2			
					625	nt	nt	22.0*	1.8	96	2			
					1250	nt	nt	12.5	1.0	98	2			
					2500	nt	nt	19.0	1.6	94	2			
					5000	nt	nt	13.0	1.1	91	2			
					MMC 0.05	nt	nt	353.0**	29.4	98	2			
Clofibrate														
Lab 1	Assay 1	–	3+21	DMSO	0	21.5	1.0	na	na	100	2		Y	NEG
					15.6	28.5	1.3	na	na	69	2			
					31.3	17.0	0.8	na	na	76	2			
					62.5	15.0	0.7	na	na	66	2			
					125	16.5	0.8	na	na	25	2	T		
					MMC 0.1	110.5**	5.1	na	na	57	2			
		–	3+45	DMSO	0	20.0	1.0	na	na	100	2		Y	NEG
					15.6	28.5	1.4	na	na	82	2			
					31.3	25.0	1.3	na	na	90	2			
					62.5	21.0	1.1	na	na	105	2			
					125	16.5	0.8	na	na	21	2	T		
					MMC 0.1	68.5**	3.4	na	na	94	2			
		–	24+0	DMSO	0	11.0	1.0	na	na	100	2		Y	NEG
					15.6	4.0	0.4	na	na	91	2			
					31.3	6.0	0.5	na	na	99	2			
					62.5	7.0	0.6	na	na	86	2			
					125	6.5	0.6	na	na	16	2	T		

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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							
Lab 1	Assay 2	–	24 + 24	DMSO	MMC 0.05	147.0**	13.4	na	na	82	2					
					0	28.0	1.0	na	na	100	2		Y	NEG		
					15.6	31.5	1.1	na	na	92	2					
					31.3	23.5	0.8	na	na	101	2					
					62.5	28.5	1.0	na	na	86	2					
					125	10.5	0.4	na	na	12	2	T				
					MMC 0.05	406.0**	14.5	na	na	86	2					
					0	24.0	1.0	11.5	1.0	100	2		Y	NEG		
					31.3	22.0	0.9	9.5	0.8	99	2					
					62.5	21.5	0.9	8.5	0.7	98	2					
					125	7.5	0.3	4.5	0.4	58	2					
					250	4.0	0.2	12.2	1.1	43	2					
					MMC 0.1	27.0	1.1	99.0**	8.6	90	2					
					0	10.0	1.0	13.0	1.0	100	2		Y	NEG		
					15.6	13.0	1.3	11.5	0.9	100	2					
		31.3	9.5	1.0	17.5	1.3	102	2								
		62.5	7.0	0.7	14.0	1.1	96	2								
		125	10.0	1.0	19.8	1.5	22	2	T							
		MMC 0.05	252.5**	25.3	465.5**	35.8	103	2								
		Lab 1	Assay 2	–	3 + 21	DMSO	0	11.5	1.0	na	na	100	2		Y	NEG
							15.6	9.5	0.8	na	na	92	2			
							31.3	18.0	1.6	na	na	104	2			
							62.5	9.5	0.8	na	na	103	2			
							125	12.0	1.0	na	na	31	2	T		
							MMC 0.1	92.5**	8.0	na	na	88	2			
							0	12.5	1.0	na	na	100	2		Y	EQ
							15.6	19.0	1.5	na	na	94	2			
							31.3	12.0	1.0	na	na	97	2			
							62.5	26.0**	2.1	na	na	98	2			
							125	22.0*	1.8	na	na	8	2	T		
							MMC 0.1	57.0**	4.6	na	na	115	2			
							0	24.0	1.0	na	na	100	2		Y	NEG
							15.6	33.0	1.4	na	na	91	2			
							31.3	31.5	1.3	na	na	94	2			
				62.5	34.0	1.4	na	na	89	2						
				125	15.5	0.6	na	na	20	2	T					
				MMC 0.05	174.5**	7.3	na	na	83	2						
				0	13.5	1.0	na	na	100	2		Y	EQ			
				15.6	18.0	1.3	na	na	91	2						
				31.3	15.0	1.1	na	na	94	2						
				62.5	28.0**	2.1	na	na	88	2						
				125	13.5	1.0	na	na	10	2	T					
				MMC 0.05	349.0**	25.9	na	na	87	2						
				0	25.0	1.0	14.0	1.0	100	2		Y	EQ			
				31.3	32.0	1.3	11.0	0.8	99	2						
				62.5	37.5*	1.5	7.5	0.5	100	2						
125	2.5			0.1	4.5	0.3	37	2	T							
MMC 0.1	30.5			1.2	64.0**	4.6	97	2								

Lab 2	Assay 1	+	24 + 18	DMSO	0	14.0	1.0	13.0	1.0	100	2	T	Y	NEG
					15.6	13.0	0.9	11.0	0.8	98	2			
					31.3	11.5	0.8	12.0	0.9	102	2			
					62.5	14.5	1.0	12.0	0.9	97	2			
					125	13.3	1.0	16.1	1.2	10	2			
					MMC 0.05	247.0**	17.6	372.5**	28.7	101	2			
	Assay 2	-	3 + 21	DMSO	0	10.0	1.0	na	na	100	1	T	N	na
					25	14.0	1.4	na	na	76	1			
					50	14.0	1.4	na	na	53	1			
					75	23.0*	2.3	na	na	10	1			
					MMC 0.1	94.0**	9.4	na	na	69	1			
					0	8.0	1.0	na	na	100	1			
		-	3 + 45	DMSO	25	9.0	1.1	na	na	110	1	T	N	na
					50	12.0	1.5	na	na	76	1			
					75	17.0	2.1	na	na	8	1			
100					40.0**	5.0	na	na	1	1				
MMC 0.1					78.0**	9.8	na	na	87	1				
0					9.0	1.0	na	na	100	1				
-	24 + 0	DMSO	25	5.0	0.6	na	na	78	1	T	Y	NEG		
			50	12.0	1.3	na	na	81	1					
			75	11.0	1.2	na	na	11	1					
			MMC 0.05	160.0**	17.8	na	na	80	1					
			0	8.0	1.0	na	na	100	1					
			25	9.0	1.1	na	na	75	1					
-	24 + 24	DMSO	50	16.0	2.0	na	na	55	1	T	N	na		
			75	21.0*	2.6	na	na	11	1					
			MMC 0.05	362.0**	45.3	na	na	54	1					
			0	11.0	1.0	20.0	1.0	100	1					
			25	18.0	1.6	22.0	1.1	99	1					
			50	19.0	1.7	18.0	0.9	102	1					
+	3 + 18	DMSO	75	10.0	0.9	22.0	1.1	106	1	T	Y	NEG		
			100	6.0	0.5	19.0	1.0	63	1					
			125	6.0	0.5	11.0	0.6	27	1					
			MMC 0.1	18.0	1.6	113.0**	5.7	86	1					
			0	5.0	1.0	14.0	1.0	100	1					
			25	9.0	1.8	19.0	1.4	95	1					
+	24 + 18	DMSO	50	8.0	1.6	14.0	1.0	103	1	T	Y	NEG		
			75	10.0	2.0	19.0	1.4	101	1					
			100	10.0	2.0	21.0	1.5	46	1					
			MMC 0.05	165.0**	33.0	471.0**	33.6	114	1					
			0	7.5	1.0	na	na	100	2					
			5	9.0	1.2	na	na	74	2					
-	3 + 21	DMSO	10	12.0	1.6	na	na	91	2	T	Y	NEG		
			30	7.5	1.0	na	na	85	2					
			50	11.0	1.5	na	na	65	2					
			70	10.5	1.4	na	na	48	2					
			MMC 0.1	93.5**	12.5	na	na	64	2					
			0	7.5	1.0	na	na	100	2					

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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+45	DMSO	0	11.5	1.0	na	na	100	2		N	na
					5	11.5	1.0	na	na	96	2			
					10	8.0	0.7	na	na	111	2			
					30	11.5	1.0	na	na	88	2			
					50	7.0	0.6	na	na	82	2			
					70	8.5	0.7	na	na	55	2			
					MMC 0.1	88.5**	7.7	na	na	82	2			
		–	24+0	DMSO	0	11.0	1.0	na	na	100	2		Y	NEG
					5	12.0	1.1	na	na	80	2			
					10	9.0	0.8	na	na	94	2			
					30	7.0	0.6	na	na	67	2			
					50	10.0	0.9	na	na	71	2			
					70	6.5	0.6	na	na	36	2	T		
					MMC 0.05	136.5**	12.4	na	na	50	2			
		–	24+24	DMSO	0	12.0	1.0	na	na	100	2		Y	NEG
					5	14.0	1.2	na	na	82	2			
					10	15.0	1.3	na	na	85	2			
					30	13.0	1.1	na	na	74	2			
					50	10.0	0.8	na	na	79	2			
					70	12.0	1.0	na	na	43	2			
					MMC 0.05	349.0**	29.1	na	na	43	2			
		+	3+18	DMSO	0	7.0	1.0	15.5	1.0	100	2		Y	EQ
					50	12.5	1.8	16.0	1.0	112	2			
					75	13.0	1.9	17.0	1.1	99	2			
					100	7.5	1.1	25.5*	1.7	47	2			
					125	6.0	0.9	21.0	1.4	16	1	T		
					MMC 0.1	15.5*	2.2	102.0**	6.6	85	2			
		+	24+18	DMSO	0	5.0	1.0	12.5	1.0	100	2		N	na
					50	9.0	1.8	17.5	1.4	111	2			
					75	9.5	1.9	25.5**	2.0	77	2			
					100	16.0**	3.2	0.0		12	1	T		
					MMC 0.05	162.5**	32.5	456.0**	36.5	112	2			
Bleomycin														
Lab 1	Assay 1	–	3+21	Medium	0	6.0	1.0	na	na	100	1		N	na
					2	241.0**	40.2	na	na	113	1			
					4	239.0**	39.8	na	na	89	1			
					8	114.0**	19.0	na	na	37	1			
					16	33.0**	5.5	na	na	147	1			
					32	18.0*	3.0	na	na	74	1			
					63	32.0**	5.3	na	na	21	1	T		
					125	24.0**	4.0	na	na	16	1	T		
					250	27.0**	4.5	na	na	26	1	T		
					500	54.0**	9.0	na	na	21	1	T		
					MMC 0.1	51.0**	8.5	na	na	32	1			
		–	3+45	Medium	0	8.0	1.0	na	na	100	1		N	na
					2	24.0**	3.0	na	na	34	1	T		
					4	176.0**	22.0	na	na	22	1	T		
					8	389.0**	48.6	na	na	13	1	T		

