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Standard Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials[1]

This standard is issued under the fixed designation F 1439; 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 is intended to assist the biomaterials testing laboratory in the conduct and evaluation of tumorigenicity tests to evaluate the potential for new materials to evoke a neoplastic response. The procedure is generally reserved only for those materials which have not previously been used for human implantation for a significant period of time.

1.2 Assessment of tumorigenicity is one of several procedures employed in determining the biological response to a material as recommended in Practice F 748. It is assumed that the investigator has already determined that this type of testing is necessary for a particular material before consulting this guide. The recommendations of Practice F 748 should be considered before a study is commenced.

1.3 Whenever possible, it is recommended that a battery of genotoxicity procedures be initiated and proposed as an alternative to an *in-vivo* tumorigenicity bioassay. Genotoxicity assays may also be considered as initial screening procedures due to the sensitivity of the assays, the significant reduction in time to gain valuable data, and the desire to reduce the use of animals for testing. Genotoxicity assays that may be considered are outlined in Guides E 1262, E 1263, E 1280, and E 2186, and Practices E 1397 and E 1398. Additionally, other genotoxicity testing which might be considered (but which do not yet have ASTM test methods) include Salmonella/Mammalian-Microsomal Plate Incorporation Mutagenicity Assay, In Vivo Cytogenetics Bone Marrow Chromosomal Damage Assay, BALB/3T3 Morphological Transformation of Mouse Embryo Cells, and the Mouse Micronucleus Assay. The investigator is advised to consider carefully the appropriateness of a particular method for his application after a review of the published literature.

1.4 *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. Referenced Documents

2.1 *ASTM Standards:* [2]

E 1262 Guide for ~~the~~ Performance of ~~the~~ Chinese Hamster Ovary Cell/Hypoxanthine Guanine Phosphoribosyl Transferase Gene Mutation Assay

E 1263 Guide for Conduct of Micronucleus Assays in Mammalian Bone Marrow Erythrocytes

E 1280 Guide for Performing the Mouse Lymphoma Assay for Mammalian Cell Mutagenicity

E 1397~~Practices for the In-Vitro Rat Hepatocyte DNA Repair Assay~~[2] Practice for ***In Vitro*** Rat Hepatocyte DNA Repair Assay

E 1398~~Practices for the In-Vivo Rat Hepatocyte DNA Repair Assay~~[2] Practice for ***In Vivo*** Rat Hepatocyte DNA Repair Assay

E 2186 Guide for Determining DNA Single-Strand Damage in Eukaryotic Cells Using the Comet Assay

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

2.2 *Other Documents:*

National Toxicology Program General Statement of Work for the Conduct of Toxicity and Carcinogenicity Studies in Laboratory Animals[3]

OECD Guidelines for Testing of Chemicals: Guideline 451, Carcinogenicity Studies[4]

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[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.

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~~[2] Annual Book of ASTM Standards, Vol 11.05.~~

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For Annual Book of ASTM Standards volume information, refer to the standard's Document Summary page on the ASTM website.

~~[3] Annual Book of ASTM Standards, Vol 13.01.~~

[3] Available from National Institute of Environmental Health Sciences, Research Triangle Park, NC, August 1988.

~~[4] Available from National Institute of Environmental Health Sciences, Research Triangle Park, NC, August 1988.~~

OECD Guidelines for Testing of Chemicals: ~~Guideline 453, CombinedChronic Toxicity/Carcinogenicity Studies[5]~~ Guideline 453, Combined Chronic Toxicity/Carcinogenicity Studies[4]

Good Laboratory Practice for Nonclinical Laboratory Studies [5]

3. Terminology

3.1 *Definitions of Terms Specific to this Standard:*

3.1.1 *carcinogenic*—a substance is considered to be carcinogenic if it can be shown to be causally related to an increased incidence of malignant neoplastic formation.

3.1.2 *maximum implantable dose*—the maximum weight or volume of the test article which can be reasonably implanted into the test site taking into account the gross distention of tissue which can occur and its possible effects on test results.

