Environmental and Molecular Mutagenesis 41:99–103 (2003)

Etoposide and Merbarone Are Clastogenic and Aneugenic in the Mouse Bone Marrow Micronucleus Test Complemented by Fluorescence In Situ Hybridization With the Mouse *minor* Satellite DNA Probe

S.M. Attia,[1,2] U. Kliesch,[1] G. Schriever-Schwemmer,[3] O.A. Badary,[2] F.M. Hamada,[2] and I.-D. Adler[1]*

[1] *Institute of Experimental Genetics, GSF-National Research Center for Environment and Health, Neuherberg, Germany*
[2] *Pharmacology and Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt*
[3] *Institute of Toxicology and Environmental Hygiene, Technical University Munich, Freising-Weihenstephan, Germany*

The topoisomerase II (topo II) inhibitors etoposide (VP-16) and merbarone (MER) were investigated with the in vivo micronucleus test (MN test) combined with fluorescence in situ hybridization (FISH) using the mouse *minor* satellite DNA probe to discriminate MN of clastogenic and aneugenic origin. All experiments were performed with male (102/ElxC3H/El) F1 mice bred in the mouse colony of the GSF Research Center. The sample size per experimental group was five animals and 2,000 polychromatic erythrocytes (PCE) were scored per animal from coded slides in the conventional MN test. A separate set of coded slides was used for the FISH analysis. All treatments consisted of single intraperitoneal injections. Colchicine (COL, 3 mg/kg) and mitomycin (MMC, 1 mg/kg) were used as a positive control aneugen and clastogen, respectively, and these compounds produced the expected responses. A dose of 1 mg/kg VP-16 induced 3.44% MNPCE (compared to the concurrent solvent control of 0.37%, $P < 0.001$) and of these 39.9% (1.4% MNPCE) showed one or more fluorescent signals. MER (7.5–60 mg/kg) increased the MNPCE frequencies in a dose-dependent manner, with 15 mg/kg being the lowest positive dose. At the highest dose of 60 mg/kg of MER, a total of 4.26% MNPCE were found (compared to 0.31% in the concurrent solvent control, $P < 0.001$) and of these 46.2% (2.0% MNPCE) contained one or more fluorescent signals. The data demonstrate that VP-16 and MER induced both clastogenic and aneugenic events despite their different modes of topo II inhibition. Environ. Mol. Mutagen. 41:99–103, 2003. © 2003 Wiley-Liss, Inc.

Key words: topoisomerase II; etoposide; merbarone; micronuclei; clastogens; aneugens

INTRODUCTION

Topoisomerase II (topo II) has many functions in cellular processes; most important, it relieves torsional stress occurring in DNA during transcription, replication, and cell division by causing transient double strand breaks [Baguley and Ferguson, 1998]. Topo II is the target for very successful anticancer drugs. Some topo II inhibitors, such as etoposide (VP-16), termed topo II poisons, stabilize covalent enzyme-cleaved DNA complexes that are normally short-lived intermediates in the catalytic cycle of topo II [Fortune and Osheroff, 2000]. These drugs generate high levels of enzyme-mediated breaks in treated cells. Other topo II-targeted drugs, such as merbarone (MER), are catalytic inhibitors of enzyme function and block topo II-mediated DNA cleavage [Fortune and Osheroff, 1998]. Fortune and Osheroff indicate that MER shares an interaction domain on topo II with cleavage-enhancing agents such as VP-16.

Many topo II inhibitors are potent mutagens and lead to major chromosomal deletions, errors in recombination, and aneuploidy [Ferguson, 1998]. The micronucleus (MN) test is able to assess both the clastogenic and aneugenic properties of a test compound. The origin of MN is determined either by immunofluorescence labeling of centromeric proteins using antikinetochore antibodies (CREST-staining) or

Grant sponsors: the Egyptian Channel Program (to SMA), the EU; Grant number: QLK4-CT-2000-00058.