				16	303.0**	37.9	na	na	13	1	T		
				32	149.0**	18.6	na	na	8	1	T		
				63	88.0**	11.0	na	na	13	1	T		
				125	22.0 [†]	2.8	na	na	3	1	T		
				250	19.0	2.4	na	na	7	1	T		
				500	30.0**	3.8	na	na	7	1	T		
				MMC 0.1	81.0**	10.1	na	na	14	1			
		–	24 + 0	0	6.0	1.0	na	na	100	1		N	na
				2	34.0**	5.7	na	na	55	1			
				4	8.0	1.3	na	na	29	1	T		
				8	3.0	0.5	na	na	42	1	T		
				16	6.0	1.0	na	na	21	1	T		
				32	4.0	0.7	na	na	21	1	T		
				63	10.0	1.7	na	na	5	1	T		
				125	15.0	2.5	na	na	5	1	T		
				MMC 0.05	63.0**	10.5	na	na	16	1			
		–	24 + 24	0	9.0	1.0	na	na	100	1		N	na
				2	112.0**	12.4	na	na	21	1	T		
				4	69.0**	7.7	na	na	36	1	T		
				8	21.0 [†]	2.3	na	na	13	1	T		
				16	5.0	0.6	na	na	23	1	T		
				MMC 0.05	63.0**	7.0	na	na	19	1			
		+	3 + 18	0	4.0	1.0	10.0	1.0	100	1		Y	POS
				2	105.0**	26.3	555.0**	55.5	83	1			
				4	78.0**	19.5	732.0**	73.2	63	1			
				8	48.0**	12.0	811.0**	81.1	25	1	T		
				16	12.0	3.0	853.0**	85.3	6	1	T		
				32	8.0	2.0	621.0**	62.1	3	1	T		
				MMC 0.1	36.0**	9.0	98.0**	9.8	85	1			
		+	24 + 18	0	9.0	1.0	10.0	1.0	100	1		N	na
				2	66.0**	7.3	940.0**	94.0	18	1	T		
				4	21.0 [†]	2.3	tox			0	1	T	
				MMC 0.05	110.0**	12.2	334.0**	33.4	78	1			
Lab 1	Assay 2	–	3 + 21	0	12.5	1.0	na	na	100	2		Y	POS
				1	245.5**	19.6	na	na	75	2			
				2	295.0**	23.6	na	na	74	2			
				4	283.5**	22.7	na	na	79	2			
				8	194.0**	15.5	na	na	49	2			
				MMC 0.1	48.0**	3.8	na	na	97	2			
		–	3 + 45	0	12.0	1.0	na	na	100	2		Y	POS
				0.25	37.5**	3.1	na	na	116	2			
				0.5	71.5**	6.0	na	na	86	2			
				1	111.5**	9.3	na	na	55	2			
				2	258.0**	21.5	na	na	48	2			
				MMC 0.1	67.0**	5.6	na	na	82	2			
		–	24 + 0	0	13.0	1.0	na	na	100	2		Y	POS
				0.5	204.0**	15.7	na	na	85	2			
				1	76.0**	5.8	na	na	53	2			
				2	16.0	1.2	na	na	64	2			
				4	6.5	0.5	na	na	47	2			
				MMC 0.05	111.0**	8.5	na	na	82	2			
		–	24 + 24	0	10.5	1.0	na	na	100	2		Y	POS
				0.5	196.0**	18.7	na	na	83	2			

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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								
Lab 2	Assay 1	+	3 + 18	Medium	1	409.5**	39.0	na	na	58	2	T	Y	POS			
					2	530.0**	50.5	na	na	52							
					4	449.0**	42.8	na	na	28							
					MMC 0.05	197.5**	18.8	na	na	77							
					0	2.5	1.0	8.5	1.0	100							
					1	88.5**	35.4	360.0**	42.4	98							
					2	90.5**	36.2	588.0**	69.2	75							
					4	38.0**	15.2	777.0**	91.4	45							
					8	31.5**	12.6	898.5**	105.7	21							
			+ 24 + 18	Medium	MMC 0.1	39.0**	15.6	186.5**	21.9	60	2						
					0	4.5	1.0	9.5	1.0	100	2						
					0.125	165.5**	36.8	341.0**	35.9	76	2						
					0.25	316.5**	70.3	598.5**	63.0	98	2						
					0.5	355.0**	78.9	706.5**	74.4	80	2						
					1	172.5**	38.3	763.5**	80.4	55	2						
					MMC 0.05	110.0**	24.4	252.0**	26.5	84	2						
					-	Medium	3 + 21	0	13.0	1.0	na	na	100	1	T	Y	POS
								2	75.0**	5.8	na	na	99				
		5.12	119.0**	9.2				na	na	79							
		12.8	201.0**	15.5				na	na	88							
		32	297.0**	22.8				na	na	60							
		80	357.0**	27.5				na	na	41							
		- 3 + 45	Medium	200			181.0**	13.9	na	na	36	1	T	Y	POS		
				500			154.0**	11.8	na	na	24						
				MMC 0.1			88.0**	6.8	na	na	61						
				0			9.0	1.0	na	na	100						
				2			36.0**	4.0	na	na	84						
				5.12			63.0**	7.0	na	na	90						
		-	Medium	24 + 0	12.8	82.0**	9.1	na	na	77	1	T	Y	POS			
					32	165.0**	18.3	na	na	55							
					80	181.0**	20.1	na	na	30							
					200	178.0**	19.8	na	na	12							
					500	306.0**	34.0	na	na	5							
					MMC 0.1	40.0**	4.4	na	na	43							
				- 24 + 24	Medium	0	17.0	1.0	na	na	100	1	T	Y	POS		
						0.81	90.0**	5.3	na	na	95						
						2	192.0**	11.3	na	na	84						
						5.12	339.0**	19.9	na	na	59						
						12.8	385.0**	22.6	na	na	41						
						32	319.0**	18.8	na	na	43						
		-	Medium	80	197.0**	11.6	na	na	26	1	T	Y	POS				
				200	74.0**	4.4	na	na	15								
MMC 0.05	148.0**			8.7	na	na	65										
0	13.0			1.0	na	na	100										
0.81	73.0**			5.6	na	na	94										
2	151.0**			11.6	na	na	64										
-	Medium	5.12	328.0**	25.2	na	na	47	1	T	Y	POS						
		12.8	508.0**	39.1	na	na	21										

