



International Cooperation on Harmonisation of Technical Requirements
for Registration of Veterinary Medicinal Products

#116

Guidance for Industry

Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Genotoxicity Testing

VICH GL23(R)

Submit comments on this revised guidance at any time. Submit electronic comments to <http://www.regulations.gov>. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All written comments should be identified with Docket No. FDA-2000-D-0598.

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Additional copies of this guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either <http://www.fda.gov/AnimalVeterinary/default.htm> or <http://www.regulations.gov>.

**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
May 2015**

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VICH GL23 (R) (SAFETY) - GENOTOXICITY
October 2014
Revision at Step 9
For consultation at Step 7

STUDIES TO EVALUATE THE SAFETY OF RESIDUES OF VETERINARY DRUGS IN HUMAN FOOD: GENOTOXICITY TESTING

Revision at Step 9

Adopted at Step 7 of the VICH Process by the VICH Steering Committee in September 2014
for implementation by October 2015.

This Guidance has been developed by the appropriate VICH Expert Working Group and has been subject to consultation by the parties, in accordance with the VICH Process. At Step 7 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and USA.

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Guidance for Industry

STUDIES TO EVALUATE THE SAFETY OF RESIDUES OF VETERINARY DRUGS IN HUMAN FOOD: GENOTOXICITY TESTING

This guidance represents the Food and Drug Administration's (FDA or Agency) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance using the contact information on the title page of this guidance.

1. INTRODUCTION

1.1. Objective of the guidance

In order to establish the safety of veterinary drug residues in human foods, a number of toxicological evaluations are recommended, including investigation of possible hazard from genotoxic activity. Many carcinogens and/or mutagens have a genotoxic mode of action and it is prudent to regard genotoxicants as potential carcinogens unless there is convincing evidence that this is not the case. Additionally, substances causing reproductive and/or developmental toxicity may have a mode of action that involves genotoxic mechanisms. The results of genotoxicity tests will not normally affect the numerical value of an acceptable daily intake (ADI), but they may influence the decision about whether an ADI can be established.

The objective of this guidance is to ensure international harmonization of genotoxicity testing.

FDA's guidance documents, including this revised guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word "should" in Agency guidances means that something is suggested or recommended, but not required.

1.2. Background

There have been differences in the genotoxicity testing requirements of the EU, Japan, and the USA for establishing the safety of veterinary drug residues in human food.

This guidance is one of a series of VICH guidances developed to facilitate the mutual acceptance of safety data necessary for the establishment of ADIs for veterinary drug residues in human food by the relevant regulatory authorities. It should be read in conjunction with the guidance on the overall strategy for the evaluation of veterinary drug residues in human food (see VICH GL33). This VICH guidance was developed after consideration of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidances for pharmaceuticals for

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human use: “Genotoxicity: A Standard Battery of Genotoxicity Testing of Pharmaceuticals” and “Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals”. Account was also taken of Organisation for Economic Co-operation and Development (OECD) Guidances for Testing of Chemicals and of national/regional guidances and the current practices for evaluating the safety of veterinary drug residues in human food in the EU, Japan, the USA, Australia, New Zealand, and Canada.

1.3. Scope of the guidance

This guidance recommends a Standard Battery of Tests that can be used for the evaluation of the genotoxicity of veterinary drugs. In most cases, the results will give a clear indication of whether or not the test material is genotoxic. However, the Standard Battery of Tests is not appropriate for certain classes of veterinary drugs. For instance, some antimicrobials may be toxic to the tester strains used in the test for gene mutation in bacteria. The guidance advises on amendments to the basic battery of tests for testing such drugs. In some instances the results of the Standard or amended Battery of Tests may be unclear or equivocal, so advice is given on the assessment and interpretation of results. Additional testing may be recommended in some instances, e.g., substances showing potential aneugenic and/or germ cell effects.

In most cases, it is the parent drug substance that is tested, although in some cases it may be recommended to also test one or more of the major metabolites that occur as residues in food. Instances when testing a metabolite may be recommended include situations in which the metabolite has structural alerts that are not present in the molecular structure of the parent drug and when the residues in food are mostly in the form of a metabolite that has a molecular structure that is fundamentally different from that of the parent drug. Salts, esters, conjugates and bound residues are usually assumed to have similar genotoxic properties to the parent drug, unless the converse can be demonstrated.