*Correspondence to: I.-D. Adler, Institute of Experimental Genetics, GSF-National Research Center for Environment and Health, Ingolstaedter Landstrasse, D-85764 Neuherberg, Germany. E-mail: adler@gsf.de

Received 11 June 2002; provisionally accepted 28 August 2002; and in final form 16 November 2002

DOI 10.1002/em.10135

by fluorescence in situ hybridization (FISH) with pan-centromeric DNA probes such as the murine-γ-satellite DNA probe [*major* probe; Vissel and Choo, 1989] or the mouse *minor* satellite DNA probe [*minor* probe; Wong and Rattner, 1988]. The satellite DNA probes hybridize to the centromeric areas of all chromosomes except the Y-chromosome. The *minor* probe is recommended for FISH detection of MN containing lagging chromosomes, as opposed to the *major* probe because it is short, hybridizes closely to the centromere, and avoids false-positives due to breaks within the centromeric heterochromatin [Chen et al., 1994; Hayashi et al., 2000; Schriever-Schwemmer and Adler, 1994].

Recently, the origin of VP-16- and MER-induced MN in TK6 cells was analyzed by CREST-staining [Wang and Eastmond, 2002]. The majority of MN were of clastogenic origin (CREST-positive to CREST-negative MN ratio of ~1:5). When this analysis was conducted on bone marrow erythrocytes of mice treated with MER, the frequencies of CREST-negative MN were increased dose-dependently, indicating only a clastogenic effect [Wang and Eastmond, 2002]. In rodent spermatocytes, VP-16 and MER (80 mg/kg) induced meiotic MN in vitro and in vivo which were analyzed in the subsequent stage of early spermatids [Kallio and Laehdetie, 1996, 1997; Laehdetie et al., 1994; Sjoblom et al., 1994]. Further analyses of meiotic MN induced in vivo by VP-16 (10 and 20 mg/kg) with CREST-staining and with FISH using the *minor* probe revealed that 58.8–74.4% were signal-positive, depending on the treated stage of meiosis [Kallio and Laedetie, 1996, 1997]. Furthermore, VP-16 induced minute signal-positive MN (7.0–12.8%). Cytogenetic analyses of different stages of the first and second meiotic divisions indicated that these minute MN were due to fragmentation of centromeric DNA [Kallio and Laehdetie, 1996]. The authors suggested that VP-16 caused clastogenic and aneugenic events in male mouse meiosis in vivo in at least two ways. First, inhibition of topo II-mediated strand religation in the decatenation process of centromeric DNA during meiosis I results in DNA fragmentation. Second, lagging of bivalents (two homologous chromosomes joined by chiasmata) during anaphase of meiosis I involves VP-16-mediated inhibition of the resolution of chiasmata which are formed between homologous chromosomes by crossing-over events. In contrast to VP-16, MER (80 mg/kg) did not induce minute MN and most of the large, medium, and small MN contained one or more CREST-signals (78.3–85.5%), indicating loss of bivalents or chromosomes during the preceding meiotic divisions. In parallel cytogenetic studies of meiotic chromosomes, the authors found increased frequencies of meiotic univalents (in meiosis I) and hypoploidies (in meiosis II) and no significant induction of structural chromosome damage [Kallio and Laehdetie, 1997]. It was concluded that MER impaired homologous chromosome separation and/or sister-chromatid separation by inhibiting the decatenation activity of topo II during both meiotic divisions. The aneugenic activity of VP-16 and MER during male meiosis in vivo was recently confirmed with the sperm FISH assay [Attia et al., 2002].

These previous reports indicate that VP-16 is clastogenic and aneugenic during mitotic as well as meiotic divisions. In contrast, in vivo exposure to MER caused only clastogenic events during mitosis and predominantly aneugenic events during meiosis. Thus, it seems that MER-induced aneuploidy by the inhibition of decatenation is restricted to meiotic division processes which involve resolution of chiasmata between homologous chromosomes (meiosis I) as well as separation of sister chromatids (meiosis II). The data of the present experiments demonstrate that this conclusion is not correct.

MATERIALS AND METHODS

Animals and Chemical Treatment

All experiments were performed with male (102/ElxC3H/El) F1 mice age 10–14 weeks weighing 25–29 g. Animals bred in the mouse colony of the GSF-Research Center were maintained on a 12-hr light/dark cycle with mouse pellet food and water ad libitum. Each treatment group and vehicle control group consisted of five randomly assigned animals.