					32	558.0**	42.9	na	na	8	1	T		
					80	371.0**	28.5	na	na	3	1	T		
					200	242.0**	18.6	na	na	1	1	T		
					MMC 0.05	193.0**	14.8	na	na	42	1			
					0	32.0	1.0	34.0	1.0	100	1		Y	POS
					2	42.0	1.3	89.0**	2.0	96	1			
					5.12	80.0**	2.5	137.0**	4.0	94	1			
					12.8	102.0**	3.2	188.0**	5.5	87	1			
					32	134.0**	4.2	263.0**	7.7	87	1			
					80	133.0**	4.2	425.0**	12.5	72	1			
					200	114.0**	3.6	488.0**	14.4	55	1			
					500	36.0	1.1	192.0**	11.3	27	1	T		
					MMC 0.1	42.0	1.3	117.0**	3.4	72	1			
					0	25.0	1.0	23.0	1.0	100	1		Y	POS
					2	140.0**	5.6	215.0**	9.3	98	1			
					5.12	304.0**	12.2	316.0**	13.7	99	1			
					12.8	414.0**	16.6	465.0**	20.2	75	1			
					32	518.0**	20.7	616.0**	26.8	62	1			
					80	395.0**	15.8	646.0**	28.1	29	1	T		
					200	248.0**	9.9	tox		14	1	T		
					MMC 0.05	214.0**	8.6	381.0**	16.6	104	1			
Lab 2	Assay 2	–	3+21	Medium	0	12.0	1.0	na	na	100	2		Y	POS
					12.8	175.0**	14.6	na	na	113	2			
					32	221.0**	18.4	na	na	85	2			
					80	389.0**	32.4	na	na	40	2			
					200	428.5**	35.7	na	na	42	2			
					MMC 0.1	123.5**	10.3	na	na	45	2			
					0	15.5	1.0	na	na	100	2		Y	POS
					12.8	126.0**	8.1	na	na	95	2			
					32	176.0**	11.4	na	na	74	2			
					80	322.5**	20.8	na	na	35	2	T		
					200	458.5**	29.6	na	na	15	2	T		
					MMC 0.1	71.5**	4.6	na	na	26	2			
					0	9.0	1.0	na	na	100	2		Y	POS
					2	142.5**	15.8	na	na	96	2			
					5.12	192.0**	21.3	na	na	79	2			
					12.8	307.5**	34.2	na	na	59	2			
					32	355.0**	39.4	na	na	42	2			
					MMC 0.05	152.0**	16.9	na	na	52	2			
					0	12.5	1.0	na	na	100	2		Y	POS
					2	175.0**	14.0	na	na	77	2			
					5.12	338.5**	27.1	na	na	51	2			
					12.8	522.5**	41.8	na	na	27	2	T		
					32	591.5**	47.3	na	na	11	2	T		
					MMC 0.05	259.0**	20.7	na	na	39	2			
					0	30.0	1.0	24.5	1.0	100	2		Y	POS
					32	105.5**	3.5	261.0**	10.7	101	2			
					80	108.0**	3.6	358.5**	14.6	81	2			
					200	87.0**	2.9	467.5**	19.1	55	2			
					500	59.0**	2.0	563.5**	23.0	28	2	T		
					MMC 0.1	51.0*	1.7	110.5**	4.5	102	2			
					0	16.7	1.0	23.0	1.0	100	2		Y	POS
					5.12	188.0**	11.3	280.0**	12.2	94	2			

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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					
					12.8	297.0**	17.8	429.0**	18.7	82	2			
					32	424.5**	25.5	577.0**	25.1	68	2			
					80	421.0**	25.3	477.0**	20.7	38	2			
					MMC 0.05	290.0**	17.4	451.0**	19.6	100	2			
5-Fluorouracil														
Lab 1	Assay 1	–	3 + 21	Medium	0	7.0	1.0	na	na	100	1		Y	POS
					150	8.0	1.1	na	na	74	1			
					500	22.0**	3.1	na	na	62	1			
					1500	78.0**	11.1	na	na	67	1			
					5000	48.0**	6.9	na	na	61	1			
					MMC 0.1	50.0**	7.1	na	na	84	1			
		–	3 + 45	Medium	0	7.0	1.0	na	na	100	1		Y	POS
					3	12.0	1.7	na	na	101	1			
					10	10.0	1.4	na	na	83	1			
					30	19.0*	2.7	na	na	51	1			
					100	9.0	1.3	na	na	37	1	T		
					MMC 0.1	40.0**	5.7	na	na	111	1			
		–	24 + 0	Medium	0	10.0	1.0	na	na	100	1		Y	POS
					150	15.0	1.5	na	na	61	1			
					500	62.0**	6.2	na	na	63	1			
					1500	57.0**	5.7	na	na	56	1			
					5000	41.0**	4.1	na	na	47	1			
					MMC 0.05	129.0**	12.9	na	na	92	1			
		–	24 + 24	Medium	0	10.0	1.0	na	na	100	1		Y	POS
					1	17.0	1.7	na	na	88	1			
					3	17.0	1.7	na	na	51	1			
					10	25.0*	2.5	na	na	40	1			
					30	46.0**	4.6	na	na	34	1	T		
					MMC 0.05	229.0**	22.9	na	na	79	1			
		+	3 + 18	Medium	0	0.0	1.0	9.0	1.0	100	1		N	na
					12.5	6.0*	6.0	12.0	1.3	45	1			
					25	4.0	4.0	9.0	1.0	33	1	T		
					50	2.0	2.0	19.0	2.1	29	1	T		
					100	4.0	4.0	16.0	1.8	28	1	T		
					MMC 0.1	11.0**	11.0	54.0**	6.0	92	1			
		+	24 + 18	Medium	0	2.0	1.0	5.0	1.0	100	1		N	na
					25	10.0*	5.0	94.0**	18.8	53	1			
					50	10.0*	5.0	143.0**	28.6	34	1	T		
					75	8.0	4.0	tox		13	1	T		
					100	7.0	3.5	tox		7	1	T		
					MMC 0.05	66.0**	33.0	173.0**	34.6	104	1			
Lab 1	Assay 2	–	3 + 21	Medium	0	9.0	1.0	na	na	100	2		Y	POS
					150	9.5	1.1	na	na	80	2			
					500	36.0**	4.0	na	na	76	2			
					1500	64.0**	7.1	na	na	72	2			
					5000	70.5**	7.8	na	na	64	2			
					MMC 0.1	45.5**	5.1	na	na	68	2			

Lab 2	Assay 1	–	3 + 45	Medium	0	5.0	1.0	na	na	100	2	Y	NEG
					3	5.5	1.1	na	na	83	2		
					10	6.5	1.3	na	na	76	2		
					30	7.5	1.5	na	na	54	2		
					100	7.0	1.4	na	na	44	2		
		–	24 + 0	Medium	MMC 0.1	32.5**	6.5	na	na	89	2	Y	POS
					0	4.5	1.0	na	na	100	2		
					150	106.0**	23.6	na	na	72	2		
					500	94.0**	20.9	na	na	59	2		
					1500	70.5**	15.7	na	na	56	2		
		–	24 + 24	Medium	5000	25.5**	5.7	na	na	39	2	N	na
					MMC 0.05	108.0**	24.0	na	na	61	2		
					0	5.5	1.0	na	na	100	2		
					1	8.5	1.6	na	na	85	2		
					3	10.0	1.8	na	na	66	2		
		+	3 + 18	Medium	10	5.0	0.9	na	na	50	2	Y	NEG
					30	18.0**	3.3	na	na	36	2		
					MMC 0.05	211.0**	38.4	na	na	59	2		
					0	10.0	1.0	12.0	1.0	100	2		
					12.5	5.0	0.5	7.0	0.6	67	2		
+	24 + 18	Medium	25	5.0	0.5	10.0	0.8	55	2	Y	POS		
			50	6.0	0.6	7.5	0.6	45	2				
			100	3.0	0.3	7.5	0.6	44	2				
			MMC 0.1	20.5**	2.1	60.0**	5.0	93	2				
			0	3.5	1.0	12.0	1.0	100	2				
–	3 + 21	Medium	25	11.0**	3.1	48.0**	4.0	87	2	T	na		
			50	8.5	2.4	150.5**	12.5	52	2				
			75	8.0	2.3	tox		16	2				
			MMC 0.05	140.5**	40.1	237.0**	19.8	122	2				
			0	13.5	1.0	na	na	100	2				
–	3 + 45	Medium	125	13.0	1.0	na	na	57	2	Y	POS		
			250	19.0	1.4	na	na	46	2				
			500	30.5**	2.3	na	na	56	2				
			1000	59.0**	4.4	na	na	56	2				
			MMC 0.1	37.5**	2.8	na	na	74	2				
–	24 + 0	Medium	0	9.0	1.0	na	na	100	2	N	na		
			1.25	15.0	1.7	na	na	72	2				
			2.5	20.0**	2.2	na	na	66	2				
			5	19.5**	2.2	na	na	42	2				
			10	32.0**	3.6	na	na	43	2				
–	24 + 0	Medium	MMC 0.1	30.5**	3.4	na	na	79	2	N	na		
			0	9.0	1.0	na	na	100	2				
			0.5	5.0	0.6	na	na	91	2				
			1	5.5	0.6	na	na	70	2				
			2	4.5	0.5	na	na	57	2				
–	24 + 0	Medium	4	7.0	0.8	na	na	64	2	N	na		
			MMC 0.05	93.0**	10.3	na	na	61	2				

