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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.*

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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~~ *in vitro* Rat Hepatocyte DNA Repair Assay

E 1398 Practices for the ~~In-Vivo~~ *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]

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

Good Laboratory Practice for Nonclinical Laboratory Studies[5–6]

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.

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

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[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, ~~Vol 11.05.~~ volume information, refer to the standard's Document Summary page on the ASTM website.

~~*Annual Book*~~

[3] Available from National Institute of ~~ASTM Standards, Vol 13.01.~~ Environmental Health Sciences, Research Triangle Park, NC, August 1988.

[4] Available from ~~National Institute of Environmental Health Sciences, Research Triangle Park, NC, August 1988.~~ Organization for Economic Cooperation and Development, 200 L St., NW, Suite 650, Washington, DC 20036–4922.

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 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.

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[6] Available from ~~Organization for Economic Cooperation and Development, 200 L~~ 21 CFR, Part 58, U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, ~~Suite 650,~~ Mail Stop: SDE, Washington, DC ~~20036-4922.~~ 20401.

[7] ~~Available from 21 CFR, Part 58, U.S. Government Printing Office, Superintendent~~

[7] The boldface numbers in parentheses refer to the list of ~~Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.~~ references at the end of this guide.

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.

10.2 In addition to the requirements for humane treatment of animals in 10.1, the facilities and environment used, as well as any postoperative drug therapies or other treatments of the animals, must be carefully considered to prevent unexpected effects on the results of the study. The recommendation in 7.1 that a housing control be considered is related to the possibility that environmental factors could provide unexpected changes in study results if adequate care is not taken to eliminate the possibility.

11. Evaluation of Results

11.1 The test and control animals should be examined on a daily basis and any remarkable observations noted. This examination should include noticeable changes in eating habits, alertness, or obvious loss in body weight and palpation at least weekly for detectable masses. A complete record should be maintained of these examinations. When a mass is detected, the date of initial observation should be recorded and the record should document subsequent growth or change in each mass. If it is determined that humane considerations require that an animal be sacrificed early, or if an animal should die before its planned sacrifice date, a complete necropsy shall be performed in accordance with 11.2-11.6.

11.2 At termination or early death or sacrifice, a complete necropsy should be performed and gross observations recorded. Any abnormalities or lesions should be noted, photographed, and evaluated by histopathology. The implant site in particular should be identified and evaluated in detail, documenting all findings carefully. The list of tissues to be evaluated histologically is divided into a minimum list (see 11.4) to be evaluated for all studies and a list of additional tissues which may be appropriate (see 11.5) depending upon the type of material being tested, the route of administration, and the anticipated end use of the test material.

11.3 The investigator should consider performing appropriate hematology and blood chemistry assays on samples taken at the time of animal termination to identify possible effects on blood elements as well as to point to organs which may have been affected by the experiment.

11.4 The tissues to be evaluated shall include (at minimum):

11.4.1 Implant site,

11.4.2 Suspect lesions,

11.4.3 Lymph nodes in region of implant,

11.4.4 Heart,

11.4.5 Kidneys,

11.4.6 Liver,

11.4.7 Lungs,

11.4.8 Brain, and

11.4.9 Other tissues appropriate to the type of implant material being tested. The choice of additional sites should be carefully considered with the intent of not missing important information.

11.5 Depending upon the particular site of implantation and type of material being evaluated, it may be necessary to evaluate other tissues microscopically. Other tissues which should be considered in determining those appropriate to a particular material or application include:

11.5.1 Adrenal glands,

11.5.2 Esophagus,

11.5.3 Femur (effects on marrow cells, cartilage, and growth plates from substances which may enter the systemic circulation),
11.5.4 Gallbladder,
11.5.5 Intestines, large and small,
11.5.6 Mammary gland with adjacent skin,
11.5.7 Muscle adjacent to implant,
11.5.8 Sciatic nerve,
11.5.9 Nasal cavity,
11.5.10 Oral cavity, larynx, and pharynx,
11.5.11 Ovaries,
11.5.12 Pancreas,
11.5.13 Parathyroid gland,
11.5.14 Pituitary gland,
11.5.15 Prostate,
11.5.16 Salivary glands,
11.5.17 Seminal vesicles,
11.5.18 Skin,
11.5.19 Spinal cord,
11.5.20 Spleen,
11.5.21 Stomach,
11.5.22 Testes,
11.5.23 Thymus,
11.5.24 Thyroid gland,
11.5.25 Tongue,
11.5.26 Trachea,
11.5.27 Urinary bladder,
11.5.28 Uterus, and
11.5.29 Vagina.