All chemical solutions were prepared 1 hr before injection. MMC and COL (both purchased from Sigma, Deisenhofen, Germany) were dissolved in double-distilled water. Stock solutions of VP-16 (Sigma) and MER (supplied by the Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD, USA) were made with 100% DMSO, at concentrations of 10 and 40 mg/ml, respectively, and were frozen at –20°C for later use (one to several weeks). The stock solutions were thawed and diluted with double-distilled water to the required concentrations immediately prior to use. All animals were injected by a single i.p. injection. Injected volumes were 0.1 ml per 10 g of body weight.

The doses for the positive controls were chosen by reference to earlier studies in our laboratory [COL: Schmid et al., 1999; MMC: Kliesch et al., 1981]. The dose of 1 mg/kg VP-16 was selected based on the data of Ashby et al. [1994]. The highest dose of MER was selected based on previous studies in mouse sperm [Attia et al., 2002]. In the first experiment, mice were treated with 1 mg/kg VP-16 or 3 mg/kg of the aneugen COL. Concurrent vehicle-control mice received equal amounts of 1% DMSO in double-distilled water (control I). In the second experiment, mice were treated with 60, 30, 15, or 7.5 mg/kg of MER, or 1 mg/kg of the clastogen MMC. Concurrent vehicle-control mice received equal amounts of 15% DMSO in double-distilled water (control II).

Conventional Micronucleus Test

The animals were sacrificed 24 hr after treatment and both femurs were removed. The bone marrow slides were prepared according to a standard technique [Adler, 1984]. At least four slides were made for each animal and allowed to dry overnight. The slides were coded by an investigator who was not involved in the scoring. Two slides per animal were stained with May-Gruenwald/Giemsa solutions for conventional assessment of the MN frequencies. For each of five animals per dose group and concurrent solvent controls, 1,000 polychromatic erythrocytes (PCE) were scored per slide (2,000 per animal, 10,000 PCE per group). Numbers of PCE were counted in microscopic fields which contained 2,000 NCE and expressed as PCE/(PCE+NCE) in percent as a measure of erythroblast proliferation. The remaining unstained slides from the animals treated with 1 mg/kg VP-16, 60 mg/kg MER, 3 mg/kg COL, and 1 mg/kg MMC as well as from the vehicle-controls were stored at –20°C for 1 day to several weeks before the FISH analysis.

TABLE I. Frequencies of Micronucleated Polychromatic Erythrocytes (MNPCE) and Polychromatic Erythrocytes (PCE) in Bone Marrow of Mice 24 hr After Treatment With Etoposide (VP-16), Merbarone (MER), Colchicine (COL), and Mitomycin C (MMC)

Chemical	Dose (mg/kg)	Individual animal data (MNPCE/2000 PCE)	% MNPCE (mean ± SE)	% PCE[a]
First Experiment				
Control I (1% DMSO in water)	0	9, 8, 7, 8, 5	0.37 ± 0.03	48.12 ± 0.38
VP-16	1	75, 62, 62, 66, 79	3.44 ± 0.14**	45.10 ± 0.33**
COL	3	18, 23, 25, 30, 18	1.14 ± 0.08**	47.06 ± 0.46
Second experiment				
Control II (15% DMSO in water)	0	5, 6, 5, 9, 6	0.31 ± 0.03	49.27 ± 0.25
MER	7.5	6, 7, 4, 5, 5	0.27 ± 0.02	47.29 ± 0.29*
MER	15	6, 16, 8, 8, 12	0.50 ± 0.06*	47.13 ± 0.27*
MER	30	21, 17, 26, 24, 13	1.01 ± 0.09**	46.60 ± 0.29*
MER	60	97, 86, 87, 71	4.26 ± 0.26**	45.19 ± 0.33**
MMC	1	43, 33, 44, 42, 40	2.02 ± 0.09**	47.24 ± 0.33*

[a]Numbers of PCE counted in microscopic fields which contained 2000 NCE, expressed as PCE/(PCE + NCE) in percent.
*$P \leq 0.05$; **$P < 0.01$, compared with the concurrent vehicle control (Mann-Whitney test).