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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 + 24	Medium	0	12.0	1.0	na	na	100	2		Y	NEG
					0.25	6.5	0.5	na	na	96	2			
					0.5	6.0	0.5	na	na	75	2			
					1	6.5	0.5	na	na	45	2			
					2	8.0	0.7	na	na	43	2			
					MMC 0.05	202.5**	16.9	na	na	60	2			
		+	3 + 18	Medium	0	8.0	1.0	9.0	1.0	100	2		Y	POS
					625	13.5	1.7	56.5**	6.3	88	2			
					1250	21.0**	2.6	103.0**	11.4	83	2			
					2500	22.0**	2.8	105.5**	11.7	81	2			
					5000	23.0**	2.9	118.0**	13.1	80	2			
					MMC 0.1	18.0**	2.3	60.5**	6.7	102	2			
		+	24 + 18	Medium	0	nt	nt	7.0	1.0	100	2		Y	POS
					10	5.5		24.0**	3.4	59	2			
					20	11.5		80.5**	11.5	52	2			
					40	43.0		116.0**	16.6	41	2			
					80	23.0		tox		20	2	T		
					MMC 0.05	nt	nt	223.5**	31.9	102	2			
Lab 2	Assay 2	–	3 + 21	Medium	0	15.5	1.0	na	na	100	2		Y	POS
					125	80.0**	5.2	na	na	74	2			
					250	127.0**	8.2	na	na	78	2			
					500	134.0**	8.6	na	na	79	2			
					1000	148.0**	9.5	na	na	74	2			
					MMC 0.1	62.0**	4.0	na	na	71	2			
		–	3 + 45	Medium	0	19.0	1.0	na	na	100	2		Y	POS
					1.25	20.0	1.1	na	na	104	2			
					2.5	26.5	1.4	na	na	59	2			
					5	23.5	1.2	na	na	59	2			
					10	38.0**	2.0	na	na	43	2			
					MMC 0.1	45.0**	2.4	na	na	91	2			
		–	24 + 0	Medium	0	11.0	1.0	na	na	100	2		N	na
					0.5	17.0	1.5	na	na	94	2			
					1	19.5*	1.8	na	na	75	2			
					2	13.5	1.2	na	na	82	2			
					4	21.5*	2.0	na	na	77	2			
					MMC 0.05	98.0**	8.9	na	na	94	2			
		–	24 + 24	Medium	0	17.0	1.0	na	na	100	2		Y	POS
					0.25	18.5	1.1	na	na	83	2			
					0.5	20.0	1.2	na	na	59	2			
					1	35.0**	2.1	na	na	43	2			
					2	38.0**	2.2	na	na	44	2			
					MMC 0.05	172.5**	10.1	na	na	100	2			
		+	3 + 18	Medium	0	27.0	1.0	10.5	1.0	100	2		Y	POS
					625	25.5	1.0	99.0**	9.4	52	2			
					1250	24.0	0.9	118.0**	11.2	52	2			
					2500	24.5	0.9	90.0**	8.6	55	2			
					5000	21.5	0.8	72.5**	6.9	53	2			
					MMC 0.1	24.5	0.9	100.0**	9.5	87	2			

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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					
Lab 1	Assay 2	–	3 + 21	Medium	0	5.0	1.0	na	na	100	2		Y	POS
					0.0625	12.5*	2.5	na	na	73	2			
					0.125	22.5**	4.5	na	na	61	2			
					0.25	48.0**	9.6	na	na	57	2			
					0.5	64.5**	12.9	na	na	44	2			
					1	52.5**	10.5	na	na	33	2	T		
					MMC 0.1	52.5**	10.5	na	na	68	2			
					0	4.5	1.0	na	na	100	2		Y	
					0.0625	9.5	2.1	na	na	83	2			
					0.125	15.0**	3.3	na	na	77	2			
					0.25	38.5**	8.6	na	na	60	2			
					0.5	101.0**	22.4	na	na	41	2			
					1	220.0**	48.9	na	na	23	2	T		
					MMC 0.1	56.5**	12.6	na	na	86	2			
					0	2.5	1.0	na	na	100	2		Y	
	0.00625	14.0**	5.6	na	na	73	2							
	0.0125	16.5**	6.6	na	na	62	2							
	0.025	42.5**	17.0	na	na	51	2							
	0.05	46.5**	18.6	na	na	42	2							
	0.1	54.0**	21.6	na	na	33	2	T						
	MMC 0.05	64.5**	25.8	na	na	74	2							
	0	5.0	1.0	na	na	100	2		Y					
	0.00625	11.5*	2.3	na	na	87	2							
	0.0125	14.0**	2.8	na	na	75	2							
	0.025	26.5**	5.3	na	na	74	2							
	0.05	56.5**	11.3	na	na	47	2							
	0.1	103.5**	20.7	na	na	38	2	T						
	MMC 0.05	177.5**	35.5	na	na	63	2							
	0	nt	nt	6.5	1.0	100	2		Y					
	0.0625	nt	nt	13.5*	2.1	99	2							
	0.125	nt	nt	12.5	1.9	96	2							
	0.25	nt	nt	28.5**	4.4	79	2							
	0.5	nt	nt	27.5**	4.2	59	2							
	1	nt	nt	73.5**	11.3	32	2	T						
	MMC 0.1	nt	nt	42.5**	6.5	94	2							
	0	nt	nt	8.0	1.0	100	2		Y					
	0.00625	nt	nt	19.5**	2.4	117	2							
	0.0125	nt	nt	18.5**	2.3	120	2							
	0.025	nt	nt	37.0**	4.6	118	2							
	0.05	nt	nt	90.0**	11.3	107	2							
	0.1	nt	nt	161.5**	20.2	114	2							
	0.2	nt	nt	172.5**	21.6	117	2							
0.4	nt	nt	230.0**	28.8	115	2								
0.8	nt	nt	330.5**	41.3	61	2								
1.6	nt	nt	422.5**	52.8	16	2	T							
MMC 0.05	nt	nt	180.5**	22.6	109	2								
Lab 2	Assay 1	–	3 + 21	Medium	0	23.0	1.0	na	na	100	1		Y	POS
					0.0625	128.0**	5.6	na	na	65	1			
					0.25	109.0**	4.7	na	na	31	1			

				1	117.0**	5.1	na	na	42	1			
				4	99.0**	4.3	na	na	38	1			
				MMC 0.1	95.0**	4.1	na	na	85	1			
	–	3 + 45	Medium	0	29.0	1.0	na	na	100	1	Y	POS	
				0.0625	100.0**	3.4	na	na	62	1			
				0.25	84.0**	2.9	na	na	52	1			
				1	82.0**	2.8	na	na	30	1			
				4	103.0**	3.6	na	na	21	1			
				MMC 0.1	54.0**	1.9	na	na	79	1			
	–	24 + 0	Medium	0	21.0	1.0	na	na	100	1	T	N	
				0.005	58.0**	2.8	na	na	26	1			
				0.01	57.0**	2.7	na	na	28	1			
				0.02	50.0**	2.4	na	na	23	1			
				0.04	33.0	1.6	na	na	21	1			
				MMC 0.05	193.0**	9.2	na	na	70	1			
	–	24 + 24	Medium	0	28.0	1.0	na	na	100	1	T	N	
				0.005	252.0**	9.0	na	na	21	1			
				0.01	181.0**	6.5	na	na	15	1			
				0.02	145.0**	5.2	na	na	15	1			
				0.04	132.0**	4.7	na	na	8	1			
				MMC 0.05	354.0**	12.6	na	na	78	1			
	+	3 + 18	Medium	0	nt	nt	38.0	1.0	100	1	N	na	
				0.0625	nt	nt	98.0**	2.6	89	1			
				0.25	nt	nt	173.0**	4.6	30	1			
				MMC 0.1	nt	nt	131.0**	3.4	92	1			
	+	24 + 18	Medium	0	nt	nt	25.0	1.0	100	1	N	na	
				0.005	nt	nt	454.0**	18.2	76	1			
				MMC 0.05	nt	nt	376.0**	15.0	69	1			
Lab 2	Assay 2	–	3 + 21	Medium	0	14.5	1.0	na	na	100	2	Y	POS
				0.0125	17.5	1.2	na	na	131	2			
				0.025	22.0	1.5	na	na	98	2			
				0.05	26.5*	1.8	na	na	90	2			
				0.1	29.0**	2.0	na	na	94	2			
				0.2	41.5**	2.9	na	na	92	2			
				MMC 0.1	75.5**	5.2	na	na	82	2			
	–	3 + 45	Medium	0	16.5	1.0	na	na	100	2	Y	POS	
				0.0125	15.5	0.9	na	na	97	2			
				0.025	18.5	1.1	na	na	91	2			
				0.05	20.5	1.2	na	na	111	2			
				0.1	18.5	1.1	na	na	82	2			
				0.2	30.0**	1.8	na	na	86	2			
				MMC 0.1	54.5**	3.3	na	na	96	2			
	–	24 + 0	Medium	0	12.5	1.0	na	na	100	2	Y	POS	
				0.00025	13.5	1.1	na	na	79	2			
				0.0005	16.0	1.3	na	na	101	2			
				0.001	14.5	1.2	na	na	77	2			
				0.002	21.0*	1.7	na	na	73	2			
				0.004	27.0**	2.2	na	na	61	2			
				MMC 0.05	149.5**	12.0	na	na	58	2			
	–	24 + 24	Medium	0	11.0	1.0	na	na	100	2	Y	POS	
				0.00025	14.5	1.3	na	na	87	2			
				0.0005	14.5	1.3	na	na	77	2			
				0.001	17.0	1.5	na	na	87	2			