11.6 In order to allow additional tissue to be evaluated if found necessary, all tissues in 11.4 and 11.5 shall be collected and should be preserved in neutral buffered formalin until the final report has been completed and approved. Retention beyond that time should be consistent with US FDA Good Laboratory Practice regulations or equivalent requirements of other countries.

12. Report

12.1 The study report should include a description of the study design in sufficient detail that it could be repeated if necessary.

12.2 A comparison of the types and incidence of tumors for the control and test series should be made and statistical techniques applied to measure the significance of any differences seen.

12.3 A complete report of the findings from the hematology and blood chemistry assays should be included, a comparison of the results for the control and test series made, and statistical techniques applied to measure the significance of any differences seen.

12.4 A complete record of the rationale for the study design decisions should be maintained as a part of the permanent record in accordance with FDA Good Laboratory Practice regulations.

12.45 The provisions of the FDA Good Laboratory Practice regulations must be followed in study design, conduct, and recordkeeping.

13. Keywords

13.1 biocompatibility; carcinogenicity testing; tumorigenicity testing

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 The medical industry in the United States is developing new materials and new applications of existing materials. Regulatory requirements have included the request for tumorigenesis bioassay for materials that have not previously seen clinical use and been recognized as safe. During the preparation of this guide, there was extensive discussion concerning the validity of this type of testing as any predictor of the tumorigenic potential of materials in humans. Among the issues raised were the Oppenheimer Effect and the effect of mechanical irritation on the production of tumors in rodents. While the occurrence of tumors due to these effects has been frequently reported in rodents, there is little evidence that similar phenomena exist in humans. The investigator is cautioned to carefully consider these possibilities in protocol development and the interpretation of results. The use of a negative control with exactly the same size and shape as the test article is strongly encouraged as an aid in the interpretation of results.

X1.2 The task force has attempted to identify the items that will need to be considered and some of the important steps to be taken in the performance of these types of studies. It is intended that this guide will be modified and made more specific as the industry and researchers gain more experience with the bioassay of solid materials.

X1.3 In the development of this guide, the possibility was raised that animals other than rats might be used for this testing. The large numbers of animals required and the current desire to reduce or eliminate the use of animals for testing would seem to suggest that canine and feline species would not be appropriate for this type of testing unless overpowering reasons can be developed for their use. The use of mice was eliminated because it was believed that mice were too small to allow the types and sizes of implants necessary for solid material testing to be implanted. The costs and logistics of such a study would seem to make it nearly impossible to use primates in these quantities. It is the feeling of the task force that rats should be used unless there is an overriding need for other species. The study should be designed to utilize the minimum number of animals consistent with adequate statistical treatment of the data.

X1.4 Typically, in the performance of tumorigenicity assays, a maximum tolerated dose is determined and that dosage level is utilized as one of the test groups. The physical effects of implanting gross amounts of solid materials into rats, such as tissue distension and its effects on the long-term results of the study, may make the definition of this maximum dosage and interpretation of the results difficult, at best. The task force determined, based upon comments received, that a maximum implantable dose should be recommended as one of the dosage levels despite the experimental problems which might result.

X1.5 There was extensive discussion about the appropriate way to address the Oppenheimer Effect relating to the tumorigenicity of plastics and the Stanton Hypothesis relating to the tumorigenicity of fibrous materials. Paragraphs 6.4 and 6.5 reflect this discussion. Much of the discussion centered around the extreme difficulty or impossibility of performing a valid study in light of these two phenomena. There was also discussion of the possibility of the development of neoplasia in rodents due to mechanical irritation.

X1.6 Data were presented during the development of this guide which reflected a possible decline in the average lifetime in the rats used for these studies. It was decided to address the issue in terms of the cause of early death. Since these strains are used because of their history of spontaneous tumor formation, it is to be expected that early deaths or termination may occur. The task force has attempted to present this concern in terms of the reasons for death rather than the absolute ratios of survivors.

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