FISH Analysis of MN

The *minor* probe p^{MKB6} was used according to a published protocol [Schriever-Schwemmer and Adler, 1994] with the following alterations. The *minor* probe was labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) using the nick translation system according to the manufacturer's instructions (GIBCO Invitrogen, Carlsbad, CA, USA). The rehydration time in 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0) was increased from 5 to 10 min and the denaturing temperature in 70% formamide was increased from 74°C to 78°C. The hybridization mix contained 20 parts of master mix 1.0 (50% formamide, 10% dextran sulfate, 2× SSC), three parts of the biotinylated *minor* probe (0.15 μg per slide), three parts of herring sperm DNA (30 μg per slide), and four parts of distilled water. The biotin-labeled *minor* probe was detected with 40 μl per slide of streptavidin-CY3 (5 μg/ml; Dianova, Hamburg, Germany) in PNBR buffer [PN buffer (0.1M sodium phosphate, 0.1% Nonidet P40, pH 8.0) plus 5% blocking reagent (Boehringer Mannheim) plus 0.02% sodium azide] for 30 min at room temperature in a moist chamber. The cells were counterstained with 40 μl per slide of 4,6-diamidino-2-phenylindole (DAPI) (0.5 μg/ml in PBS; Roche Diagnostics, Mannheim, Germany) for 10 min at room temperature and coverslipped in Vectashield mounting medium (Sigma). The slides were stored at 4°C in the dark before scoring. A fluorescence microscope with a dual bandpass filter was used for analysis of fluorescent signals in MN; one filter had a wavelength of 365–450 nm for observation of DAPI fluorescence and the second had a wavelength of 546–590 nm for observation of CY3-streptavidin fluorescence.

The slides from animals treated with COL and MC as well as from two animals of each concurrent vehicle-control group were used to verify the scorer's (SMA) ability to efficiently and correctly detect MN with fluorescent signals (data not shown). Once that was established, 100–200 MN were scored from a group of coded slides representing five animals treated with 1 mg/kg of VP-16, three animals treated with 60 mg/kg of MER and five animals from control I (two animals) and control II (three animals).

Statistical Analysis

Significant differences of mean frequencies of MNPCE between individual treatment groups and corresponding solvent controls or between the two solvent control groups were calculated on an animal-to-animal basis by the nonparametric Mann-Whitney *U*-test [Sachs, 1984]. To compare the frequencies of signal-positive MN between the two treatment groups (1 mg/kg of VP-16 and 60 mg/kg of MER), the χ^2 test was used [Sachs, 1984]. Results were considered significantly different if $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of the conventional MN tests are presented in Table I. The frequencies of micronucleated PCE (MNPCE) in vehicle control I of 0.37% and in vehicle control II of 0.31% were not significantly different, so an average of 0.34% was calculated. This value is at the upper limit of the range regarded as acceptable for negative controls [3.4/1,000; Salamone and Marvournin, 1994]. In the first experiment, COL showed the expected positive effect [Schriever-Schwemmer and Adler, 1994]. Similarly, VP-16 at a dose of 1 mg/kg significantly increased the frequency of MNPCE, as expected [Ashby et al., 1994]. VP-16 significantly decreased the percent PCE, indicating a reduction in erythroblast proliferation most likely by mitotic arrest. In the second experiment, MMC showed the expected significant increase in the frequency of MNPCE [Kliesch et al., 1981]. MER increased the MN frequencies dose-dependently, as described by Wang and Eastmond [2002]. However, the lowest positive dose in the present study was determined to be 15 mg/kg ($P < 0.05$, Mann-Whitney *U*-test) as opposed to 40 mg/kg in the study by Wang and Eastmond [2002]. The statistical treatment of the data may be responsible for the difference. Unfortunately, the data by Wang and Eastmond [2002] are only presented as graphs, so that a comparative statistical analysis is not possible. The highest dose of MER (60 mg/kg) significantly decreased the percent PCE, indicating an inhibition of erythroblast proliferation most likely by mitotic arrest.