3.1.3 *mutagenic*—a substance is said to be mutagenic if it induces alterations in the genetic code of the cell.

3.1.4 *tumorigenic*—a substance is said to be tumorigenic if it can be shown to be causally related to an increased incidence of neoplastic formation whether malignant or benign.

4. Significance and Use

4.1 This guide is not intended to specify the exact method of conducting a test for any particular material but only to present some of the criteria that should be considered in method design and possible problems that could lead to misleading results. In the development of the actual test protocol, it is recommended that recognized tumorigenesis bioassay procedures be consulted.

4.2 The recommendations given in this guide may not be appropriate for all applications or types of implant materials. These recommendations should be utilized by experienced testing personnel in conjunction with other pertinent information and the requirements of the specific material application.

5. Choice of Animal Model

5.1 These types of bioassays for chemical substances have traditionally been performed in mice or rats, or both, because of their small size, relative cost factors, and lifespan. For the testing of biomaterials, mice are not recommended because the small animal size is not conducive to the placement of solid implants. The investigator should seriously consider the use of one of the traditional models in order to draw upon the extensive information available about typical tumor formation rates and sites in control animals. The National Toxicology Program[3] recommends the use of Fischer 344 (F344/N) rats. However, other readily available species and strains may also be acceptable for the performance of these studies. Other rat species which have been recommended include Sprague-Dawley, Long-Evans, and Wistar. Some investigators have recommended the use of Long-Evans or Wistar Rats because of the difficulty of achieving a two-year lifespan for Fischer and Sprague-Dawley rats.

5.2 The currently accepted level of testing in a particular site of implantation or medical specialty should be carefully researched and regulatory requirements determined before a study design is finalized to ensure acceptability of the final results.

5.3 The appropriate choice of male or female animals or a combination should be carefully considered in light of the particular material and application being investigated. If the device will ultimately be used only in the male or female, only one sex may need to be evaluated. Otherwise, both sexes should be used.

5.4 The decision to use other species for study should be carefully documented in terms of a clear need. The use of species which have not previously been used may reduce the amount of comparative data available on control animals. Typical tumor rates for hamsters, rats, and mice have been tabulated and are available in Refs. **(1, 2, 3)**.[6]

6. Selection of Size and Form of Implant

6.1 Tumorigenicity bioassays have traditionally been performed using chemical substances as the challenge. The evaluation of implant materials requires that solid material be implanted in some form. It is important to realize that the down-sized implants necessary for use in animals will have a greater surface area to volume ratio, and this difference must be considered in experimental design.

6.2 It may be important to determine the site of administration of the test material that is most appropriate to the end use before determining implant size. The site of implantation should be the paravertebral muscle unless the size of the implant causes this site to be unacceptable. Alternatively, the site of implantation should mimic the anticipated end use, if possible. Where a specific material may be utilized in more than one type of device, multiple sites of administration should be considered if different types of tissue will be contacted. (For instance, materials that may be in contact with bone or implanted into internal organ tissue might be tested in both tissues.)

6.3 It should be recognized that the response of the test animal to an extract of a material may not fully represent the response that might be seen if the material itself were to be implanted. In general, an extract should not be used as a substitute for the actual material of interest.

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[4] Available from Organization for Economic Cooperation and Development, 200 L St., NW, Suite 650, Washington, DC 20036–4922.

~~[5] Available from Organization for Economic Cooperation and Development, 200 L St., NW, Suite 650, Washington, DC 20036–4922.~~

[5] Available from 21 CFR, Part 58, U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

~~[6] Available from 21 CFR, Part 58, U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.~~

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

6.4 The physical form of the test material should be representative of that intended for use in human patients and should consider potential material debris, if appropriate. The investigator should be aware that tests have shown **(4)** that powdered polymeric materials may not elicit a tumorigenic response subcutaneously even when prepared from polymers that do induce tumors when implanted in the form of a film. The impact of physical form and surface properties on tumorigenesis must be carefully considered, in making decisions about the physical form of the implants **(5, 6, 7, 8, 9, 10)** .