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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					
					0.002	17.0	1.5	na	na	68	2			
					0.004	20.5*	1.9	na	na	74	2			
					MMC 0.05	424.5**	38.6	na	na	42	2			
		+	3 + 18	Medium	0	nt	nt	28.5	1.0	100	2		Y	POS
					0.0125	nt	nt	32.0	1.1	103	2			
					0.025	nt	nt	34.0	1.2	99	2			
					0.05	nt	nt	36.5	1.3	100	2			
					0.1	nt	nt	46.0**	1.6	98	2			
					0.2	nt	nt	50.5**	1.8	102	2			
					MMC 0.1	nt	nt	189.5**	6.6	90	2			
		+	24 + 18	Medium	0	nt	nt	26.5	1.0	100	2		Y	POS
					0.00025	nt	nt	34.5	1.3	102	2			
					0.0005	nt	nt	36.5	1.4	104	2			
					0.001	nt	nt	38.0*	1.4	106	2			
					0.002	nt	nt	38.0*	1.4	100	2			
					0.004	nt	nt	38.0*	1.4	102	2			
					MMC 0.05	nt	nt	459.5**	17.3	98	2			
Urethane														
Lab 1	Assay 1	–	3 + 21	Medium	0	19.0	1.0	na	na	100	2		Y	NEG
					320	20.0	1.1	na	na	144	2			
					800	23.0	1.2	na	na	108	2			
					2000	21.5	1.1	na	na	134	2			
					5000	24.5	1.3	na	na	99	2			
					MMC 0.1	78.5**	4.1	na	na	82	2			
		–	3 + 45	Medium	0	12.5	1.0	na	na	100	2		Y	NEG
					320	8.0	0.6	na	na	100	2			
					800	9.0	0.7	na	na	102	2			
					2000	9.0	0.7	na	na	119	2			
					5000	8.5	0.7	na	na	96	2			
					MMC 0.1	30.5**	2.4	na	na	102	2			
		–	24 + 0	Medium	0	19.0	1.0	na	na	100	2		Y	POS
					320	25.5	1.3	na	na	85	2			
					800	32.5**	1.7	na	na	79	2			
					2000	45.0**	2.4	na	na	73	2			
					5000	44.0**	2.3	na	na	35	2			
					MMC 0.05	172.0**	9.1	na	na	73	2			
		–	24 + 24	Medium	0	20.5	1.0	na	na	100	2		N	na
					320	21.0	1.0	na	na	90	2			
					800	20.0	1.0	na	na	72	2			
					2000	28.5	1.4	na	na	54	2			
					5000	46.0**	2.2	na	na	27	2			
					MMC 0.05	212.5**	10.4	na	na	59	2			
		+	3 + 18	Medium	0	nt	nt	17.5	1.0	100	2		Y	NEG
					625	nt	nt	21.0	1.2	101	2			
					1250	nt	nt	21.0	1.2	99	2			
					2500	nt	nt	20.0	1.1	102	2			
					5000	nt	nt	19.5	1.1	92	2			
					MMC 0.1	nt	nt	65.5**	3.7	92	2			

Lab 1	Assay 2	+	24 + 18	Medium	0	nt	nt	17.0	1.0	100	2	Y	POS	
					1481	nt	nt	18.5	1.1	101	2			
					2222	nt	nt	25.5	1.5	91	2			
					3333	nt	nt	28.0*	1.6	74	2			
					5000	nt	nt	35.0**	2.1	50	2			
				MMC 0.05	nt	nt	253.5**	14.9	109	2				
		Assay 2	-	3 + 21	Medium	0	9.0	1.0	na	na	100	2	Y	NEG
	320					10.0	1.1	na	na	111	2			
	800					11.5	1.3	na	na	158	2			
	2000					8.5	0.9	na	na	127	2			
	5000					7.5	0.8	na	na	161	2			
				MMC 0.1	66.0**	7.3	na	na	98	2				
		Assay 2	-	3 + 45	Medium	0	11.0	1.0	na	na	100	2	Y	NEG
	320					8.0	0.7	na	na	102	2			
	800					13.0	1.2	na	na	108	2			
	2000					14.0	1.3	na	na	96	2			
	5000					13.5	1.2	na	na	102	2			
				MMC 0.1	55.0**	5.0	na	na	78	2				
		Assay 2	-	24 + 0	Medium	0	10.5	1.0	na	na	100	2	Y	NEG
	320					7.0	0.7	na	na	144	2			
800	12.5					1.2	na	na	132	2				
2000	10.5					1.0	na	na	117	2				
5000	14.0					1.3	na	na	94	2				
			MMC 0.05	109.0**	10.4	na	na	129	2					
	Assay 2	-	24 + 24	Medium	0	6.5	1.0	na	na	100	2	Y	EQ	
320					16.5**	2.5	na	na	84	2				
800					16.5**	2.5	na	na	96	2				
2000					8.0	1.2	na	na	79	2				
5000					19.0**	2.9	na	na	67	2				
			MMC 0.05	226.5**	34.8	na	na	84	2					
	Assay 2	+	3 + 18	Medium	0	1.5	1.0	21.5	1.0	100	2	Y	EQ	
625					3.5	2.3	37.0**	1.7	100	2				
1250					3.5	2.3	31.0	1.4	101	2				
2500					5.5	3.7	42.0**	2.0	102	2				
5000					5.0	3.3	31.0	1.4	97	2				
			MMC 0.1	11.5**	7.7	92.0**	4.3	98	2					
	Assay 2	+	24 + 18	Medium	0	2.0	1.0	7.0	1.0	100	2	Y	EQ	
1481					2.0	1.0	10.5	1.5	99	2				
2222					1.5	0.8	9.5	1.4	98	2				
3333					1.0	0.5	20.0**	2.9	96	2				
5000					1.0	0.5	9.0	1.3	97	2				
			MMC 0.05	5.5	2.8	265.0**	37.9	105	2					
Lab 2	Assay 1	-	3 + 21	Medium	0	10.0	1.0	na	na	100	1	Y	NEG	
					625	3.0	0.3	na	na	72	1			
					1250	7.0	0.7	na	na	102	1			
					2500	10.0	1.0	na	na	81	1			
					5000	6.0	0.6	na	na	77	1			
				MMC 0.1	71.0**	7.1	na	na	80	1				
		Assay 1	-	3 + 45	Medium	0	9.0	1.0	na	na	100	1	Y	NEG

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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					
					625	4.0	0.4	na	na	89	1			
					1250	5.0	0.6	na	na	102	1			
					2500	6.0	0.7	na	na	98	1			
					5000	5.0	0.6	na	na	92	1			
					MMC 0.1	21.0*	2.3	na	na	98	1			
		–	24+0	Medium	0	6.0	1.0	na	na	100	1		Y	NEG
					625	3.0	0.5	na	na	95	1			
					1250	3.0	0.5	na	na	88	1			
					2500	8.0	1.3	na	na	71	1			
					5000	8.0	1.3	na	na	71	1			
					MMC 0.05	130.0**	21.7	na	na	70	1			
		–	24+24	Medium	0	7.0	1.0	na	na	100	1		Y	NEG
					625	7.0	1.0	na	na	99	1			
					1250	7.0	1.0	na	na	96	1			
					2500	4.0	0.6	na	na	79	1			
					5000	6.0	0.9	na	na	42	1			
					MMC 0.05	315.0**	45.0	na	na	57	1			
		+	3+18	Medium	0	28.0	1.0	20.0	1.0	100	1		Y	NEG
					625	6.7	0.2	17.0	0.9	101	1			
					1250	25.6	0.9	20.0	1.0	100	1			
					2500	9.2	0.3	10.0	0.5	100	1			
					5000	6.8	0.2	14.0	0.7	95	1			
					MMC 0.1	59.9	2.1	135.0**	6.8	93	1			
		+	24+18	Medium	0	0.0	0.0	10.0	1.0	100	1		Y	EQ
					625	0.0	0.0	9.0	0.9	96	1			
					1250	0.0	0.0	16.0	1.6	96	1			
					2500	0.0	0.0	10.0	1.0	91	1			
					5000	8.0	8.0	24.0*	2.4	67	1			
					MMC 0.05	103.0**	103.0	298.0**	29.8	107	1			
Lab 2	Assay 2	–	3+21	Medium	0	10.0	1.0	na	na	100	2		Y	NEG
					625	9.0	0.9	na	na	93	2			
					1250	9.0	0.9	na	na	85	2			
					2500	12.0	1.2	na	na	83	2			
					5000	12.5	1.3	na	na	79	2			
					MMC 0.1	85.5**	8.6	na	na	84	2			
		–	3+45	Medium	0	13.5	1.0	na	na	100	2		Y	NEG
					625	12.5	0.9	na	na	105	2			
					1250	12.0	0.9	na	na	73	2			
					2500	12.0	0.9	na	na	102	2			
					5000	11.0	0.8	na	na	103	2			
					MMC 0.1	46.0**	3.4	na	na	112	2			
		–	24+0	Medium	0	11.5	1.0	na	na	100	2		Y	NEG
					625	11.0	1.0	na	na	96	2			
					1250	12.0	1.0	na	na	109	2			
					2500	10.5	0.9	na	na	109	2			
					5000	10.0	0.9	na	na	83	2			
					MMC 0.05	90.0**	7.8	na	na	103	2			

