Designation: E 1263 – 97 (Reapproved 2003)

Standard Guide for Conduct of Micronucleus Assays in Mammalian Bone Marrow Erythrocytes[1]

This standard is issued under the fixed designation E 1263; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide provides recommended guidelines for performing the mammalian *in vivo* bone marrow micronucleus assay. Under appropriate test conditions, measurement of the frequency of newly formed micronucleated erythrocytes in bone marrow provides a convenient index of chromosomal damage in nucleated erythrocyte precursor cells. The rationale for the occurrence of micronuclei in conjunction with chromosomal damage has been described previously **(1)**.[2] This guide describes conditions under which the frequency of micronucleated erythrocytes in mammalian bone marrow is an appropriate measure of *in vivo* chromosomal damage, and provides guidelines for the design and technical execution of assays employing this endpoint.

1.2 The following guidelines for mammalian bone marrow erythrocyte micronucleus assays have been published by organizations concerned with the evaluation of genotoxicity test data. These references should be consulted for recommendations on details not covered in depth by this guide and for requirements of specific organizations or government agencies **(2-6)**.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Summary of Guide

2.1 Animals are exposed either acutely or chronically to a test substance. At predetermined times after or during exposure, animals are sacrificed and the bone marrow is extracted, spread on slides, and stained. The frequency of micronucleated cells among the newly-formed (RNA-containing) erythrocytes is determined, and this frequency is compared among treatment groups. The newly-formed erythrocytes are identified by staining the residual RNA which remains in the newly-formed cells for about 2 days after enucleation. Cells that stain uniformly positive for RNA are referred to as polychromatic, or polychromatophilic, erythrocytes (PCEs). Cells that do not stain positively for RNA are referred to as normochromatic erythrocytes (NCEs). An increase in the frequency of micronucleated PCEs relative to the vehicle control group indicates that the test substance induced structural chromosomal damage or lagging chromosomes aneuploidy in the nucleated erythrocytic cells.

3. Significance and Use

3.1 This guide provides guidelines for the selection of animal species, dosage and sampling conditions, sampling and scoring methods, statistical design, and analysis of genotoxicity assays in which the endpoint measured is the frequency of micronucleated erythrocytes in mammalian bone marrow.

4. Animal Selection and Care

4.1 Laboratory species that are suitable for use in this assay include the mouse (*Mus musculus*), rat (*Rattus rattus*), and Chinese hamster (*Cricetulus griseus*) **(1)**. Other species probably are equally suitable. If species or strains not previously used are employed, it must be established that the preparation procedure adequately visualizes RNA-containing erythrocytes and micronuclei, that potential artifacts such as aggregated RNA and mast cell granules do not interfere with the identification of micronuclei under the conditions employed, and that the micronucleus frequency is responsive to known clastogens and aneuploidy-inducing agents in that species and strain.

4.2 In choosing the species and strain of test animal, consideration should be given both to the availability of historical data on the response of that species and strain to known genotoxins and to the availability of other toxicity data on the same test material in the species and strain chosen. Choice of the same strain to be used in other genotoxicity assays of the same test material, or in long-term toxicity or carcinogenicity bioassays, has the advantage that the micronucleus frequency can be directly compared with other endpoints. The species for which the largest data base on known genotoxins is available is the mouse **(1)**.

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

Current edition approved Sept. 10, 2003. Published September 2003. Originally approved in 1988. Last previous edition approved in 1997 as E 1263 – 97.

[2] The boldface numbers in parentheses refer to the list of references at the end of this guide.

4.3 Animals should be obtained from a recognized source of laboratory animals and should be acclimated to laboratory conditions prior to use. Upon arrival, the age, sex, weight, and health of each animal should be documented. Only healthy animals should be used. Animal care and housing should conform to prevailing guidelines for the country and institution where the work is conducted. General information on guidelines for animal care and use can be obtained from the American Association for Accreditation of Laboratory Animal Care.[3] For any given experiment, all animals should be from the same source and should be approximately the same age (within one week for young adults). In the absence of special requirements for a particular age and sex, young adults of both sexes are recommended. Data from each sex should be analyzed independently.

