Available online at www.sciencedirect.com
ScienceDirect
Mutation Research 608 (2006) 82–87
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Micronucleus studies in the peripheral blood and bone marrow of mice treated with jet fuels, JP-8 and Jet-A

Vijayalaxmi [a], Andrew D. Kligerman [b,*], Thomas J. Prihoda [c], Stephen E. Ullrich [d]

[a] *Department of Radiation Oncology, The University of Texas Health Science Center, San Antonio, TX 78229, USA*
[b] *National Health and Environmental Effects Research Laboratory, Environmental Carcinogenesis Division, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA*
[c] *Department of Pathology, The University of Texas Health Science Center, San Antonio, TX 78229, USA*
[d] *Department of Immunology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, USA*

Received 21 April 2006; received in revised form 15 May 2006; accepted 19 May 2006
Available online 3 July 2006

Abstract

The potential adverse effects of dermal and inhalation exposure of jet fuels are important for health hazard evaluation in humans. The genotoxic potential of jet fuels, JP-8 and Jet-A, was investigated in an animal model. Mice were treated dermally with either a single or multiple applications of these jet fuels. Peripheral blood and bone marrow smears were prepared to examine the incidence of micronuclei (MN) in polychromatic erythrocytes (PCEs). In all experiments, using several different exposure regimens, no statistically significant increase in the incidence of MN was observed in the bone marrow and/or peripheral blood of mice treated with JP-8 or Jet-A when compared with those of untreated control animals. The data in mice treated with a single dose of JP-8 or Jet-A did not confirm the small but statistically significant increase in micronuclei reported in our previous study.
Published by Elsevier B.V.

Keywords: Mice; Jet fuel; JP-8; Jet-A; Micronuclei; Genotoxicity

1. Introduction

Each year several billion gallons of petroleum-derived middle distillate jet fuels, including JP-8 and Jet-A, are manufactured, transported, stored and used to power vehicles, operate machinery and equipment in industrial and commercial establishments, and heat commercial, industrial and residential buildings. JP-8 is used primarily by the department of defense to power aircraft and land vehicles; whereas, Jet-A is commercial aviation fuel. JP-8 is comprised of Jet-A with additional icing and corrosion inhibitors, and a static dissipater [1]. The jet fuels contain complex mixtures of aliphatic, aromatic and other substituted naphthalene hydrocarbon compounds [2]. The human health effects of exposures to JP-8 and other related jet fuels on cardiovascular, developmental, immune, renal, neuronal, respiratory tract and reproductive systems as well as carcinogenicity were reviewed recently [3]. In some investigations, 'potential' adverse effects were reported in the respiratory tract, immune and nervous systems in animals as well as in humans. With respect to genotoxicity, *in vitro* investigations failed to demonstrate mutagenic effects induced by jet fuels [4–9]; whereas, recent studies reported significant increases in DNA single-strand breaks in mammalian cells exposed *in vitro* and *in vivo* to petroleum derivatives, engine exhausts and jet fuels, including Jet-A and JP-8 [10–13]. Considering the apparently contra-

* Corresponding author. Tel.: +1 919 541 4254; fax: +1 919 541 0694.
E-mail address: Kligerman.andrew@epa.gov (A.D. Kligerman).

1383-5718/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.mrgentox.2006.05.005

dictory reports related to the genotoxic effects of JP-8 and other related jet fuels, the National Research Council recommended that future studies in animals should include evaluation of *in vivo* genotoxicity [3].

In an earlier investigation [14] we examined the incidence of micronuclei (MN) in mice treated dermally with a single dose of 300 μl (240 mg) of jet fuel, JP-8 or Jet-A, per mouse. Peripheral blood smears were prepared at the start of the experiment ($t = 0$), and at 24, 48 and 72 h following treatment with JP-8 or Jet-A. Bone marrow smears were also prepared when the animals were sacrificed at 72 h following the treatment with jet fuels. In both tissues, the extent of genotoxicity was determined from the incidence of MN in polychromatic erythrocytes (PCE). The frequency of MN in the peripheral blood of mice treated with jet fuels increased over time and reached a small but statistically significant increase at 72 h compared with concurrent untreated animals. The incidence of MN in the bone marrow of jet-fuel-treated mice was also higher when compared with controls [14].