The results of the FISH analysis of MN are shown in Table II and Figure 1. In the solvent-control group, the frequency of 43.1% signal-positive MN and the distribution of signals per MN were consistent with previous experi-

TABLE II. Results of FISH Analyses With the Mouse *minor* Satellite DNA Probe on VP-16 or MER-Induced Micronucleated Polychromatic Erythrocytes (MNPCE)

		No. of signal-positive MNPCE		Distribution of FISH-signals per MN		
Chemical	No. of MN scored	Total	%	1 signal	2 signals	≥3 signals
Control*	102	44	43.1	24 (54.6%)	17 (38.6%)	3 (6.8%)
VP-16	208	83	39.9	49 (59.0%)	26 (31.3%)	8 (9.6%)
MER	199	92	46.2	41 (44.6%)	41 (44.6%)	10 (10.8%)

*The control consisted of two animals from the first experiment and three animals from the second experiment injected with 1% and 15% DMSO in double-distilled water, respectively. VP-16 = etoposide (1 mg/kg), MER = merbarone (60 mg/kg).

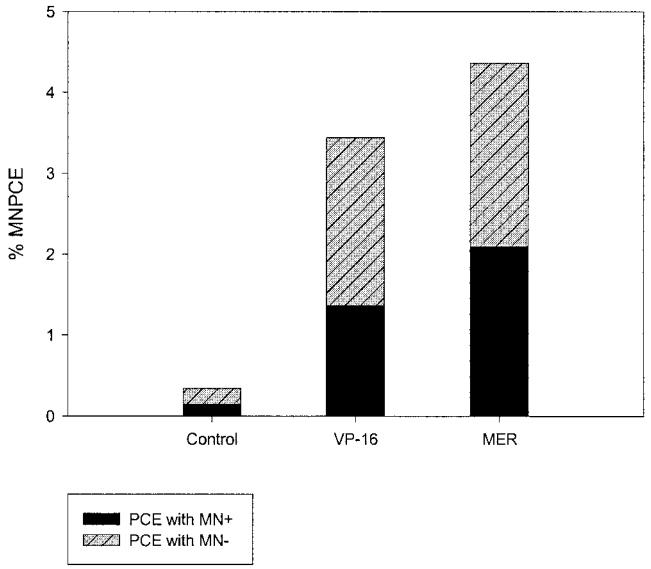

Fig. 1. Percentage of polychromatic erythrocytes (PCE) with signal-positive MN (solid boxes) and signal-negative MN (slashed boxes) in mouse bone marrow after treatment with etoposide (VP-16, 1 mg/kg) or merbarone (MER, 60 mg/kg). Data combined from Tables I and II. The control consisted of two animals from the first experiment and three animals from the second experiment injected with 1% and 15% DMSO in double-distilled water, respectively.

ments [Schriever-Schwemmer and Adler, 1994]. In the VP-16 group, a total of 208 MN were analyzed by FISH and 83 MN (39.9%) were signal-positive. This result is similar to findings obtained by CREST-staining of VP-16-induced MN in Chinese hamster cells [Hermine et al., 1997] and contrasts with data from neonatal human lymphocytes [Slavotinek et al., 1993] and the human lymphoblastoid cell line TK6 [Wang and Eastmond, 2002]. The differences between the three in vitro results cannot be attributed to different drug concentrations in the cell cultures since Slavotinek et al. and Wang and Eastmond both used 0.2 μM as their lowest concentration and Slavotinek et al. and Hermine et al. both used concentrations above 1 μM. Also, the repair capacities of the various cell types used cannot explain the discrepancies. Hermine et al. [1997] found no significant differences in the responses to VP-16 between repair-competent V79 cells and repair-deficient V79 cell mutants. Thus, other technical features of the test procedures may have produced the low frequencies of CREST-positive MN in two of the studies.