5. Route of Administration and Choice of Vehicle

5.1 The choice of exposure route depends on the objective of the experiment. The objective of most micronucleus assays is to determine if the test substance induces types of chromosomal damage known to result in the formation of micronuclei. In this case, it is desirable to choose a route of administration and a vehicle that maximize the dose delivered to the target tissue. For this purpose, intraperitoneal and oral routes have been used most commonly, although others may also be appropriate. In other cases, the objective may be to evaluate specifically *in vivo* activity under conditions based upon known exposure routes in man. In such cases, the appropriate route is the one that provides the best experimental model of the expected exposure route in man.

5.2 The choice of a solvent or vehicle is influenced by several factors, including the chemical nature and solubility of the test substance, its toxicity to the test organism, and the route of exposure. In all cases care must be taken to ensure that the vehicle selected will not produce measurable toxicity or interfere with the normal uptake and metabolism of the test substance at the dose employed. In particular, the vehicle should not alter the spontaneous micronucleus frequency. If possible, it is desirable to use isotonic saline for parenteral administration and water or isotonic saline for oral administration. For oral administration of organic substances not readily soluble in aqueous solution, a pharmaceutical grade of corn or other vegetable oil may be used. Vegetable oil is less suitable for intraperitoneal administration because it is poorly absorbed from the peritoneal cavity. Other acceptable choices of vehicle include carboxymethylcellulose or suspension in gum arabic. Dimethylsulfoxide (DMSO) is an effective solvent for a wide range of substances and has frequently been used in experiments with mice, although there are a few reports of foreign intermediates being produced by interaction of DMSO with certain test substances **(7)** and one unconfirmed report that DMSO increases the frequency of chromosomal aberrations in the rat **(8)**.

[3] American Association for Accreditation of Laboratory Animal Care, 208A North Cedar Rd., New Lenox, IL 60451.

6. Dose Selection

6.1 The doses to be employed should be selected on the basis of either toxicity data obtained in the same laboratory or published toxicity data, if available. Preliminary range-finding experiment(s) should employ a minimum of two animals per dose group and should use the solvent and route of exposure to be employed in the final experiment. The highest dose level should be chosen to meet one or more of the following criteria in the experiment carried out with the full test group:

6.1.1 It should cause a marked and significant increase in the micronucleus frequency in the target cell population.

6.1.2 It should produce a statistically significant suppression of the frequency of RNA-positive erythrocytes.

6.1.3 It should cause compound-related signs of toxicity or significantly reduce survival.

6.1.4 It should be the maximum practical dose that can be administered. The maximum practical dose of a nontoxic test material is determined by the physical bulk and solubility. Testing at such a maximum dose level has been referred to as a “limit test” in OECD and EPA/TSCA testing guidelines. This dose will vary with test agent, but will generally be in the range 5 to 10 g/kg for acute oral or intraperitoneal (i.p.) administration **(3, 5)**.

6.2 The doses employed should include a minimum of two, and preferably three, doses, at least one of which does not severely reduce the frequency of RNA-positive erythrocytes (the frequency should be at least 10 to 20 % of the control value) and which does not significantly reduce the survival of the test animals. The rationale for selecting test doses has previously been discussed in the U.S. Environmental Protection Agency Gene-Tox Program report on the bone marrow polychromatic erythrocyte assay **(1)** and by Salamone and Heddle **(9)**. Because the maximum cytogenetic effect is likely to be found at doses near the maximum tolerated dose (MTD), the lower doses should be spaced at relatively small increments below the highest dose (for example, no more than ½ and ¼ of the upper dose).

7. Controls

7.1 *Vehicle or Solvent Control*—A vehicle or solvent control shall be included for each sampling condition (dose, time, sex) in each experiment. Animals are treated with the solvent or vehicle in the absence of the test substance. The quantity of solvent or vehicle administered should be equivalent to the maximum given to the animals receiving the test substance. This control helps discriminate any test-substance effect from any that may have been induced by the solvent.

7.2 *Untreated Control*—The use of untreated animals is generally not necessary during routine testing. It is important, however, that each laboratory determine the frequency of micronucleated cells in animals treated with the vehicle or solvent control relative to the spontaneous frequency in untreated animals, so that any effect of the vehicle or solvent is known.

7.3 *Positive Control Substance*—A positive control substance, that is, a substance known to induce micronuclei in bone marrow, should be included with each experiment to confirm that all features of the protocol have been carried out

correctly. The positive control agent preferably should be one that is chemically related to the test substance and preferably administered by the same route as the test article. In addition, the agent or dose should be chosen to produce a mild or weakly positive result. This provides a better evaluation of the sensitivity of the assay than does the use of a high dose of a potent clastogen which would almost always be detected regardless of whether or not the sensitivity of the assay were optimal.