In the current investigation, we have expanded upon these initial studies and treated mice with either a single dermal application or three applications (on consecutive days or weeks) of JP-8 or Jet-A to determine whether significant increases in the incidence of MN could be observed.

2. Materials and methods

C3H/HeNCr (MTV-) pathogen-free female mice, 8–10 weeks old, were obtained from the National Cancer Institute Frederick Cancer Research Facility Animal Production Area, Frederick, Maryland. The animals were maintained at the M.D. Anderson Cancer Center, Houston, Texas, in facilities that are approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, in accordance with current regulations and standards of the US Department of Agriculture, Department of Health and Human Services, National Institutes of Health, and the National Toxicology Program. The protocols used for handling of animals were reviewed and approved by the University of Texas M.D. Anderson Cancer Center Institutional Animal Care and Use Committee. JP-8 (Lot# 3509) and Jet-A (Lot# 3404) were supplied by the Operational Toxicology Branch, Air Force Research Laboratory, Wright Patterson Air Force Base, Dayton, OH. Both jet fuels were stored and used in a chemical fume hood.

2.1. Experimental protocol

All animal exposures were conducted at the M.D. Anderson Cancer Center, Houston, TX. Mice were acclimatized for 7 days and then randomized to different groups. The mean body weights of the animals in different groups were not significantly different (22.0 ± 0.5 g). Two hours before the start of the experiment(s), an area of approximately 8 cm^2 on the backs of all mice was shaved. Ten mice in Group 1 received no further treatment and were used as untreated controls. Ten mice in Group 2 (five only in one experiment) were injected with cyclophosphamide (CP—dissolved in sterile phosphate-buffered saline, single intra-peritoneal injection) to give a final dose of 40 mg/kg body weight, which served as positive controls. Ten mice in each of Groups 3 and 4 were treated on the skin with JP-8 and Jet-A, respectively (see below). The highest single dose of 300 μl (240 mg/mouse) jet fuel was chosen because, in previous studies, application of 300 μl of JP-8 and Jet-A was found to be immunotoxic (contact- and delayed-hypersensitivity response to a bacterial antigen) [15,16]. The undiluted Jet-A and JP-8 was applied directly to the shaved dorsal skin with a micropipette. The mice were then caged individually in a chemical fume hood for the next 3 h. This prevented cage mates from grooming and ingesting the fuel. After 3 h the residual fuel had been either absorbed through the skin of the mice or had evaporated. The animals were then returned to standard housing in an SPF-barrier facility.

- Experiment 1
  - Group 3a: JP-8, 50 μl each on three consecutive days (total 150 μl).
  - Group 3b: JP-8, 100 μl each on three consecutive days (total 300 μl).
  - Group 3c: JP-8, 300 μl each on three consecutive days (total 900 μl).
  - Group 4a: Jet-A, 50 μl each on three consecutive days (total 150 μl).
  - Group 4b: Jet-A, 100 μl each on three consecutive days (total 300 μl).
  - Group 4c: Jet-A, 300 μl each on three consecutive days (total 900 μl).
  - Blood and bone marrow smears were prepared at 24 h after the last treatment [17].
- Experiment 2
  - Group 3a: JP-8, 300 μl, single treatment.
  - Blood smears were prepared at 72 h after treatment [14].
  - Group 3b: JP-8, 300 μl each on three consecutive days (total 900 μl).
  - Blood smears were prepared at 24 h after the last treatment, as in Experiment 1.
  - Group 4a: Jet-A, 300 μl, single treatment.
  - Blood smears were prepared at 72 h after treatment.
  - Group 4b: Jet-A, 300 μl each on three consecutive days (total 900 μl).
  - Blood smears were prepared at 24 h after the last treatment, as in Experiment 1.
- Experiment 3
  - Group 3a: JP-8, 300 μl each on three consecutive days (total 900 μl).
  - Blood smears were prepared at 24 h after the last treatment, as in Experiment 1.
  - Group 3b: JP-8, 300 μl each, three weekly treatments, 7 days apart, on the same mouse (total 900 μl).

- First treatment—week 1.
- Blood smears were prepared at 72 h after the first treatment [14].
- Second treatment—week 2.
- Blood smears were prepared at 72 h after the second treatment.
- Third treatment—week 3.
- Blood smears were prepared at 72 h after the third treatment.