Colchicine Lab 1	Assay 1	–	24 + 24	Medium	0	6.0	1.0	na	na	100	2	Y	EQ	
					625	9.0	1.5	na	na	88	2			
					1250	9.5	1.6	na	na	89	2			
					2500	8.5	1.4	na	na	78	2			
					5000	14.5*	2.4	na	na	48	2			
		MMC 0.05	228.0**	38.0	na	na	74	2						
		+	3 + 18	Medium	0	31.4	1.0	12.5	1.0	100	2	Y	NEG	
					625	25.5	0.8	14.5	1.2	98	2			
					1250	27.3	0.9	19.5	1.6	99	2			
					2500	29.1	0.9	15.5	1.2	98	2			
					5000	27.0	0.9	11.0	0.9	97	2			
		+	24 + 18	Medium	0	45.5	1.4	133.5**	10.7	98	2	Y	NEG	
					625	14.7	1.0	15.0	1.0	100	2			
					1250	6.2	4.2	16.5	1.1	101	2			
					2500	21.5	1.5	15.0	1.0	101	2			
	5000				16.5	1.1	24.0	1.6	100	2				
	MMC 0.05	180.2**	4.8	499.5**	33.3	95	2							
	Assay 2	–	3 + 21	Medium	0	5.0	1.0	na	na	100	1	Y	POS	
					10	74.0**	14.8	na	na	53	1			
					30	83.0**	16.6	na	na	54	1			
					100	79.0**	15.8	na	na	48	1			
					300	124.0**	24.8	na	na	38	1			
			MMC 0.1	72.0**	14.4	na	na	95	1					
			–	3 + 45	Medium	0	9.0	1.0	na	na	100	1	Y	POS
						0.1	5.0	0.6	na	na	91	1		
						0.3	18.0	2.0	na	na	63	1		
						1	32.0**	3.6	na	na	49	1		
						3	25.0**	2.8	na	na	42	1		
			–	24 + 0	Medium	0	40.0**	4.4	na	na	96	1	Y	POS
						0.01	12.0	1.0	na	na	100	1		
						0.03	9.0	0.8	na	na	103	1		
						0.1	11.0	0.9	na	na	90	1		
		0.3				220.0**	18.3	na	na	45	1			
		–	24 + 24	Medium	0	124.0**	10.3	na	na	30	1	T		
					MMC 0.05	71.0**	5.9	na	na	73	1			
					0	5.0	1.0	na	na	100	1			
0.01					10.0	2.0	na	na	97	1				
0.03					12.0	2.4	na	na	91	1				
–		24 + 24	Medium	0.1	72.0**	14.4	na	na	28	1	T			
				0.3	72.0**	14.4	na	na	20	1				
				MMC 0.05	145.0**	29.0	na	na	62	1				
				0	14.0	1.0	7.0	1.0	100	1				
				10	114.0**	8.1	24.0**	3.4	58	1				
+		3 + 18	Medium	30	128.0**	9.1	44.0**	6.3	52	1	Y	POS		
				100	140.0**	10.0	66.0**	9.4	37	1				
				300	98.0**	7.0	tox	tox	4	1				
				MMC 0.1	10.0	0.7	96.0**	13.7	78	1				
				0	14.0	1.0	10.0	1.0	100	1				
+		24 + 18	Medium	0	14.0	1.0	10.0	1.0	100	1	Y	POS		

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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					
					0.01	22.0	1.6	10.0	1.0	99	1			
					0.03	26.0	1.9	13.0	1.3	98	1			
					0.1	196.0**	14.0	53.0**	5.3	79	1			
					0.3	182.0**	13.0	tox	tox	45	1			
					MMC 0.05	94.0**	6.7	270.0**	27.0	110	1			
Lab 1	Assay 2	–	3 + 21	Medium	0	8.0	1.0	na	na	100	2		Y	POS
					25	92.0**	11.5	na	na	50	2			
					50	88.5**	11.1	na	na	53	2			
					100	87.5**	10.9	na	na	52	2			
					200	98.5**	12.3	na	na	48	2			
					MMC 0.1	67.5**	8.4	na	na	84	2			
		–	3 + 45	Medium	0	9.0	1.0	na	na	100	2		Y	POS
					0.25	7.5	0.8	na	na	92	2			
					0.5	10.5	1.2	na	na	74	2			
					1	22.5**	2.5	na	na	57	2			
					2	39.0**	4.3	na	na	46	2			
					MMC 0.1	35.5**	3.9	na	na	100	2			
		–	24 + 0	Medium	0	7.0	1.0	na	na	100	2		Y	POS
					0.025	5.0	0.7	na	na	103	2			
					0.05	9.5	1.4	na	na	100	2			
					0.1	37.5**	5.4	na	na	87	2			
					0.2	138.5**	19.8	na	na	49	2			
					MMC 0.05	127.5**	18.2	na	na	73	2			
		–	24 + 24	Medium	0	8.0	1.0	na	na	100	2		Y	POS
					0.025	11.0	1.4	na	na	119	2			
					0.05	9.5	1.2	na	na	101	2			
					0.1	36.5**	4.6	na	na	57	2			
					0.2	87.0**	10.9	na	na	48	2			
					MMC 0.05	199.5**	24.9	na	na	72	2			
		+	3 + 18	Medium	0	6.0	1.0	10.0	1.0	100	2		Y	POS
					12.5	167.0**	27.8	33.5**	3.4	61	2			
					25	158.0**	26.3	45.5**	4.6	62	2			
					50	154.0**	25.7	47.5**	4.8	62	2			
					100	148.0**	24.7	51.5**	5.2	48	2			
					MMC 0.1	8.0	1.3	66.0**	6.6	96	2			
		+	24 + 18	Medium	0	9.0	1.0	6.5	1.0	100	2		Y	POS
					0.025	7.0	0.8	7.5	1.2	97	2			
					0.05	6.0	0.7	9.5	1.5	99	2			
					0.1	25.0**	2.8	33.5**	5.2	88	2			
					0.2	66.0**	7.3	60.5**	9.3	60	2			
					MMC 0.05	180.0**	20.0	199.5**	30.7	110	2			
Lab 2	Assay 1	–	3 + 21	Medium	0	8.0	1.0	na	na	100	1		Y	POS
					0.1	2.0	0.3	na	na	75	1			
					0.3	19.0	2.4	na	na	64	1			
					1	41.0**	5.1	na	na	46	1			
					3	55.0**	6.9	na	na	40	1			
					10	62.0**	7.8	na	na	37	1			
					30	70.0**	8.8	na	na	41	1			

				100	78.0**	9.8	na	na	35	1	T		
				300	90.0**	11.3	na	na	37	1	T		
				1000	114.0**	14.3	na	na	30	1	T		
				MMC 0.1	93.0**	11.6	na	na	79	1			
	–	3 + 45	Medium	0	10.0	1.0	na	na	100	1		N	na
				0.1	10.0	1.0	na	na	73	1			
				0.3	9.0	0.9	na	na	47	1			
				1	21.0	2.1	na	na	36	1	T		
				3	26.0*	2.6	na	na	30	1	T		
				10	39.0**	3.9	na	na	24	1	T		
				30	43.0**	4.3	na	na	27	1	T		
				100	32.0**	3.2	na	na	23	1	T		
				300	44.0**	4.4	na	na	23	1	T		
				1000	80.0**	8.0	na	na	13	1	T		
				MMC 0.1	53.0**	5.3	na	na	76	1			
	–	24 + 0	Medium	0	7.0	1.0	na	na	100	1		Y	POS
				0.001	6.0	0.9	na	na	99	1			
				0.003	11.0	1.6	na	na	97	1			
				0.01	3.0	0.4	na	na	111	1			
				0.03	23.0**	3.3	na	na	87	1			
				0.1	153.0**	21.9	na	na	29	1	T		
				0.3	126.0**	18.0	na	na	29	1	T		
				MMC 0.05	140.0**	20.0	na	na	63	1			
	–	24 + 24	Medium	0	6.0	1.0	na	na	100	1		Y	POS
				0.001	11.0	1.8	na	na	67	1			
				0.003	4.0	0.7	na	na	125	1			
				0.01	3.0	0.5	na	na	119	1			
				0.03	31.0**	5.2	na	na	47	1			
				0.1	99.0**	16.5	na	na	17	1	T		
				MMC 0.05	360.0**	60.0	na	na	41	1			
	+	3 + 18	Medium	0	14.0	1.0	12.0	1.0	100	1		Y	POS
				0.1	13.0	0.9	11.0	0.9	94	1			
				0.3	53.0**	3.8	14.0	1.2	88	1			
				1	49.0**	3.5	38.0**	3.2	76	1			
				3	133.0**	9.5	55.0**	4.6	76	1			
				10	129.0**	9.2	56.0**	4.7	81	1			
				30	164.0**	11.7	46.0**	3.8	88	1			
				100	134.0**	9.6	71.0**	5.9	75	1			
				300	136.0**	9.7	123.0**	10.3	59	1			
				1000	85.0**	6.1	117.0**	9.8	15	1	T		
				MMC 0.1	22.0	1.6	59.0**	4.9	94	1			
	+	24 + 18	Medium	0	13.0	1.0	5.0	1.0	100	1		Y	POS
				0.1	248.0**	19.1	209.0**	41.8	110	1			
				0.3	365.0**	28.1	299.0**	59.8	97	1			
				MMC 0.05	183.0**	14.1	208.0**	41.6	108	1			
Lab 2	Assay 2	–	3 + 21	Medium	0	3.5	1.0	na	na	100	2	Y	POS
				0.375	11.5**	3.3	na	na	62	2			
				0.75	31.0**	8.9	na	na	56	2			
				1.5	52.5**	15.0	na	na	47	2			
				3	58.5**	16.7	na	na	42	2			
				MMC 0.1	67.5**	19.3	na	na	76	2			
	–	3 + 45	Medium	0	6.0	1.0	na	na	100	2		Y	POS
				0.125	7.0	1.2	na	na	97	2			