8. Number of Animals/Sex

8.1 It is desirable to have data for both sexes. For routine screening, both sexes should be tested using a minimum of five animals of each sex at each test dose. If a positive result is obtained in one sex, a test agent may be classified as active without data from the other sex, but both sexes must be tested to verify a negative result.

9. Treatment and Sampling Schedule

9.1 The main requirement of the treatment/sampling schedule is to obtain at least one sample at or near the time of the maximum incidence of micronucleated cells among the RNA-positive erythrocytes in bone marrow. The time of maximum incidence varies with the test agent, dose, and treatment schedule.

9.2 *Treatment Schedule*:

9.2.1 Treatment protocols using single, double, and multiple treatments have been reported **(9)**. Although each of these treatment schedules has been reported to be advantageous with specific test agents, there is insufficient evidence at present to support the exclusive use of a specific treatment schedule for all test substances. Accordingly, the choice of single, multiple, or continuous dosing protocols must be made by the investigator, based on the specific objectives of a particular study and the available knowledge of the pharmacokinetic behavior of the test substance. The use of a single- or double-dose treatment has the advantage that these protocols have been most often employed in studies reported to date, so a larger comparative data base will be available if these treatment schedules are used.

9.2.2 Although the interval between multiple treatments can affect the response obtained, little data are available to support the choice of an optimum interval. Since historical data on multiple-treatment schedules in the mouse and rat are based primarily on a 24-h dosing interval, it appears best to use this interval until definitive data supporting an alternative are available.

9.3 *Sampling Schedule*:

9.3.1 Following each treatment, there is a particular time interval during which micronucleated RNA-positive erythrocytes, if induced, would be present. Since micronuclei are formed during division of the nucleated erythropoietic cells but scored in the anucleate mature erythrocyte, micronuclei cannot appear earlier after treatment than the interval between completion of the final erythroblast mitosis and enucleation. In the mouse, this minimum time between treatment and appearance of micronuclei is about 5 h **(10)**. For most chemicals, substantial increases in the micronucleus frequency have not been found earlier than 9 to 12 h after treatment. Since the life span of the RNA-positive erythrocyte within the bone marrow has been reported to be between 10 and 30 h in the mouse and rat (for review, see **(9)**), any micronucleated RNA-positive erythrocytes formed will remain in the bone marrow for at least 10 to 12 h. It is therefore not necessary to sample earlier than 19 to 24 h after the first treatment.

9.3.2 Due to differences between test agents in the time after treatment at which the peak frequency of micronuclei occurs, it is important that two or more samples be taken if only one or two treatments are given. Available data indicate that this peak frequency usually occurs between 24 and 48 h after treatment, but that in certain cases it may occur as late as 72 h after treatment **(9)**. The interval between samples should be shorter than the time it would take a clastogen-affected cell population to pass through the scorable stage of erythropoiesis. This time period is approximately 24 to 36 h in mice and rats. Since a clastogen may affect more than a single erythroblast cell cycle, the period during which micronucleated PCEs are observable may be longer than 24 to 36 h **(9)**. However, the micronucleated PCE frequency usually is not constant during this period, but rises to a maximum and then declines. Because it is desirable to sample as near as possible to the time of the maximum micronucleated PCE frequency, it is recommended that the time between samples not exceed approximately 24 h.

9.3.3 Based on these considerations, the following sampling schedules are recommended for experiments with mice and rats.

9.3.3.1 If one treatment is employed, a minimum of three samples should be obtained between 20 and 72 h after the treatment.

9.3.3.2 If two treatments are employed, a minimum of two samples should be obtained between 20 and 48 h after the last dose. If only two samples are taken, sampling times of approximately 20 and 48 h after the last dose would be suitable for detection of most chemicals currently known to induce micronuclei.

9.3.3.3 If three or more consecutive daily treatments are given, a single sample obtained approximately 18 to 24 h after the last dose should be sufficient.