2.2. Data collection

Peripheral blood and bone marrow samples were collected at specified times as described above, and smears were prepared on microscope slides. These were then fixed in absolute methanol. Each slide was labeled with a random number that would not give the identity of the treatment group during microscopic evaluations. Coded slides were sent to the Department of Radiation Oncology, University of Texas Health Science Center (UTHSC), San Antonio, TX. All slides were stained with acridine orange (0.01 mg/ml in 0.2 M sodium phosphate buffer, pH 7.4) before microscopic examination. One complete set was used for microscopic examination in the Department of Radiation oncology, UTHSC, San Antonio, TX (UTHSC); whereas, the second set was mailed to the Environmental Carcinogenesis Division, US Environmental Protection Agency (USEPA), Research Triangle Park, NC for microscopic evaluation. Thus, two independent investigators (UTHSC and USEPA) assessed the incidence of MN in the blood and bone marrow cells. Each investigator used a fluorescence microscope fitted with appropriate filters for acridine orange stain. For each mouse, (a) the percentages of PCE were obtained from the examination of 1000 erythrocytes in the blood and 200 erythrocytes in the bone marrow (UTHSC only), and (b) the incidence of MN was determined from the examination of 2000 consecutive PCE in blood and bone marrow smears (UTHSC and USEPA). The results were decoded after complete microscopic evaluations.

2.3. Statistical analyses

A SAS User's Guide [18] and Version 9.1.2 for Windows of the software was used for statistical analyses. The data were subjected to the analysis of variance (ANOVA) test for repeated measures to assess significant differences between groups, tissue collection times, investigators, and doses of jet fuels. The combined data obtained from duplicate slides analyzed by two investigators were also evaluated to determine whether the pooled data analyses were valid for comparison of different groups. Pair-wise comparisons were done with the LSD method. A square root transformation of the data was used when the conditions for such transformations were warranted. Residuals were analyzed for homogeneity of variance and normality of distributions. Statistical significance was taken at a level of $p < 0.05$.

3. Results

The incidence of MN/2000 PCE in the blood and bone marrow smears of mice treated with JP-8 or Jet-A is presented in Tables 1–3. The tables show the means and 95% confidence intervals (C.I.) for the combined data from the two laboratories. In all experiments, the incidences of MN/2000 PCE in individual mice and in individual groups recorded in UTHSC and US EPA were slightly different; however, the average group indices were not significantly different. In four instances (Table 2: 300 μl Jet-A, 3× 300 μl Jet-A; and Table 3: 3× 300 μl JP-8, 300 μl Jet-A week 1), one laboratory found significant

Table 1
Experiment 1: incidence of MN in PCEs in the blood and bone marrow of mice exposed to JP-8 or Jet-A

Group	# Mice studied	Blood % PCEs ± S.D. (UTHSC only)	Blood MN/2000 PCEs group mean ± S.D. (UTHSC + USEPA) (95% C.I.)	Bone marrow % PCEs ± S.D. (UTHSC only)	Bone marrow MN/2000 PCEs group mean ± S.D. (UTHSC + USEPA) (95% C.I.)
JP-8					
Control	10	2.5 ± 0.5	4.4 ± 1.8 (3.1–5.7)	64.0 ± 6.8	5.1 ± 1.5 (4.0–6.2)
3× 50 μl	10	2.5 ± 0.6	4.8 ± 1.4 (3.8–5.8)	63.5 ± 5.9	4.2 ± 1.8 (2.9–5.5)
3× 100 μl	10	2.4 ± 0.5	4.6 ± 1.1 (3.8–5.4)	64.0 ± 5.9	4.6 ± 0.7 (4.1–5.0)
3× 300 μl	10	2.4 ± 2.7	5.0 ± 1.3 (4.1–5.9)	65.1 ± 6.8	4.8 ± 0.8 (4.2–5.4)
CP	10	2.0 ± 0.9	28 ± 4.8*** (24.5–31.5)	48.1 ± 9.7	25.2 ± 8.2*** (19.3–31.0)
Jet-A					
Control	10	2.8 ± 0.5	4.3 ± 1.5 (3.2–5.3)	63.2 ± 6.5	4.5 ± 1.3 (3.5–5.4)
3× 50 μl	10	2.7 ± 0.5	5.4 ± 1.8 (4.1–6.7)	65.0 ± 6.0	3.9 ± 2.9 (1.9–5.9)
3× 100 μl	10	2.6 ± 0.6	4.6 ± 1.1 (3.8–5.3)	64.9 ± 5.9	4.2 ± 0.7 (3.7–4.7)
3× 300 μl	10	2.5 ± 0.6	4.8 ± 1.7 (3.6–6.0)	64.9 ± 6.8	3.9 ± 1.2 (3.0–4.8)
CP	10	2.0 ± 0.8	26.4 ± 3.0*** (24.2–28.6)	48.6 ± 8.8	22.9 ± 7.1*** (17.8–27.9)