Of the VP-16-induced signal-positive MN in the present study (Table II), 59.0% had one signal, 31.3% contained two signals, and 9.6% had ≥3 signals. This distribution indicates that more than half of the signal-positive MN were formed by a single lagging chromosome and the rest of the signal-positive MN contained several lagging chromosomes or nondisjoined chromatids. These possibilities cannot be discriminated because the *minor* probe often shows a hybridization signal on each of the two chromatids in mitotic metaphases [Schriever-Schwemmer and Adler, 1993]. Since we did not differentiate MN by size, we cannot exclude the possibility that some of the MN with multiple signals contained fragmented centromeric DNA, as observed by Kallio and Laehdetie [1996] for meiotic MN. However, from all the published evidence discussed in the Introduction and here, there is no doubt that VP-16 has clastogenic as well as aneugenic activity.

A total of 199 MN from the 60 mg/kg MER-treatment group were analyzed by FISH and 92 MN (46.2%) were signal-positive. In contrast, Wang and Eastmond [2002] found no significant increase in the frequency of CREST-positive MN in the bone marrow of mice treated with the same dose of MER. Comparing the two centromere-labeling procedures used in these studies [Schriever-Schwemmer and Adler, 1994; Chen et al., 1994], it is unlikely that the difference is due to the modes of centromere detection, i.e., CREST vs. FISH with the *minor* probe. Additionally, it is somewhat inexplicable that CREST-negative MN frequencies in mouse spermatid MN were as low as 14.7–21.7% after MER-exposure of male mice, indicating very low incidences of clastogenicity during meiosis [Kallio and Laehdetie, 1997].

Of the MER-induced signal-positive MN in the present study, 44.6% had one signal and 44.6% contained two signals, while 10.9% had ≥3 signals (Table II). The frequencies of MN with 1 and >1 signal observed for MER-treatment are not significantly different from the frequencies obtained with VP-16-treatment ($\chi^2 = 3.66$ (one degree of freedom), $P > 0.05$). Therefore, we conclude that both topo

II inhibitors caused lagging chromosomes that formed signal-positive MN. Similarly, VP-16 and MER induced 60.1% and 53.8% signal-negative MN, respectively, indicating that they were formed by acentric fragments and represented clastogenic activity.

In conclusion, by using the *minor* probe for FISH analysis of mouse erythrocyte MN it was shown that VP-16 and MER are aneugens as well as clastogens in somatic cells in vivo despite their different modes of topo II inhibition. While the mechanism for the clastogenicity of VP-16 is understood as an accumulation of enzyme-cleaved DNA complexes, the clastogenicity of MER may be a secondary effect of the inhibition of decatenation. Finally, the present data contradict the impression that the aneugenic activity of MER is restricted to meiotic cell divisions I and II.

ACKNOWLEDGMENTS

The authors thank Dr. Robert J. Schultz (National Cancer Institute, Bethesda, MD) for providing the Merbarone sample, and Helga Gonda and Isa Sybille Otten for technical assistance.

REFERENCES

Adler I-D. 1984. Cytogenetic tests in mammals. In: Venitt S, Parry JM, editors. Mutagenicity testing: a practical approach. Oxford: IRI. p: 275–306.

Ashby J, Tinwell H, Glover P, Poorman-Allen P, Krehl R, Callander RC, Clive D. 1994. Potent clastogenicity of the human carcinogen etoposide to the mouse bone marrow and mouse lymphoma L5178Y cells: comparison to Salmonella responses. Environ Mol Mutagen 24:51–60.

Attia SM, Schmid TE, Badary OA, Hamada FM, Adler I-D. 2002. Molecular cytogenetic analysis in mouse sperm of chemically induced aneuploidy: studies with topoisomerase II inhibitors. Mutat Res 520:1–13.

Baguley BC, Ferguson LR. 1998. Mutagenic properties of topoisomerase-targeted drugs. Biochim Biophys Acta 1400:213–222.

Chen HW, Tomar R, Eastmond DA. 1994. Detection of hydroquinone-induced non-random breakage in the centromeric heterochromatin of mouse bone marrow cells using multicolour fluorescence in situ hybridisation with the major and minor satellite probes. Mutagenesis 9:563–569.