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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					
					0.25	6.0	1.0	na	na	81	2			
					0.5	12.5*	2.1	na	na	60	2			
					1	19.0**	3.2	na	na	42	2			
					MMC 0.1	29.5**	4.9	na	na	96	2			
		–	24+0	Medium	0	4.5	1.0	na	na	100	2		Y	POS
					0.001	5.5	1.2	na	na	101	2			
					0.003	4.0	0.9	na	na	96	2			
					0.05	16.0**	3.6	na	na	86	2			
					0.1	74.5**	16.6	na	na	72	2			
					MMC 0.05	129.0**	28.7	na	na	61	2			
		–	24+24	Medium	0	6.5	1.0	na	na	100	2		N	na
					0.00625	9.0	1.4	na	na	105	2			
					0.01	7.0	1.1	na	na	97	2			
					0.025	9.5	1.5	na	na	90	2			
					0.05	45.0**	6.9	na	na	37	2	T		
					MMC 0.05	215.0**	33.1	na	na	59	2			
		+	3+18	Medium	0	13.0	1.0	12.0	1.0	100	2		Y	POS
					50	120.0**	9.2	50.0**	4.2	84	2			
					100	125.5**	9.7	48.5**	4.0	80	2			
					200	133.0**	10.2	62.0**	5.2	77	2			
					400	119.5**	9.2	95.0**	11.1	44	2			
					MMC 0.1	14.0	1.1	57.5**	4.8	92	2			
		+	24+18	Medium	0	6.5	1.0	5.5	1.0	100	2		Y	POS
					0.0375	24.0**	3.7	15.5**	2.8	96	2			
					0.075	107.0**	16.5	42.0**	7.6	96	2			
					0.15	259.0**	39.8	106.0**	30.3	107	2			
					0.3	384.0**	59.1	140.0**	46.7	122	2			
					MMC 0.05	159.5**	24.5	225.5**	41.0	108	2			
Lab 2	Assay 3	–	24+0	Medium	0	9.0	1.0	na	na	100	2		Y	POS
					0.0125	4.0	0.4	na	na	93	2			
					0.025	8.0	0.9	na	na	91	2			
					0.05	47.5**	5.3	na	na	75	2			
					0.1	88.0**	9.8	na	na	66	2			
					0.2	220.5**	24.5	na	na	41	2			
					MMC 0.05	135.0**	15.0	na	na	67	2			
Diethylstilboestrol														
Lab 1	Assay 1	–	3+21	Ethanol	0	12.0	1.0	na	na	100	1		N	na
					3.125	10.0	0.8	na	na	91	1			
					6.25	8.0	0.7	na	na	103	1			
					12.5	14.0	1.2	na	na	83	1			
					25	3.0	0.3	na	na	81	1			
					MMC 0.1	168.0**	14.0	na	na	63	1			
		–	3+45	Ethanol	0	10.0	1.0	na	na	100	1		N	na
					3.125	7.0	0.7	na	na	113	1			
					6.25	10.0	1.0	na	na	104	1			
					12.5	13.0	1.3	na	na	107	1			
					25	15.0	1.5	na	na	85	1			
					MMC 0.1	136.0**	13.6	na	na	103	1			

Lab 1	Assay 2	–	24 + 0	Ethanol	0	7.0	1.0	na	na	100	1	Y	POS						
					3.125	9.0	1.3	na	na	81	1								
					6.25	79.0**	11.3	na	na	61	1								
					12.5	338.0**	48.3	na	na	33	1			T					
					25	267.0**	38.1	na	na	29	1			T					
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		–	24 + 24	Ethanol	0	11.0	1.0	na	na	100	1	Y	POS						
					3.125	9.0	0.8	na	na	100	1								
					6.25	33.0**	3.0	na	na	40	1								
					12.5	200.0**	18.2	na	na	25	1			T					
					25	124.0**	11.3	na	na	30	1			T					
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		+	3 + 18	Ethanol	0	12.0	1.0	18.0	1.0	100	1	N	na						
					3.125	14.0	1.2	19.0	1.1	100	1								
					6.25	9.0	0.8	21.0	1.2	107	1								
					12.5	21.0	1.8	18.0	1.0	107	1								
					25	19.0	1.6	24.0	1.3	99	1								
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		+	24 + 18	Ethanol	0	18.0	1.5	113.0**	6.3	90	1	Y	POS						
					3.125	9.0	2.3	15.0	1.5	97	1								
					6.25	44.0**	11.0	25.0*	2.5	109	1								
					12.5	332.0**	83.0	208.0**	20.8	81	1								
					25	357.0**	89.3	175.0**	17.5	75	1								
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		–	3 + 21	Ethanol	0	10.0	1.0	na	na	100	2	Y	EQ						
					10	14.0	1.4	na	na	91	2								
					20	11.5	1.2	na	na	76	2								
					30	13.5	1.4	na	na	68	2								
					40	88.0**	8.8	na	na	51	2								
					–	–	–	–	–	–	–			–	–	–	–	–	–
					–	3 + 45	Ethanol	0	12.0	1.0	na			na	100	2	N	na	
								10	17.5	1.5	na			na	105	2			
								20	12.0	1.0	na			na	81	2			
								30	13.5	1.1	na			na	66	2			
		40	124.5**	10.4				na	na	18	2	T							
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		–	24 + 0	Ethanol	0	8.5	1.0	na	na	100	2	Y	POS						
					3.125	11.5	1.4	na	na	101	2								
					6.25	58.0**	6.8	na	na	85	2								
					12.5	278.5**	32.8	na	na	47	2								
					25	176.5**	20.8	na	na	44	2								
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		–	24 + 24	Ethanol	0	17.0	1.0	na	na	100	2	Y	POS						
					3.125	12.0	0.7	na	na	116	2								
					6.25	50.0**	2.9	na	na	46	2								
					12.5	131.0**	7.7	na	na	28	2			T					
					25	91.5**	5.4	na	na	27	2			T					
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		+	3 + 18	Ethanol	0	10.0	1.0	15.0	1.0	100	2	N	na						
					10	15.5	1.6	17.5	1.2	107	2								
					20	18.5*	1.9	19.5	1.3	97	2								
					30	13.5	1.4	23.5	1.6	76	2								
					40	8.5	0.9	63.0**	4.2	35	2								
		–	–	–	–	–	–	–	–	–	–	–	–	–					
		+	24 + 18	Ethanol	0	15.5	1.6	101.0**	6.7	86	2	Y	POS						
					0	7.0	1.0	16.0	1.0	100	2								

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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						
Lab 2	Assay 1	–	3 + 21	Ethanol	10	221.0**	31.6	132.0**	8.3	85	2				
					20	212.5**	30.4	128.0**	8.0	81	2				
					MMC 0.05	183.0**	26.1	456.0**	28.5	110	2				
					0	10.0	1.0	na	na	100	1		Y	NEG	
					2.5	10.0	1.0	na	na	85	1				
					5	15.0	1.5	na	na	95	1				
					10	16.0	1.6	na	na	60	1				
					20	15.0	1.5	na	na	45	1				
					MMC 0.1	47.0**	4.7	na	na	60	1				
		–	3 + 45	Ethanol	0	11.0	1.0	na	na	100	1			N	na
					2.5	16.0	1.5	na	na	103	1				
					5	11.0	1.0	na	na	99	1				
					10	16.0	1.5	na	na	96	1				
					20	12.0	1.1	na	na	67	1				
					MMC 0.1	26.0*	2.4	na	na	89	1				
					0	9.0	1.0	na	na	100	1			Y	POS
					2.5	27.0**	3.0	na	na	81	1				
					5	132.0**	14.7	na	na	86	1				
		–	24 + 24	Ethanol	10	352.0**	39.1	na	na	48	1				
					20	tox	tox	na	na	10	1				
					MMC 0.05	147.0**	16.3	na	na	71	1				
					0	15.0	1.0	na	na	100	1			N	na
					2.5	21.0	1.4	na	na	67	1				
					5	109.0**	7.3	na	na	26	1			T	
					10	130.0**	8.7	na	na	30	1			T	
					20	tox	tox	na	na	20	1			T	
					MMC 0.05	174.0**	11.6	na	na	74	1				
+	3 + 18	Ethanol	0	nt	nt	17.0	1.0	100	1				N	na	
			2.5	nt	nt	19.0	1.1	100	1						
			5	nt	nt	24.0	1.4	102	1						
			10	nt	nt	17.0	1.0	97	1						
			20	nt	nt	29.0	1.7	102	1						
			MMC 0.1	nt	nt	62.0**	3.7	102	1						
			0	13.0	1.0	15.0	1.0	100	1				Y	POS	
			2.5	17.0	1.3	27.0	1.8	86	1						
			5	133.0**	10.2	115.0**	7.7	71	1						
+	24 + 18	Ethanol	10	236.0**	18.2	70.0**	4.7	40	1						
			MMC 0.05	nt	nt	282.0**	18.8	40	1						
			0	11.0	1.0	na	na	100	2			N	na		
			5	17.0	1.5	na	na	102	2						
			10	14.0	1.3	na	na	91	2						
			20	11.0	1.0	na	na	73	2						
			30	15.0	1.4	na	na	78	2						
			MMC 0.1	51.0**	4.6	na	na	87	2						
			Lab 2	Assay 2	–	3 + 21	Ethanol	0	11.0	1.0	na	na	100	2	