10. Sample Preparation and Staining

10.1 *Harvesting Bone Marrow and Preparation of Smears*:

10.1.1 The principal requirements of the method of obtaining bone marrow and preparing a cell smear are the following:

10.1.1.1 A representative sample of bone marrow is obtained from each animal.

10.1.1.2 Normal cell morphology is preserved during sample preparation.

10.1.1.3 The cells are spread in a thin layer that allows individual cells to be visualized.

10.1.1.4 The cell preparation is suitable for staining in a manner that allows differentiation of the RNA-containing erythrocytes from the older erythrocytes lacking RNA, and the unequivocal identification of chromatin-containing micronuclei.

10.1.2 The most commonly used method of obtaining marrow is to remove the femur, flush the shaft with serum, concentrate the cells by gentle centrifugation, and spread the cells on a standard microscope slide **(11)**. A more rapid method is to push the marrow directly onto the slide through a small

opening in the iliac end of the femur by inserting a pin from the epiphysial end, and then to mix the marrow with serum and disperse the cells with the edge of a second clean slide which is subsequently used to spread the smear **(9)**. Care should be taken to obtain a uniformly mixed marrow sample, since it has been reported that micronucleated cells are not uniformly distributed within the marrow (J. Ashby, unpublished). Following preparation of the smear, slides are normally air dried and fixed for 2 to 5 min in absolute methanol **(9, 11)**. Other techniques that meet the stated requirements are also suitable.

10.2 *Staining*—The stain employed shall allow clear visualization of the chromatin-containing micronucleus and of the RNA-containing erythrocytes. Routine screening may be carried out satisfactorily in the mouse with standard Giemsa-based blood stains if the observer is alert to the potential occurrence of artifacts. Satisfactory Giemsa-based staining methods have been described **(11, 12)**. The presence of the two major potential artifacts, clumped cellular RNA and basophilic granules from ruptured leukocytes, is readily recognized by an experienced observer. Preparations from some species, such as the rat, often contain so many basophilic leukocyte granules that a more specific stain which differentiates chromatin from basophilic granules and RNA is required for reliable scoring. A number of stains which provide this differentiation are available (for example, acridine orange **(13)** and Hoechst 33258/pyronin Y **(14)**). The characteristics of micronuclei and of the common artifacts which may interfere with the assay have been described **(11)**.

11. Scoring

11.1 Prior to scoring, slides should be randomly coded so that the scorer is unaware of the treatment group from which each slide originated. A few slides should be randomly chosen and examined to appraise the quality and uniformity of the stain. The RNA-positive erythrocytes should be readily distinguishable from the RNA-negative erythrocytes, nuclei should be clearly stained with a visible chromatin structure, and the slides should be free of potential artifacts such as debris, stain precipitate, and basophilic granules outside of cells. If the quality of the staining and cell morphology is not good, the slides should be restained or the experiment repeated.

11.2 The criteria which distinguish micronuclei from artifacts have been described by **(11)**. Any structure that is refractile when in focus should not be scored as a micronucleus. If a nonspecific stain such as Giemsa is used for routine testing, an increase in micronucleus frequency found to be caused by an agent not previously known to induce micronuclei should be confirmed by staining at least one representative dose group with a stain that differentiates chromatin from RNA and from basophilic leukocyte granules.

11.3 For each sample, the number of micronucleated PCEs among a predetermined number of PCEs is determined. The number of cells to be scored depends on the required power of the test, the spontaneous frequency of micronucleated cells, and the number of animals in each treatment group. The minimum number of cells scored per sample should be chosen to minimize the proportion of zero class samples. At a spontaneous frequency of 1 to 2 micronucleated cells per thousand, at least 1000 cells should be scored from each sample.

11.4 Since the frequency of micronucleated cells among NCEs does not increase as markedly as that among PCEs following acute exposures, it is not necessary to score micronucleated NCEs if acute exposure protocols are used. It may, however, be useful to score this parameter for purposes of quality control, since artifacts in any given slide will produce apparent increases in the frequencies of micronuclei in both NCEs and PCEs and the incidence of artifacts will generally fail to follow the time course through the erythrocyte subpopulations expected for true micronuclei.

11.5 In addition to the frequency of micronucleated PCEs, the ratio of PCEs to other erythrocytes should be determined. This ratio may be determined by counting the number of PCEs among 100 to 200 total erythrocytes. A marked reduction in the frequency of PCEs indicates that division and maturation of the nucleated erythropoietic cells have been inhibited.

