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VICH GL23 (R) (SAFETY) - GENOTOXICITY
December 2012
Revision at Step 9
For consultation at Step 4

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

Revision at Step 9

Recommended for consultation at Step 4 of

the VICH Process by the VICH Steering Committee

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

Please note that the comments provided during the public consultation procedure will be published by VICH.

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

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1. INTRODUCTION

1.1. Objective of the guideline

In order to establish the safety of veterinary drug residues in human foods, a number of toxicological evaluations are required including investigation of possible hazard from genotoxic activity. Many carcinogens 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 guideline is to ensure international harmonisation of genotoxicity testing.

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 guideline is one of a series of VICH guidelines 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 guideline on the overall strategy for the evaluation of veterinary drug residues in human food (see VICH GL33). This VICH guideline was developed after consideration of the existing ICH guidelines for pharmaceuticals for 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 OECD Guidelines for Testing of Chemicals and of national/regional guidelines 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 guideline

This guideline 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 guideline advises on amendments to the basic battery of tests that are needed for the testing of 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 required 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 necessary to also test one or more of the major metabolites that occur as residues in food. Instances when the need to test a metabolite may be required 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:

- A test for gene mutation in bacteria.

- 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.

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

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), WP2*uvr*A (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).

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.

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 required 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 there may be a need for modifications to the choice of tests or to the protocols of the individual tests undertaken. The physicochemical properties of a substance (e.g. volatility, pH, solubility, stability, etc.) can sometimes make standard test conditions inappropriate. It is essential 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 Guidelines 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 may be taken. Drugs tested using alternative batteries of genotoxicity tests will 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.

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 may 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 test

A bacterial reverse mutagenicity test should be performed accordingto the protocol set out in OECD Test Guideline 471[1].

4.2. In vitro test for chromosomal effects in mammalian cells

Chromosome aberration tests should be performed according to OECD Test Guideline 473[2]. 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 Guideline 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 Guideline 4732. The *in vitro* mammalian cell micronucleus test allows the detection of 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 Guideline 476[5] 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 Guideline 476[5].

4.4. In vivo test for chromosomal effects

Either a mammalian erythrocyte micronucleus test (OECD Test Guideline 474[3]) or a mammalian bone marrow chromosome aberration test (OECD Test Guideline 475[4]) may be performed as part of the initial battery of genotoxicity tests. The mammalian erythrocytemicronucleus test may 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, will 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, it will be necessary to confirm whether it is genotoxic or not with another *in vivo* genotoxicity test using a target tissue other than bone marrow. The most appropriate test will need to be chosen on a case-by-case basis.

In the case of other positive or equivocal results in the Standard Battery of Tests the need for further tests should be decided on a case–by-case basis.

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1. OECD. 1997. Test Guideline 471. Bacterial Reverse Mutation Test.In: OECD Guideline for Testing of Chemicals. Paris, Organization for Economic Cooperation & Development.

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4. OECD. 1997. Test Guideline 475. Mammalian Bone Marrow Chromosome Aberration Test. In: OECD Guideline for Testing of Chemicals. Paris, Organization for Economic Cooperation & Development.

5. OECD. 1997. Test Guideline 476. *In Vitro* Mammalian Cell Gene Mutation Test.In: OECD Guideline for Testing of Chemicals. Paris, Organization for Economic Cooperation & Development.

6. OECD. 2010. Test Guideline 487. *In Vitro* Mammalian Cell Micronucleus Test In: OECD Guideline for the Testing of Chemicals. Paris, Organization for Economic Cooperation & Development.

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 may 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 may 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.