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# SFTG international collaborative study on in vitro micronucleus test V. Using L5178Y cells

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**Abstract**

In this report, results are presented from an international study of the in vitro micronucleus assay using mouse lymphoma L5178Y 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, 5-fluorouracil, colchicine and griseofulvin. 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 mouse lymphoma L5178Y cells. Mannitol was the only exception, being tested in only one laboratory. Mannitol was negative, while bleomycin induced a concentration-dependent increase in micronucleated cells. Equivocal results were obtained for 5-fluorouracil, colchicine and griseofulvin. High levels of cytotoxicity interfered with the assessment of aneuploidy for colchicine and griseofulvin, preventing the ability to obtain clear results in all the treatment schedules. Experiments with 5-fluorouracil, colchicine and griseofulvin showed that both short and long treatment times are required as each compound was detected using one or more treatment protocol. No clear differences were seen in the sensitivity or accuracy of the responses in the presence of absence of cytochalasin B. It was also found that a recovery period may help to detect compounds which induce a genotoxicity associated to a reduction in cell number or cell proliferation. Overall, the results of the present study show that mouse lymphoma L5178Y cells are suitable for the in vitro micronucleus assay.


*Keywords:* In vitro micronucleus assay; Mouse lymphoma cells; L5178Y cells; Cytochalasin B; Clastogen; Aneugen

## 1. Introduction

The mouse lymphoma cell line L5178Y is widely used for regulatory genotoxicity testing with the mouse

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lymphoma tk assay [1]. Although the tk assay demonstrated some ability to detect aneugens [2], their identification is expected to be improved with the in vitro micronucleus assay. Moreover, the use of this cell line in both genotoxicity systems allows comparisons and could be useful for a better understanding of mechanisms of action. Finally, as these cells grow in suspension, a miniaturisation of the technique has been developed [3,4]. The use of L5178Y cells allows also parallel studies of mutagenic, clastogenic and aneugenic activity on the same cell line [5,6]. Indeed, various complex clastogens were detected in combined mouse lymphoma tk and micronucleus assays on L5178Y cells [7–12]. For all these reasons, the L5178Y mouse lymphoma cell line is increasingly used for screening purposes. In this collaborative study conducted by the SFTG (the French branch of the European Environmental Mutagen Society), the performances of the in vitro micronucleus assay using L5178Y were evaluated with presumed negative and positive compounds. The results described in this paper were also compared to the results obtained with CHO, CHL cells and primary cultures of human lymphocytes in the general publication in this issue [13].

## 2. Materials and methods

The general conditions of the study are detailed in a previous paper in this issue [13].

### 2.1. Cells

The L5178Y TK+/− cell line (sub-clone 3.7.2-C) used in this study was established and described by Clive et al. [1]. It was originally provided by the American Type Culture Collection (ATCC, MD, USA).

### 2.2. Culture media

RPMI1640 Dutch modified supplemented with penicillin–streptomycin (50 UI/mL–50 μg/mL), glutamine (2 or 3 mM) or Glutamax I and 10% (v/v) heat-inactivated horse 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 for details). They were: mannitol (CAS no. 69-65-8), bleomycin (CAS no. 9041-93-4), 5-fluorouracil (CAS no. 51-21-8), colchicine (CAS no. 64-86-8), and griseofulvin (CAS no. 126-07-8). Mitomycin C (CAS no. 50-07-7) was used as the positive control.

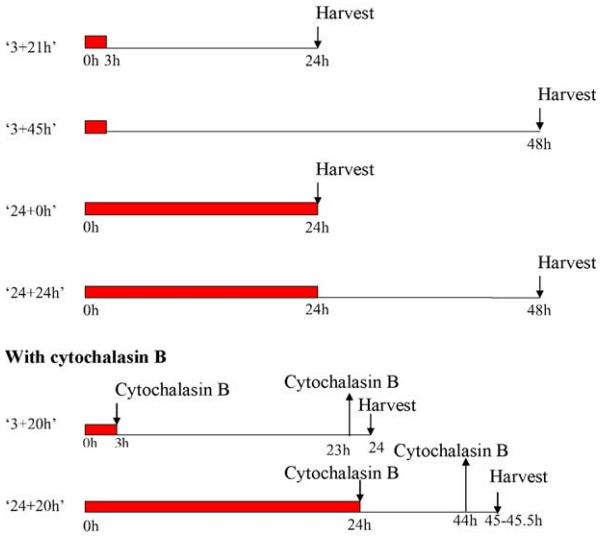


Fig. 1. Treatment and recovery schedules: (■) treatment with a chemical; (—) recovery in the fresh medium.

### 2.4. Culture conditions

Cells were incubated at 37 °C with 5% $CO_2$.

Duplicate cultures were used in at least one assay. When using cytochalasin B, if cell counts were used as a complementary evaluation to ratio of multinucleated cells, a separate third culture was used.

The day before treatment, the cells were seeded at $2 \times 10^5$ cells/mL. Under these conditions, cells were actively growing at the start of treatment.

### 2.5. Treatment and recovery times

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

Prior to treatment, the growth medium was replaced by fresh medium and the cells were incubated with the compound under agitation. At the end of the treatment period, cells were rinsed with buffer or culture medium.

When using cytochalasin B, after the 3-h period ('3 + 20 h' schedule) or the 24-h treatment period ('24 + 20 h' schedule), cells were incubated for 20 h with cytochalasin B (3 or 6 μg/mL). Cells were allowed to recover following incubation with cytochalasin B for an additional 1.5 h before harvest (24.5 h from the beginning of the treatment) to improve the cellular morphology and micronucleus scoring.

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

Mitomycin C was used as a positive control at 0.0625 μg/mL for all the 24-h treatments and at 0.125 μg/mL for the 3-h treatments followed by a 20–21-h recovery period