ISO/FDIS 10993-3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

A Manager's Quiz

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Welcome to Clinical Device Group's web publications for medical devices. Today, we'll take a manager-level look at the new ISO/FDIS 10993-Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity. We will also look at some of the basic science behind the standard.

You can find the answers in our book "Biocompatibility Testing & Management, Fourth Edition", or you may email us to obtain the answers at cdginc@clinicaldevice.com.

ISO/FDIS 10993-3: Tests for Genotoxicity, Carcinogenicity and Reproductive Toxicity

This presentation…

- **Is based on the ISO/FDIS standard of June 2003 and the basic science behind the standard.**
- **The final draft international standard may be purchased from AAMI, ISO, or your country's standards body.**
- **www.AAMI.org**
- **www.iso.ch/iso/en/ISOOnline.openerpage**

The final draft international standard was published in June of 2003 and is available for purchase from AAMI (Association for the Advancement of Medical Instrumentation, Washington, DC.) or ISO (International Organization for Standardization, Geneva).

OECD

The OECD…

- **Stands for "Organization for Economic Cooperation and Development".**
- **Has offices in Paris and Washington, DC.**
- **Writes the test methods for genotoxicity, carcinogenicity, and reproductive toxicity testing.**
- **May be found at www.oecd.org.**

The OECD is the Organization for Economic Cooperation and Development. The OECD plays a prominent role in fostering good governance in the public service and in corporate activity. The OECD produces internationally agreed instruments, decisions and recommendations to promote rules of the game in areas where multilateral agreement is necessary for individual countries to make progress in a globalized economy. The OECD writes the test methods that we follow for genotoxicity, carcinogenicity, and reproductive toxicity testing.

Genotoxicity

True or false: Genotoxicity…

[ ] Is the state of being toxic to a gene, i.e., causing damage to the DNA.

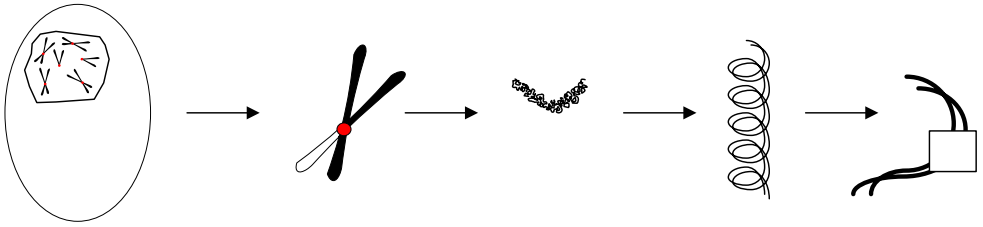
[ ] Is of concern because it may lead to cancer.

[ ] May result from exposure to certain viruses, chemicals, or physical events (such as radiation).

Let's get started with our quiz. What does the word "genotoxicity" actually mean and why do we care about it? Are there other kinds of genotoxic agents or events besides chemicals?

By the way, the first chemical carcinogen was identified by a British surgeon, Sir Percival Pott (1713-1788). He observed a high incidence of scrotal skin cancer among patients who had been chimney sweeps as boys—the carcinogen was coal tar. It's interesting that the most common chemical carcinogen today is a related compound—tobacco smoke.

Genes and Chromosomes



True or false: Polypeptides…

[ ] May be enzymes, hormones, structural proteins, or have many other cellular functions.

Chromosomes are massive molecular structures found in the nucleus of the cell (for reference, the yolk of the egg you had for breakfast was the nucleus of a cell). The chromosomes are made up of DNA plus a host of attending molecules such as proteins and lipids. The double-stranded DNA is made up for four repeating nucleic acids or nucleotides. A sequence of nucleotides is called a gene. Read in groups of three, the nucleotides in a gene provide an alphabet that codes for polypeptides. This leads us to a basic rule of biology: one gene, one polypeptide.

Mammalian Cell with

Mutations

True or false: Mutations…

[ ] Are damage to the structure or integrity of DNA.

[ ] Are caused by genotoxic agents.

[ ] Are a necessary initiation event to cancer.

[ ] Only rarely result in cancer because the body has an elaborate mechanism for grooming and repairing its DNA.

Genotoxic agents are also known as mutagens, the word for agents or events that cause mutations. Mutations are alterations to DNA that result in an incorrectly expressed gene, i.e., a dysfunctional polypeptide. If the polypeptide is critical to life, then the cell will die. If the new polypeptide is beneficial to life, the mutation could result in a stronger cell; if the cell is a sperm or egg, the mutation could result in a stronger species. If the dysfunctional polypeptide alters the cell's ability to regulate its own growth, the mutation could result in cancer.

Mutations are natural events, occurring tens or hundreds of times a day in an organism. Most mutations are repaired by the cell's grooming and repair mechanisms. But the mutation may be expressed when the repair is missed. A single mutational event could become a clinically detectable cancer in about six years.

Genotoxicity Tests

True or false: Genotoxicity tests…

[ ] May use mammalian or bacterial cells.

[ ] May be conducted in vitro or in vivo.

[ ] May test for gene mutations, changes in chromosomal structure, or other DNA or gene changes.

While we can test for either genotoxicity or carcinogenicity, genotoxicity testing is much quicker and less expensive: a few months and ~$100,000 versus 24 months and ~$1,000,000. It is also more humane because it does not cause suffering to live animals. There are many kinds of genotoxicity tests: sixteen or so are described by the OECD.