12. Statistics

12.1 An appreciation of the behavior of spontaneous or control data is crucial to a discussion of alternative statistical analyses. The published literature on control data is in good agreement. Salamone and Heddle **(9)** and Amphlett and Delow **(15)** have presented evidence that their control data for the frequency of micronucleated polychromatic erythrocytes (MPCEs) on an individual animal basis are well described by the Poisson distribution, a common statistical model for rare events (see **(16)** pp. 223–226, for a discussion of this model). Hart and Engberg-Pedersen **(17)**, on the other hand, proposed a binomial model for their control frequencies of MPCEs. With a MPCE frequency of approximately 2/1000, and with 500 to 1000 PCEs being scored in a typical experiment, the Poisson and binomial models are essentially identical **(17)**. In the remaining discussion, reference will be made to the binomial model, with the understanding that it and the Poisson model are interchangeable in this context. Further support for the binomial model for MPCE control data can be found in unpublished data of Ishidate involving 269 control mice with 1000 PCEs scored per animal, and in the extensive analyses by Margolin and Risko **(18)** of the large control database derived from mouse bone marrow experiments conducted by 15 laboratories in a recent international collaborative study organized by the International Programme on Chemical Safety (IPCS). The lone report in the literature that seemingly contradicts the applicability of the binomial model for MPCE control data is that of Mackey and MacGregor **(19)**, who report deviation from the Poisson distribution when control and test data are grouped. No reports based solely on control data have demonstrated significant deviation from the binomial model. The available evidence therefore suggests that the frequency of MPCEs in control animals is binomially distributed, and that the rate of formation in controls is homogeneous both across animals and across moderate periods of time.

12.2 Each laboratory in which the micronucleus assay is performed should verify the applicability of the binomial model for its control data via a formal test of dispersion **(15,**

16, 18), a statistical procedure particularly sensitive to variability in excess of the binomial model.

12.3 The range of spontaneous frequencies reported among different laboratories is illustrated by the control data from 15 laboratories that performed the mouse bone marrow micronucleus assay in the IPCS study alluded to here, which ranged from 1.2 to 4.9 MPCEs per 1000 PCEs **(18)**. Each laboratory must develop its own historical control database; use of rates quoted in the literature as a basis for selecting a statistical model or for comparison with treatment groups is strongly discouraged. A laboratory that reports a control frequency significantly higher than the reported range should present sufficient evidence to demonstrate that artifacts such as basophilic leukocyte granules and stain precipitates are excluded from the scores.

12.4 When a laboratory has accumulated an adequate historical control database and has verified the applicability of the binomial model for its data, it is possible to assess the consistency of concurrent control data with historical controls. To do this, one simply aggregates the data in the following tabular format:

	Historical Controls	Concurrent Controls
Number of MPCEs	x	y
Number of PCEs without micronuclei	z	w
Total PCEs	N	M

One then computes a Pearson chi-square test (see **(16)**, pp. 215–219). Concurrent control data that exhibit inconsistency with historical control data at the $p \leq 0.01$ level by the Pearson test should be carefully scrutinized and serious consideration given to replicating the experiment.

12.5 Statistical analyses should consider both dose-response trends and elevation of individual values above the control values whenever possible. Each analysis should be performed separately for male and female animal data.

12.5.1 For data that are best described by a binomial model, the Cochran-Armitage test for trend in binomial proportions (see **(16)**, pp. 246–248) is a powerful test for a dose response. Further discussion of this trend test, its formula and illustrations of its use drawn from cytogenetic analyses are found in **(18, 20)**. This statistical procedure uses all the treatment data simultaneously in one test for dose response.

12.5.2 A second analysis complementary to the test for dose response can be performed to determine which individual doses are statistically elevated above controls. Such an analysis is best performed with the normal test for equality of proportions **(21)**, which itself is a special case of the Cochran-Armitage trend test when there is only a single dose group and a single control. Both the trend test and the normal test should be performed as one-tailed tests unless there is interest *a priori* in determining anti-clastogenic agents. In executing the normal test one must be cognizant of the possibility of inflated false-positive rates caused by the multiplicity of comparisons of individual dose groups with the control. Corrections for this inflation are given by Salamone and Heddle **(9)**, or can be achieved by a Bonferroni adjustment **(20)**. If historical control data are shown to be binomially distributed and homogeneous across time, they can be aggregated with the concurrent controls to strengthen the inference **(22)**.