2. STANDARD BATTERY OF TESTS

The following battery of three tests is recommended for use as a screen of veterinary drugs for genotoxicity:

2.1. A test for gene mutation in bacteria.

For the bacterial gene mutation test, a very extensive database has been built up for bacterial reverse mutation tests for gene mutation in strains of *Salmonella typhimurium* and *Escherichia coli*. The best-validated strains are *Salmonella typhimurium* strains TA1535, TA1537 (or TA97 or TA97a), TA98 & TA100. These strains may not detect some oxidising mutagens and cross-linking agents, so to correct for this, *Escherichia coli* strains WP2 (pKM101), WP2uvrA (pKM101) or *Salmonella typhimurium* TA102 should also be used in the bacterial test. However, the bacterial gene mutation test, whilst being an efficient primary screen for detecting compounds with inherent potential for inducing gene mutations, does not detect all compounds with mutagenic potential. Some clastogenic compounds do not produce mutations in the *Salmonella* test (e.g., inorganic arsenic compounds).

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2.2. A cytogenetic test for chromosomal damage (the *in vitro* metaphase chromosome aberration test or *in vitro* micronucleus test), or an *in vitro* mouse lymphoma tk gene mutation assay

The second test should evaluate the potential of a chemical to produce chromosomal effects. This can be evaluated using one of the following three tests: (1) an *in vitro* chromosomal aberrations test using metaphase analysis, which detects both clastogenicity and aneugenicity; (2) an *in vitro* mammalian cell micronucleus test, which detects the activity of clastogenicity and aneugenicity; or (3) a mouse lymphoma test, which, with modification, can detect both gene mutation and chromosomal damage.

2.3. An *in vivo* test for chromosomal effects using rodent haematopoietic cells.

A third test has been added to the Standard Battery of Tests in order to give added assurance that the Standard Battery of Tests will detect all potential mutagens. The VICH was aware that, for the testing of some classes of chemicals, some authorities recommend the use of an initial battery of mutagenicity tests that consists solely of *in vitro* tests, with *in vivo* testing recommended only if the *in vitro* battery gives a positive or equivocal result. The VICH considered this approach but chose to include an *in vivo* test in its basic battery of tests in order to achieve harmony with the requirements of ICH for testing human drugs for genotoxicity. This could be either a micronucleus test or a cytogenetics test.

3. MODIFICATIONS TO THE STANDARD BATTERY

For most substances the Standard Battery of Tests should be sufficient, but in a few instances modifications to the choice of tests or to the protocols of the individual tests undertaken may be appropriate. The physicochemical properties of a substance (e.g., volatility, pH, solubility, stability, etc.) can sometimes make standard test conditions inappropriate. It is recommended that this be given due consideration before tests are conducted. Modified protocols should be used where it is evident that standard conditions will give a false negative result. The OECD Guidances for Testing of Chemicals for the genotoxicity tests give some advice on the susceptibility of the individual tests to the physical characteristics of the test material and they give some advice on compensatory measures that can be taken. Drugs tested using alternative batteries of genotoxicity tests should be considered on a case-by-case basis. A scientific justification should be given for not using the Standard Battery of Tests.

3.1. Antimicrobials

Some antimicrobial substances are excessively toxic to bacteria and therefore difficult to test in bacterial tests. In this case, it would be appropriate to perform a bacterial test using concentrations up to the limit of cytotoxicity and to supplement the bacterial test with an *in vitro* test for gene mutation in mammalian cells.

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3.2. Metabolic activation

The *in vitro* test should be performed in the presence and absence of a metabolic activation system. The most commonly used metabolic activation system is S9 mix from the livers of rats treated with an enzyme inducing agent (Aroclor 1254 or a combination of phenobarbital and beta-naphthoflavone). However, other systems can be used. A scientific rationale should be given to justify the choice of an alternative metabolic activation system.

4. THE CONDUCT OF TESTS

4.1. Bacterial Mutation Test

A bacterial reverse mutagenicity test should be performed according to the protocol set out in OECD Test Guidance 471¹.

4.2. *In Vitro* Test for Chromosomal Effects in Mammalian Cells

Chromosome aberration tests should be performed according to OECD Test Guidance 473². These cytogenetic tests should detect clastogenicity and may also detect heteroploidy. To detect induction of polyploidy, longer (e.g., 3 normal cell cycles) continuous treatment can give higher sensitivity. Limited information on potential aneugenicity can be obtained by recording the incidences of hyperploidy, polyploidy and/or modification of mitotic index in the cytogenetic test. If there are indicators of aneugenicity (e.g., induction of polyploidy) then this should be confirmed using appropriate staining procedures such as FISH (fluorescence *in situ* hybridisation) or chromosome painting. As apparent loss of chromosomes can occur artifactually, only hyperploidy should be regarded as a clear indication of induced aneuploidy.

An *in vitro* mammalian micronucleus test (OECD Test Guidance 487⁶) can be performed as part of the initial battery of genotoxicity tests as a substitute for the chromosome aberration tests described in OECD Test Guidance 473². The *in vitro* mammalian cell micronucleus test can detect both clastogenicity and aneugenicity, and can simultaneously detect mitotic delay, apoptosis, chromosome breakage, and chromosome loss.