Ferguson LR. 1998. Inhibitors of topoisomerase enzymes: a unique group of environmental mutagens and carcinogens. Mutat Res 400:271–278.

Fortune JM, Osheroff N. 1998. Merbarone inhibits the catalytic activity of human topoisomerase IIalpha by blocking DANN cleavage. J Biol Chem 10:17643–17650.

Fortune JM, Osheroff N. 2000. Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice. Prog Nucleic Acid Res Mol Biol 64:221–253.

Hayashi M, MacGregor JT, Gatehouse DG, Adler ID, Blakey DH, Dertinger D, Krishna G, Morita T, Russo A, Sutou S. 2000. In vivo rodent erythrocytes micronucleus assay. II. Some aspects of protocol design including repeated treatments, integration with toxicity testing and automated scoring. Environ Mol Mutagen 35:234–252.

Hermine T, Jones NJ, Parry JM. 1997. Comparative induction of micronuclei in repair-deficient and -proficient Chinese hamster cell lines following clastogen or aneugen exposures. Mutat Res 392:151–163.

Kallio M, Laehdetie J. 1996. Fragmentation of centromeric DNA and prevention of homologous chromosome separation in male mouse meiosis in vivo by the topoisomerase II inhibitor etoposide. Mutagenesis 11:435–443.

Kallio M, Laehdetie J. 1997. Effects of the DNA topoisomerase II inhibitor merbarone in male mouse meiotic divisions in vivo: cell cycle arrest and induction of aneuploidy. Environ Mol Mutagen 29:16–27.

Kliesch U, Danford N, Adler I-D. 1981. Micronucleus test and bone marrow chromosome analysis. A comparison of two methods in vivo for evaluating chemically induced chromosome alterations. Mutat Res 80:321–332.

Laehdetie J, Keiski A, Suutari A, Toppari J. 1994. Etoposide (VP-16) is a potent inducer of micronuclei in male rat meiosis: spermatid micronucleus test and DNA flow-cytometry after etoposide treatment. Environ Mol Mutagen 24:192–202.

Sachs L. 1984. Angewandte Statistik, 6. Auflage. Berlin: Springer.

Salamone MF, Mavournin KH. 1994. Bone marrow micronucleus assay: a review of the mouse stocks used and their published mean spontaneous micronucleus frequencies. Environ Mol Mutagen 23:239–273.

Schmid TE, Wang X, Adler I-D. 1999. Detection of aneuploidy by multicolor FISH in mouse sperm after in vivo treatment with acrylamide, colchicine, diazepam or thiabendazole. Mutagenesis 14: 173–179.

Schriever-Schwemmer G, Adler I-D. 1993. A mouse stock with 38 chromosomes derived from the reciprocal translocation T(7;15)33Ad. Cytogenet Cell Genet 64:122–127.

Schriever-Schwemmer G, Adler I-D. 1994. Differentiation of micronuclei in mouse bone marrow cells: a comparison between CREST staining and fluorescent in situ hybridization with centromeric and telomeric DNA probes. Mutagenesis 9:333–340.

Sjoblom T, Parvinen M, Lahdetie J. 1994. Germ-cell mutagenicity of etoposide: induction of meiotic micronuclei in cultured rat seminiferous tubules. Mutat Res 323:41–45.

Slavotinek A, Perry PE, Sumner AT. 1993. Micronuclei in neonatal lymphocytes treated with the topoisomerase II inhibitors amsacrine and etoposide. Mutat Res 319:215–222.

Vissel B, Choo KH. 1989. Mouse major (gamma) satellite DNA is highly conserved and organized into extremely long tandem arrays: implications for recombination between homologous chromosomes. Genomic 5:407–414.

Wang L, Eastmond DA. 2002. Catalytic inhibitors of topoisomerase II are DNA-damaging agents: induction of chromosomal damage by merbarone and ICRF-187. Environ Mol Mutagen 39:348–356.

Wong AKC, Rattner JB. 1988. Sequence organization and cytological localization of the minor satellite of mouse. Nuclei Acid Res 16:11645–11661.

Accepted by—
R.K. Elespuru