Mice were treated dermally with JP-8 or Jet-A on three consecutive days. Blood and bone marrow smears were prepared at 24 h after the last treatment. Details of data collection are given in the text. C.I.: confidence intervals at 95% level of the means.
*** Significant difference between CP and all other groups, $p < 0.0001$.

Table 2
Experiment 2: incidence of MN in PCEs in the blood of mice exposed to JP-8 or Jet-A

Group	# Mice studied	Blood % PCEs ± S.D. (UTHSC only)	Blood MN/2000 PCEs group mean ± S.D. (UTHSC + USEPA) (95% C.I.)
Control	10	2.7 ± 0.5	4.4 ± 1.4 (3.4–5.4)
300 μl JP-8[a]	10	2.7 ± 0.6	4.9 ± 0.8 (4.3–5.4)
3× 300 μl JP-8[b]	10	2.6 ± 0.5	4.9 ± 1.5 (3.9–5.9)
300 μl Jet-A[a]	10	2.6 ± 0.6	5.8 ± 1.1 (5.0–6.5)
3× 300 μl Jet-A[b]	10	2.5 ± 0.7	6.5 ± 1.5 (5.4–7.6)
CP	10	2.0 ± 0.9	31.1 ± 5.1*** (27.4–34.7)

[a] Mice were treated dermally with JP-8 or Jet-A on a single day. Blood smears were prepared at 72 h after the treatment.
[b] Mice were treated dermally with JP-8 or Jet-A on three consecutive days. Blood smears were prepared at 24 h after the last treatment. Details of data collection are given in the text. C.I.: confidence intervals at 95% level of the means.
*** Significant difference between CP and all other groups, $p + A9 < 0.0001$.

differences; whereas, the other laboratory did not. This was mainly due to differences in control values between the two laboratories. Overall, there were no systematic and significant differences in scoring between UTHSC and USEPA. The overall (pooled) results in all three experiments in mice treated with JP-8 or Jet-A, whether the jet fuel was applied dermally once, on three consecutive days, or on a weekly basis showed no statistically significant increases in the incidence of MN/2000 PCE compared to those observed in concurrent untreated control animals. As expected, in all three experiments, significantly elevated frequencies of MN/2000 PCE were observed in positive control (CP-injected) animals. The absence of a statistically significant increase in micronucleated PCEs in mice treated with three consecutive 300 μl doses of JP-8 or Jet-A (Experiment 1) was confirmed in repeat Experiments 2 and 3. Similarly, the absence of a significant increase in micronucleated PCEs in mice treated with a single 300 μl dose of JP-8 or Jet-A (Experiment 2) was also confirmed in Experiment 3.

The percentages of PCEs in the blood and bone marrow tissues of mice treated with JP-8 or Jet-A were not significantly different from those in concurrent untreated control animals (Tables 1–3). In contrast, positive control mice injected with CP exhibited decreased percentages of PCEs (Tables 1–3).

4. Discussion

The assessment of the genotoxic potential of any biological, chemical or physical agent is important in terms of evaluating its potential to be carcinogenic [19,20]. The rodent MN assay has been widely applied as an *in vivo* assay for detecting genotoxic agents. The assay became a standard test system for genotoxicity evaluations in regulatory agencies in several countries [21–24]. In this study, the genotoxic potential of jet fuels, JP-8 and Jet-A, was investigated using various exposure protocols, including repeated application of jet fuels. This is of concern for fuel handlers and aircraft maintenance personnel because of the possibility of chronic exposure.