12.6 Margolin and Risko **(18)** present results from a small Monte Carlo study of the sensitivity (statistical power) of the micronucleus assay when the data are analyzed with the Cochran-Armitage trend test, a matter of particular importance in studies with negative findings. The assumptions in this study were that the response of each animal is binomial and that this binomial response is homogeneous within a dose group. The discussion of historical control data at the beginning of this section supports these assumptions for control data. Margolin and Risko **(18)** offer empirical support for scoring 5000 PCEs per dose group to attain an adequate level of sensitivity; five animals per group are assumed for the evaluation. The improvement of the Cochran-Armitage trend test over the Pearson chi-square test of homogeneity for the purpose of detecting a dose response is also demonstrated. Each investigator must define the power of test sufficient to meet the objectives of the experiments being analyzed. When negative data are reported, the statistical power of the test should always be specified.

12.7 Mackey and MacGregor **(19)** indicated that their data exhibit variability greater than that described by the binomial model when data from animals treated with strongly clastogenic agents were included. If treated animals exhibit heterogeneity in their response to a clastogenic agent, one could anticipate reduced sensitivity. When this occurs with a strongly clastogenic agent the net effect is minimal, since the responses observed hardly need statistical analysis because they are so obviously positive. With weaker clastogenic agents, the published evidence for serious heterogeneity of response among treated animals is limited. Should evidence of this phenomenon accumulate, there would be a need to extend the Monte Carlo results to this case.

12.8 The analyses proposed here are recommendations; they are meant to provide yardsticks against which alternative analyses can be judged, not to exclude other possible analyses.

13. Interpretation of Data

13.1 The finding that a test substance increases the frequency of micronucleated erythrocytes indicates that the substance has interfered with nuclear division in the bone marrow erythroblasts in such a way that chromatin fragments or whole chromosomes have lagged at anaphase and have failed to be incorporated into one of the daughter nuclei. Agents which break chromosomes or interfere with spindle assembly or function are known to induce micronuclei as a result of the anaphase lag of acentric fragments, bridged translocated chromosomes, or detached whole chromosomes. The micronucleus assay provides a convenient index of these types of damage and a rapid method of identifying agents with the potential to induce these types of damage. An elevated frequency of micronucleated cells strongly suggests that one of these types of damage has occurred.

13.2 If the frequency of micronucleated cells is not elevated, it can be concluded that the types of damage described above have not occurred in the dividing bone marrow erythroblasts under the conditions of treatment employed.

13.3 Thus, the micronucleus assay is a rapid screening assay for cytogenetic damage in bone marrow that allows the classification of test substances into two categories: those that do not cause chromosomal breaks or aberrations under the

conditions tested, and those that have a high probability of causing cytogenetic damage. The much more time-consuming bone marrow chromosomal aberration assays need only be carried out on those agents that are positive in the micronucleus assay. When it is known that micronuclei arise from a particular type of damage, the frequency of micronucleated cells can be used as an indirect measure of that type of damage.

14. Reporting Data

14.1 Reports of the results of micronucleus assays should include the following information:

14.1.1 The species, strain, sex, age, and weight of the test animals, the laboratory or supplier from which the animals were obtained, the housing conditions, and the diet employed.

14.1.2 The common name and an unequivocal identification number of the test substance, if available (preferably the Chemical Abstracts Service Registry Number (CAS xxxx), the NIOSH Registry of Toxic Effects of Chemical Substance Number (RTECS xxxx), or the United Nations identification number (UN xxxx)).

14.1.3 The source and purity of the test substance.

14.1.4 Any toxicity data on the test substance that are directly relevant to the study and the doses administered.

14.1.5 The route of administration of the test substance, the dose(s) administered, the solvent or vehicle used, and the exposure schedule.

14.1.6 The negative and positive controls used and the doses of each administered.

14.1.7 The number and sex of animals treated at each dose, the sampling times, and the number of animals that survived to sampling.

14.1.8 Details of the experimental protocol, including specific reference to dosing and sampling procedures, slide preparation and staining, criteria for identification of micronuclei and RNA-positive erythrocytes, and statistical design and analysis.

14.1.9 The data and a quantitative analysis of the data. As a minimum, the data should include, or permit calculation of, the number of cells and micronucleated cells scored in each sample, the frequency of micronucleated cells for each erythrocyte scored in each sample group, the ratio of PCEs to total erythrocytes, and the total number of cells upon which this ratio is based.

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