If the mouse lymphoma tk test is conducted, it should be with a protocol amended to include measurements of both small and large colonies. The protocol should conform to the criteria set out in OECD Test Guidance 476⁵ and should include the use of appropriate positive controls (clastogens).

4.3. *In Vitro* Test for Gene Mutation in Mammalian Cells

When an *in vitro* mammalian cell gene mutation test is used, it should be performed according to OECD Test Guidance 476⁵.

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4.4. *In Vivo* Test for Chromosomal Effects

Either a mammalian erythrocyte micronucleus test (OECD Test Guidance 474³) or a mammalian bone marrow chromosome aberration test (OECD Test Guidance 475⁴) can be performed as part of the initial battery of genotoxicity tests. The mammalian erythrocyte micronucleus test can be conducted by analysis of either bone marrow or peripheral blood. If it is conducted using peripheral blood, the test species should be the mouse and not the rat, as the spleen of the latter removes circulating micronucleated erythrocytes.

These tests are designed to give a qualitative answer to the question of whether or not a substance may express genotoxicity *in vivo*, not to establish no-effect levels.

5. ASSESSMENT OF TEST RESULTS

The assessment of the genotoxic potential of a compound should take into account the totality of the findings and acknowledge the intrinsic values and limitations of both *in vitro* and *in vivo* tests.

Clearly negative results for genotoxicity in a series of tests, including the Standard Battery of Tests, should usually be taken as sufficient evidence of an absence of genotoxicity.

If a substance gives clearly positive result(s) for genotoxicity *in vitro* but a clearly negative result in the *in vivo* genotoxicity test(s) performed using bone marrow, another *in vivo* genotoxicity test using a target tissue other than bone marrow should be used to confirm whether it's genotoxic or not. The most appropriate test should be decided on a case-by-case basis.

In the case of other positive or equivocal results in the Standard Battery of Tests the decision of whether further tests are warranted should be made on a case-by-case basis.

REFERENCES

1. OECD. 1997. Test Guidance 471. Bacterial Reverse Mutation Test. In: OECD Guidance for Testing of Chemicals. Paris, Organisation for Economic Cooperation and Development.
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4. OECD. 1997. Test Guidance 475. Mammalian Bone Marrow Chromosome Aberration Test. In: OECD Guidance for Testing of Chemicals. Paris, Organisation for Economic Cooperation and Development.
5. OECD. 1997. Test Guidance 476. *In Vitro* Mammalian Cell Gene Mutation Test. In: OECD Guidance for Testing of Chemicals. Paris, Organisation for Economic Cooperation and Development.
6. OECD. 2010. Test Guidance 487. *In Vitro* Mammalian Cell Micronucleus Test. In: OECD Guidance for the Testing of Chemicals. Paris, Organisation for Economic Cooperation and Development.

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GLOSSARY

- Aneugenicity: The ability to cause aneuploidy.
- Aneuploidy: Numerical deviation of the modal number of chromosomes in a cell or organism, other than an extra or reduced number of complete sets of chromosomes.
- Clastogen: An agent that produces structural changes of chromosomes, usually detectable by light microscopy.
- Clastogenicity: The ability to cause structural changes of chromosomes (chromosomal aberrations).
- Cytogenetics: Chromosome analysis of cells, normally performed on dividing cells when chromosomes are condensed and visible with a light microscope after staining.
- Gene mutation: A detectable permanent change within a single gene or its regulating sequences. The change could be a point mutation, insertion, deletion, etc.
- Genotoxicity: A broad term that refers to any deleterious change in the genetic material regardless of the mechanism by which the change is induced.
- Heteroploidy: Any abnormal number of chromosomes in a cell or organism. This is a general term that covers polyploidy, aneuploidy, hyperploidy, etc.
- Hyperploidy: An increase over the normal number of chromosomes in a cell or organism.
- Micronucleus: Particle in a cell that contains microscopically detectable nuclear DNA; it might contain a whole chromosome(s) or a broken centric or acentric part(s) of chromosome(s). The size of a micronucleus is usually defined as less than 1/5 but more than 1/20 of the main nucleus.
- Mutagenicity: The capacity to cause a permanent change in the amount or structure of the genetic material in an organism or cell that may result in change in the characteristics of the organism or cell. The alteration can involve changes to the sequence of bases in the nucleic acid (gene mutation), structural changes to chromosomes (clastogenicity) and/or changes to the number of chromosomes in cells (aneuploidy or polyploidy).
- Polyploidy: An extra or reduced number of complete sets of chromosomes.