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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 and E 1280 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:*

E 1262 Guide for the Performance of the Chinese Hamster Ovary Cell/Hypoxanthine Guanine Phosphoribosyl Transferase Gene Mutation Assay[2]

E 1280 Guide for Performing the Mouse Lymphoma Assay for Mammalian Cell Mutagenicity[2]

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

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

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices[3]

2.2 *Other Documents:*

National Toxicology Program General Statement of Work for the Conduct of Toxicity and Carcinogenicity Studies in Laboratory Animals[4]

OECD Guidelines for Testing of Chemicals: Guideline 451, Carcinogenicity Studies[5]

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

Good Laboratory Practice for Nonclinical Laboratory Studies[6]

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.

[1] This guide is under the jurisdiction of ASTM Committee F-4 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.

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

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

[5] Available from Organization for Economic Cooperation and Development, Washington, DC.

[6] Available from 21 CFR, Part 58, U.S. Government Printing Office, Washington, DC.

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[4] 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 assure 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)**.[7]

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.

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

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