Griseofulvin Lab 1	Assay 1	–	3 + 45	Ethanol	0	13.0	1.0	na	na	100	2	N	na					
					5	12.0	0.9	na	na	102	2							
					10	14.5	1.1	na	na	90	2							
					20	15.0	1.2	na	na	104	2							
					30	13.5	1.0	na	na	99	2							
					MMC 0.1	32.0**	2.5	na	na	112	2							
					–	24 + 0	Ethanol	0	15.0	1.0	na			na	100	2	Y	POS
								1.25	16.5	1.1	na			na	82	2		
								2.5	36.0**	2.4	na			na	105	2		
								5	109.0**	7.3	na			na	56	2		
								10	337.0**	22.5	na			na	23	2		
								MMC 0.05	103.5**	6.9	na			na	84	2		
		–	24 + 24	Ethanol	0	19.5	1.0	na	na	100	2	N	na					
					1.25	14.5	0.7	na	na	100	2							
					2.5	22.5	1.2	na	na	70	2							
					5	63.0**	3.2	na	na	30	2							
					10	146.5**	7.5	na	na	13	2							
					MMC 0.05	192.0**	9.8	na	na	83	2							
		+	3 + 18	Ethanol	0	nt	nt	20.5	1.0	100	2	Y	EQ					
					2.5	nt	nt	28.0	1.4	100	2							
					5	nt	nt	27.0	1.3	100	2							
					10	nt	nt	30.5	1.5	100	2							
					20	nt	nt	28.0	1.4	95	2							
					30	nt	nt	35.0**	1.7	81	2							
					MMC 0.1	nt	nt	81.5**	4.0	95	2							
					+	24 + 18	Ethanol	0	12.5	1.0	15.5			1.0	100	2	Y	POS
								10	323.0**	25.8	150.0**			9.7	72	2		
								20	282.0**	22.6	130.0**			8.4	59	2		
30	15.0							1.2	48.0**	3.1	32			2				
MMC 0.05	nt							nt	245.0**	15.8	102			2				
Griseofulvin Lab 1	Assay 1	–	3 + 21	DMSO	0	12.0	1.0	na	na	100	2	Y	POS					
					1.4	17.0	1.4	na	na	88	2							
					4.1	14.0	1.2	na	na	154	2							
					12.3	16.5	1.4	na	na	142	2							
					37	17.5	1.5	na	na	146	2							
					111	36.0**	3.0	na	na	150	2							
					333	116.5**	9.8	na	na	108	2							
					1000	223.5**	18.7	na	na	142	2							
					MMC 0.1	68.5**	5.8	na	na	169	2							
					–	3 + 45	DMSO	0	12.0	1.0	na			na	100	2	Y	POS
								1.4	11.0	0.9	na			na	88	2		
								4.1	11.5	1.0	na			na	154	2		
		12.3	11.0	0.9				na	na	142	2							
		37	10.0	0.8				na	na	146	2							
		111	26.5**	2.3				na	na	150	2							
		333	276.0**	23.0				na	na	108	2							
		1000	284.9**	23.8				na	na	142	1							
		MMC 0.1	32.0**	2.7				na	na	169	2							

Appendix A (Continued)

Lab number	Assay number	Cyt B ^a	Schedule: treatment + recovery (h)	Solvent	Concentration (µg/ml) ^b	Micronucleated mononucleate cells ^c		Micronucleated binucleate 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								
Lab 1	Assay 2	–	24 + 0	DMSO	0	11.5	1.0	na	na	100	2		Y	POS			
					1.4	12.0	1.0	na	na	65	2						
					4.1	18.0	1.6	na	na	77	2						
					12.3	146.4**	12.7	na	na	64	2						
					37	407.0**	35.4	na	na	42	2						
					111	227.5**	19.8	na	na	28	2	p, T					
					333	43.5**	3.8	na	na	14	2	p, T					
					1000	338.0**	29.4	na	na	40	2	p					
					MMC 0.05	91.5**	8.0	na	na	92	2						
			–	24 + 24	DMSO	0	11.0	1.0	na	na	100	2		Y	POS		
						1.4	14.5	1.4	na	na	65	2					
						4.1	19.0	1.7	na	na	77	2					
						12.3	105.2**	9.6	na	na	64	2					
						37	205.0**	18.6	na	na	42	2					
						111	171.0**	15.5	na	na	28	2	T				
						333	141.7**	12.9	na	na	14	2	p, T				
						1000	292.4**	26.6	na	na	40	2	p				
						MMC 0.05	168.0**	15.3	na	na	92	2					
		+	3 + 18	DMSO	0	nt	nt	25.0	1.0	100	2		N	na			
					1.4	nt	nt	15.0	0.6	106	2						
					4.1	nt	nt	14.5	0.6	103	2						
					12.3	nt	nt	23.5	1.0	113	2						
					37	nt	nt	17.5	0.7	105	2						
					111	nt	nt	14.0	0.6	99	2						
					333	nt	nt	tox	tox	27	2	T					
					MMC 0.1	nt	nt	76.0**	3.0	101	2						
					0	nt	nt	26.5	1.0	100	2		Y	POS			
		+	24 + 18	DMSO	1.4	nt	nt	22.5	0.9	112	2						
					4.1	nt	nt	46.0**	1.7	118	2						
					12.3	nt	nt	157.9**	6.0	176	2						
					37	nt	nt	324.7**	12.3	161	2						
					111	nt	nt	286.0**	10.8	137	2						
					1000	nt	nt	275.2**	10.4	179	2	p					
					MMC 0.05	nt	nt	292.5**	11.0	190	2						
					–	3 + 21	DMSO	0	13.7	1.0	na	na	100	2		Y	POS
								25	11.5	0.8	na	na	141	2			
		50	12.5	0.9				na	na	155	2						
		100	12.0	0.9				na	na	109	2						
		200	81.0**	5.9				na	na	78	2						
		400	376.5**	27.5				na	na	56	2	p					
		MMC 0.1	40.0**	2.9				na	na	159	2						
		0	11.0	1.0				na	na	100	2		Y	POS			
		25	13.0	1.2				na	na	85	2						
		–	3 + 45	DMSO		50	12.5	1.2	na	na	73	2					
						100	32.5**	3.0	na	na	55	2					
						200	329.2**	29.9	na	na	33	2	T				
						400	487.6**	44.3	na	na	27	2	p, T				
						MMC 0.1	26.5**	2.4	na	na	97	2					

–	24+0	DMSO	0	12.5	1.0	na	na	100	2	Y	POS
			3.1	19.5	1.5	na	na	98	2		
			6.3	75.0**	6.0	na	na	82	2		
			12.5	174.0**	13.9	na	na	81	2		
			25	435.5**	34.8	na	na	53	2		
			50	235.0**	18.8	na	na	63	2		
–	24+24	DMSO	MMC 0.05	49.0**	3.9	na	na	104	2	Y	POS
			0	13.5	1.0	na	na	100	2		
			25	187.5**	13.9	na	na	54	2		
			50	145.0**	10.7	na	na	31	2		
			100	200.3**	14.8	na	na	17	2		
			200	294.3**	21.8	na	na	15	2		
+	3+18	DMSO	400	320.9**	23.8	na	na	23	2	Y	POS
			MMC 0.05	224.0**	16.6	na	na	110	2		
			0	16.0	1.0	12.5	1.0	100	2		
			25	44.0**	2.8	12.0	0.9	108	2		
			50	11.1	0.7	10.5	0.8	108	2		
			100	23.0	1.4	12.0	0.9	99	2		
+	24+18	DMSO	200	45.6**	2.8	60.5**	4.8	77	2	Y	POS
			400	64.0**	4.0	53.4**	4.3	6	2		
			MMC 0.1	13.5	0.9	38.0**	3.0	102	2		
			0	7.0	1.0	13.0	1.0	100	2		
			25	414.0**	59.1	238.1**	18.3	120	2		
			50	227.0**	32.4	120.7**	9.3	33	2		
+	24+18	DMSO	100	220.0**	31.4	271.0**	20.8	81	2	Y	POS
			200	71.8**	10.3	89.5**	6.9	64	2		
			400	257.9**	36.8	366.2**	28.2	59	2		
			MMC 0.05	112.5**	16.1	217.5**	16.7	118	2		
			25	414.0**	59.1	238.1**	18.3	120	2		
			50	227.0**	32.4	120.7**	9.3	33	2		

–: lower than the control—statistical analysis not done. nt: not tested; nd: not determined; na: not applicable; tox: not evaluated due to cytotoxicity.

^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 in the text for the formula). NS: not statistically higher than controls (Yates χ^2 -test).

^d p: precipitate; low cells: low number of cells; mm: 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.

^e Y: assay accepted; N: assay not accepted (see in the 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.

* Statistically higher than controls at $p < 0.05$.

** Statistically higher than controls at $p < 0.01$.

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