The overall results obtained in three experiments indicated that mice treated dermally with JP-8 or Jet-A did not exhibit significant increases in the incidence of

Table 3
Experiment 3: incidence of MN in PCEs in the blood of mice exposed to JP-8

Group	# Mice studied	Blood % PCE ± S.D. (UTHSC only)	Blood Mn/2000 PCEs group mean ± S.D. (UTHSC + USEPA) (95% C.I.)
Control	10	2.5 ± 0.4	4.0 ± 1.3 (3.0–4.9)
3× 300 μl JP-8[a]	10	2.5 ± 0.6	5.3 ± 2.5 (3.5–7.0)
300 μl JP-8 week 1[b]	10	2.6 ± 0.6	5.4 ± 1.9 (4.0–6.7)
300 μl JP-8 week 2[b]	10	2.6 ± 0.5	4.5 ± 1.8 (3.2–5.8)
300 μl JP-8 week 3[b]	10	2.6 ± 0.6	4.4 ± 1.8 (3.1–5.6)
CP	5	1.8 ± 0.8	36.0 ± 7.3*** (29.6–42.4)

[a] Mice were treated dermally with JP-8 on three consecutive days. Blood smears were prepared at 24 h after the last treatment.
[b] Mice were treated dermally with JP-8 at weekly interval. Blood smears were prepared treatment at 72 h after each week. C.I.: confidence intervals at 95% level of the means.
*** Significant difference between CP and all other groups, $p < 0.0001$.

micronucleated PCEs in the blood and bone marrow smears compared with concurrent untreated control animals. Also, the data obtained in Experiments 2 and 3, where a single 300 μl treatment with JP-8 or Jet-A was used, did not confirm the small but significant increase in micronucleated PCEs reported earlier [14]. The reason(s) for this discrepancy are not clear. More than likely, the observations presented in the earlier study [14] were "false positives". This can happen even in well-designed experiments using adequate sample size. Shelby et al. [25] reported that 13 of the 49 chemicals tested (27%) in mouse bone marrow MN test produced contradictory results in repeat experiments, and the final conclusion was that the data were "inconclusive". The investigators stated "the results of a single test are not always definitive or reproducible" [25]. Thus, the small but significant increases in the incidence of MN in mice treated dermally with jet fuels, JP-8 and Jet-A, which were reported in an earlier investigation [14], were not definitive and not reproducible in the current investigations. Thus, the overall MN data in jet fuel investigations can be "inconclusive". However, it is more credible to conclude that JP-8 and Jet-A do not have the potential to induce genotoxicity based on our current study in which (a) higher amounts of jet fuel were applied to the mice than in the previous study, and (b) several different protocols were used, all of which indicated no significant induction of MN in blood and bone marrow tissues.

Acknowledgments

This work is supported by the US Air Force Office of Scientific Research through research grants, F49620-03-1-0079 (V) and F49620-1-0102 (SEU). We thank Dr. Errol Zeiger, Errol Zeiger Consulting, Chapel Hill, NC, and Dr. David DeMarini, National Health and Environmental Effects Research Laboratory (NHEERL), US Environmental Protection Agency (US EPA) for reviewing the manuscript and for useful comments. We also thank Dr. D. Doerfler, NHEERL, USEPA for help with the statistical analyses. The manuscript was reviewed by the NHEERL, US EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of the trade names or commercial products constitute endorsement or recommendation for use.