6.5 Researchers have found that the aspect ratio (length/diameter) of fiber materials may play a role in the tumorigenesis of a particular material **(11, 12)**. When new fibrous materials are being tested, the actual fiber length to be anticipated in practice should be studied. If fragmentation can be anticipated or is a worse case possibility, an attempt should be made to document a clinically relevant fiber length.

6.6 The material to be tested should originate from sample(s) representative of all processing including surface finishing, passivation, and sterilization or other final processing that will occur to a finished device.

6.7 *Dosage*:

6.7.1 In most materials, the ratio between the surface area of the implant and the body weight of the animal or person will have an effect on the amount of extractable substances (if any) which leach out of the material. The total weight or volume of material used in each animal should be in excess of the anticipated dosages to be seen in clinical practice when calculated based upon the ratio of surface area of sample to body weight of the animal. Consideration should be given to using the maximum implantable dose as the dosage or as one of multiple dosage levels. For the special case of degradable materials, the sample size should be calculated based on the ratio of sample weight to animal body weight.

6.7.2 Whenever possible, more than one exposure level should be considered to evaluate a dose-response effect.

7. Choice of Control

7.1 Control groups for this type of study will usually consist of identical animals that have not received an implant of the test material but have been subjected to the remainder of the surgical procedures. Additional groups such as housing (animals which receive no treatment but are housed with the test animals) and reference control groups may be included in the study design.

7.2 The investigator should consider a negative control group in addition to the sham or untreated controls. These animals would receive an implant or treatment identical to the test animals but the implant would be manufactured from a selected negative reference material. This group would then serve to isolate any results due to the implant trauma or mechanically induced changes.

8. Size of Test Groups

8.1 The test group and the control group should each contain enough animals which will be scheduled to survive to the end of the study to allow statistically valid conclusions to be drawn from the study. If both male and female animals are being used, each group should contain an equal number of animals of each sex. The National Toxicology Program[3] requires 60 animals/sex/group for chemical studies with ten animals being sacrificed earlier than two years. Other international organizations recommend 50 animals/sex/group.[4]The investigator should ascertain that the number of animals in each group is adequate for statistical and regulatory purposes before proceeding. In order to ensure valid data analysis, the animals should be randomly assigned to control and experimental groups. Considerations specific to the particular implant application or medical specialty may mandate a greater number of animals in each group. Additional animals in interim sacrifice groups or satellite groups may be added.

8.2 The number of test animals in each group shall be determined based upon a sound statistical analysis of the scientific questions to be addressed by the study. This analysis should take into account predicted survival rates (if available) for the species being used as well as being consistent with responsible use of experimental animals. If a statistically valid experiment can be performed with fewer than the usual number of animals per group, that fact should be documented and the study design should proceed accordingly.

9. Duration of Study

9.1 Recommended durations for evaluation of tumorigenicity in rats is two years.

9.2 Depending upon the material being evaluated, the early results may suggest that the study can be terminated earlier than two years without compromising the validity of the study. Examples might include studies in which a significantly increased rate of tumor formation or toxicity is being seen in the test animals or in one or more dosage groups.

9.3 At the termination of the study, a majority of the animals in each group should have survived for euthanasia or been terminated early for study-related reasons such as increased tumor incidence, spontaneous tumors, or toxicity of the test article. It is expected that a minimum of 50 % of the animals per sex and per group should survive until final study termination barring the above reasons. Moreover, the number of survivors or study-related terminations should be sufficient for detection of effects at the $p < 0.05$ level of significance. If attrition is occurring due to reasons which cannot be attributed to the test articles or spontaneous tumor formation, other factors should be considered such as environmental and food and water problems. This type of attrition can adversely affect the validity of a study and the investigator should be cognizant of the importance of prompt investigation of attrition in animal numbers.

10. Housing and Postoperative Care

10.1 The animals shall be housed and care provided in accordance with the *Guide for the Care and Use of Laboratory Animals* **(13)** or other appropriate guidelines.