Indications for Tests

True or false: Genotoxicity tests are indicated by ISO/FDIS 10993-3…

[ ] When the device may contain compounds that may interact with genetic material.

[ ] When the chemical composition of the device is unknown.

[ ] Only for permanent implant devices.

Manufacturers often ask if they need to conduct genotoxicity tests. It isn't necessary to conduct this testing when you are using well-characterized materials in your device, and the materials have a known track record of being non-genotoxic. It isn't necessary for most limited exposure (<24 hours) or skin contact products, either.

Genotoxicity Test Strategies

True or false: ISO/FDIS 10993-3 suggests…

[ ] Two options for genotoxicity test strategies.

[ ] Option 1 is three tests: a) gene mutation in bacteria, b) gene mutation in mammalian cells, and c) clastogenicity test in mammalian cells.

[ ] Option 2 is two tests: a) gene mutation in bacteria, and b) mouse lymphoma assay incorporating colony number and size determinations.

If you must conduct genotoxicity testing, you'll want to conduct the least expensive and fastest combination of tests possible. But many materials or technologies are acquired with some history of testing, so the ISO/FDIS standard suggests two different options for test strategies. The key is that both bacterial and mammalian cells are utilized as test systems, and both gene mutation and clastogenicity are evaluated as endpoints.

By the way, a clastogen is an agent that causes breaks in chromosomes, so we are looking for effects at both the gene level and the chromosome level.

In vivo tests

True or false: In vivo tests…

[ ] Should be performed if in vitro tests are positive.

True or false: In vivo tests include…

[ ] The micronucleus test. The test sample is given to live mice. Their bone marrow is tested for micronuclei after they are sacrificed.

[ ] A metaphase analysis test in rodent bone marrow.

[ ] An unscheduled DNA synthesis test with mammalian liver cells.

In vitro tests are tests performed in cellular systems, in vivo tests are tests performed in whole, living animals. In vitro tests are preferred to in vivo tests because they do not expose the whole animal to pain or suffering.

Carcinogenicity Testing

True or false: Carcinogenicity testing is recommended if:

[ ] The materials are resorbable and there is not adequate existing information.

[ ] The materials are permanent implants and there is not adequate existing information.

[ ] Should not be conducted if genotoxic tests are positive.

Carcinogenicity tests should not be undertaken lightly; they are expensive, time-consuming, and require a lot of animals. They should only be conducted if there are legitimate toxicity questions which cannot be addressed by a risk analysis. They should not be conducted if genotoxic tests are positive; materials which test positive for genotoxicity should be considered to be carcinogenic. This rule stems from a second basic rule of biology: mutagens are carcinogens, cancer begins with a mutation.

Carcinogenicity Tests

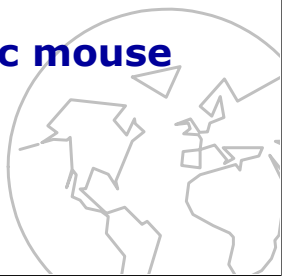
True or false: Carcinogenicity tests…

[ ] Are usually conducted in mice or rats, are two-year studies, and may cost over $1,000,000.

[ ] May be conducted in transgenic mice, these are six-month studies and cost around $500,000.

[ ] Include the rasH2 transgenic mouse model.

[ ] Include the p53(+/-) transgenic mouse model.



Carcinogenicity testing is most likely to be required when the device contains new, uncharacterized materials or combinations of materials. FDA offers manufacturers a choice, you may conduct either the standard, older, carcinogenicity test or one of the new transgenic mouse test. If you elect to conduct a transgenic mouse test, FDA will work with you individually to develop the protocol.

Solid-State Tumors

True or false: sometimes implanted materials…

[ ] Induce tumors but are not themselves carcinogenic materials.

[ ] These tumors are called "solid-state" tumors.

[ ] Solid-state tumors are common in rodents and may confound carcinogenicity test results.

While the rule that cancers are caused by mutations and all mutagens are also carcinogens is mostly true, there is one exception. Sometimes implanted, solid materials cause tumors, especially in rodents. The presence of solid-state tumors may confound the results of carcinogenicity testing for medical devices.

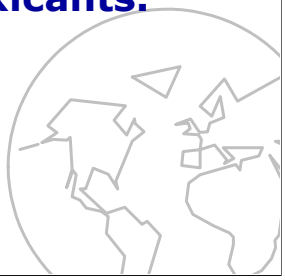
Reproductive Toxicity

True or false: Reproductive toxicity tests…

[ ] Are recommended for materials that permanently contact the reproductive organs.

[ ] Shall be justified via a risk assessment.

[ ] Are not necessary if there are adequate and reassuring data that the material or leachates are not reproductive toxicants.



Reproductive toxicity testing is rarely conducted and should be justified by a risk assessment that presents an argument that reproductive or developmental toxicity may occur.

The Commercial

- **True or False:**

[ ] You can learn more about strategic planning for biological safety by taking the seminar "Biocompatibility Testing & Management" from Clinical Device Group.

[ ] You can learn more about managing biological safety by buying the book of the same title.

[ ] You can get assistance with your safety evaluations by contracting with our experts who specialize in device biological safety.

If you found this slide presentation useful, you should consider taking the two-day seminar "Biocompatibility Testing & Management" from Clinical Device Group, or consider purchasing the book by the same name. Both will help you with strategically planning your biological safety evaluations. On the other hand, you can get personal assistance with your safety evaluations by contracting with our experts.

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