References

[1] J.R. Cooper, D.R. Mattie, Developmental toxicity of JP-8 fuel in the rat, Appl. Toxicol. (1996) 97–200.
[2] J.E. Riviere, J.D. Brooks, N.A. Monteiro-Rivieri, K. Budsaba, C.E. Smith, Dermal absorption and distribution of topically dosed jet fuels Jet-A, JP-8 and JP-8 (100), Toxicol. Appl. Pharmacol. 160 (1999) 60–75.
[3] National Research Council (NRC), Toxicologic Assessment of Jet-Propulsion Fuel, vol. 8, National Academy Press, Washington, DC, 2003.
[4] D.J. Brusick, D.W. Matheson, Mutagen and Oncogen Study on JP-8, Aerospace Medical Research Laboratory, Tech. Rep. 78-20, Wright-Patterson Air Force Base, OH, 1978.
[5] D.J. Brusick, D.W. Matheson, Mutagen and Oncogen Study on JP-4, Aerospace Medical Research Laboratory, Tech. Rep. 78-24, Wright-Patterson Air Force Base, OH, 1978.
[6] NTP, National Toxicology and Carcinogenesis Studies of Marine Diesel Fuel and JP-5 Navy fuel (CAS No. 8008-20-6) in B6C3Fa Mice (Dermal Studies), NTP 310, NIH 86-2566, Research Triangle Park, NC, 1986.
[7] R.H. McKee, R.T. Plutnick, R.T. Przygoda, The carcinogenic initiating and promoting properties of a lightly refined paraffinic oil, Fundam. Appl. Toxicol. 12 (1989) 748–756.
[8] R.H. McKee, M.A. Amoruso, J.J. Freeman, R.T. Przygoda, Evaluation of the genetic toxicity of middle distillate fuels, Environ. Mol. Mutagen. 23 (1984) 234–238.
[9] C.S. Nessel, J.J. Freeman, R.C. Forgash, R.H. McKee, The role of dermal irritation in the skin tumor promoting activity of petroleum middle distillates, Toxicol. Sci. 49 (1999) 48–55.
[10] M. Pitarque, A. Creus, R. Marcos, J.A. Hughes, D. Anderson, Examination of various biomarkers measuring genotoxic endpoints from Barcelona airport personnel, Mutat. Res. 440 (1999) 195–204.
[11] G.M. Grant, K.M. Shaffer, W.Y. Kao, D.A. Stenger, J.J. Pancrazio, Investigation of in vitro toxicity of jet fuels JP-8 and jet A, Drug Chem. Toxicol. 23 (2000) 279–291.
[12] G.M. Grant, S.M. Jackman, C.J. Kolanko, D.A. Stenger, JP-8 jet fuel-induced DNA damage in H4IIE rat hepatoma cells, Mutat. Res. 490 (2001) 67–75.
[13] S.M. Jackman, G.M. Grant, C.J. Kolanko, D.A. Stenger, J. Nath, DNA damage assessment by comet assay of human lymphocytes exposed to jet propulsion fuels, Environ. Mol. Mutagen. 40 (2002) 18–23.
[14] Vijayalaxmi, A.D. Kligerman, T.J. Prihoda, S.E. Ullrich, Cytogenetic studies in mice treated with the jet fuels, Jet-A and JP-8, Cytogenet. Genome Res. 104 (2004) 371–375.
[15] S.E. Ullrich, Dermal application of JP-8 jet fuel induces immune suppression, Toxicol. Sci. 52 (1999) 61–67.
[16] G. Ramos, D.X. Nghiem, J.P. Walterscheid, S.E. Ullrich, Dermal application of jet fuel suppresses secondary immune reactions, Toxicol. Appl. Pharmacol. 180 (2002) 136–144.
[17] R.R. Tice, G.L. Erexson, M.D. Shelby, The induction of micronucleated polychromatic erythrocytes in mice using single and multiple treatments, Mutat. Res. 234 (1990) 187–193.
[18] SAS, User's Guide: Statistics, vol. 2, Version 6.12, 6th ed., SAS Institute, Cary, NC, 1977.
[19] R.W. Tennant, E. Zeiger, Genetic toxicology: current status of methods of carcinogen identification, Environ. Health Perspect. 100 (1993) 307–315.
[20] E. Zeiger, Genetic toxicity tests for predicting carcinogenicity, in: W.N. Choy (Ed.), Genetic Toxicology and Cancer Risk Assessment, Marcel Dekker, Inc., New York, 2001, pp. 29–45.
[21] T. Sofuni, Japanese guidelines for mutagenicity testing, Environ. Mol. Mutagen. 21 (1993) 2–7.

[22] D.J. Kirkland, Genetic toxicology testing requirements: official and unofficial views from Europe, Environ. Mol. Mutagen. 21 (1993) 8–14.
[23] A.E. Auletta, K.L. Dearfield, M.C. Cimino, Mutagenicity test schemes and guidelines: US EPA office of pollution prevention and office of pesticide programs, Environ. Mol. Mutagen. 21 (1993) 38–45.
[24] Health Protection Branch Genotoxicity Committee, The assessment of mutagenicity: health protection branch mutagenicity guidelines, Environ. Mol. Mutagen. 21 (1993) 15–37.
[25] M.D. Shelby, G.L. Erexson, G.J. Hook, R.R. Tice, Evaluation of a three-exposure mouse bone marrow micronuclei protocol: results with 49 chemicals, Environ. Mol. Mutagen. 21 (1993) 160–179.