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Guidance for Industry

Safety Studies for Veterinary Drug Residues in Human Food: Genotoxicity Studies VICH GL23

DRAFT GUIDANCE

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This draft guidance recommends a basic battery of tests that can be used for the evaluation of the genotoxicity of veterinary drugs submitted for approval to the European Union, Japan, and the United States.

Comments and suggestions regarding this draft document should be submitted to Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the Docket No. 00D-1631.

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SAFETY STUDIES FOR VETERINARY DRUG RESIDUES IN HUMAN FOOD: GENOTOXICITY STUDIES

Recommended for Consultation
at Step 4 of the VICH Process
on 14 -16 June 2000
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THIS GUIDANCE 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.

SAFETY STUDIES FOR VETERINARY DRUG RESIDUES IN HUMAN FOOD: GENOTOXICITY STUDIES

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1. INTRODUCTION

1. 1. Objective of the guidance

The use of veterinary drugs in food producing animals can cause the presence in food of small amounts of drug residues. Thus, consumers of foods derived from animals may sometimes be exposed to veterinary drug residues.

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 genotoxicity. Many carcinogens have a genotoxic mode of action and it is prudent to regard in vivo genotoxins as potential carcinogens unless there is convincing evidence that this is not the case. Some reproductive toxicants have a mode of action that involves genotoxicity. 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 that genotoxicity testing is carried out according to a harmonized guidance.

1.2. Background

There have been differences in the genotoxicity testing practices 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 (VICH Guidance on General Testing Approach will be made available at a later time). It was developed after consideration of the existing ICH guidances 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 Organisation for Economic Co-operation and Development (OECD) methodological guidances and of the current practices for evaluating the safety of veterinary drug residues in human food in the EU, Japan, the USA, Australia and New Zealand.

1.3. Scope of the guidance

This guidance recommends a basic battery of tests that can be used for the evaluation of the genotoxicity of veterinary drugs. In most cases, the results from the battery of tests will give a clear indication of whether or not the test material is genotoxic. However, the basic battery of tests is not appropriate for all classes of veterinary drugs, so this guidance advises on adjustments to the basic battery of tests that are recommended for the testing of such drugs. In some cases, the results of the initial testing in the basic battery of tests (or an amended battery of tests) may be unclear, so

advice is given on the assessment and interpretation of results. Additional testing may be recommended in some instances.

In most cases, it is the parent drug substance that is tested, although in some cases it may be appropriate to also test one or more of the major metabolites that occur as residues in food. 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:

I. A test for gene mutation in bacteria.

II. An in vitro test for chromosomal damage in mammalian cells.

III. An in vivo test for chromosomal damage using rodent haematopoietic cells.

3. MODIFICATIONS TO THE STANDARD BATTERY

For most substances the standard battery of tests should be sufficient, but in a few modifications to the choice of tests or to the protocols of the individual tests undertaken may be appropriate. 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

In the case of antimicrobial substances that may be toxic to the bacterial test strains, it may be acceptable to substitute a validated mammalian cell mutagenicity assay for the bacterial assay. Some antibiotics are excessively toxic to bacteria and therefore difficult to test in bacterial assays. In this case, it would be appropriate to perform a bacterial assay using concentrations up to the limit of cytotoxicity and to supplement the bacterial assay with a validated in vitro test for gene mutation in mammalian cells.

3.2. Metabolic activation

The in vitro assays should be performed in the presence and absence of a metabolic activation system. The most commonly used metabolic activation system is S9 from the livers of rats treated with Aroclor 1254 or with a combination of phenobarbital and beta-naphthoflavone, but 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 ASSAYS

4.1. Bacterial assay

A bacterial reverse mutagenicity test should be performed according to the protocol set out in OECD Guidance 471.

4.2. In vitro assay for chromosomal effects in mammalian cells

Chromosome aberration assays should be performed according to OECD Guidance 473. These cytogenetics assays should detect clastogenicity and may also detect changes to ploidy. An increase in the number of polyploid cells should be regarded as a potential for aneugenicity which may need to be followed up by further studies.

The mouse lymphoma tk assay with a protocol amended to include measurements of both small and large colonies has been proposed as an alternative method to detect clastogens. Some authorities accept this test as a useful alternative to the in vitro cytogenetics assay. If it should become internationally accepted for this use it may provide a useful alternative to the in vitro cytogenetics assay. If a mouse lymphoma assay protocol is used in place of an in vitro cytogenetics evaluation, it should efficiently detect small colonies (caused by clastogens). This can be done by use of appropriate positive controls. The protocol used should also conform to the criteria set out in OECD Guidance 476.

4.3. In vitro assay for gene mutation in mammalian cells

When an in vitro mammalian cell gene mutation assay is used, it should be performed according to OECD Guidance 476.

4.4. In vivo assay

Either a mammalian erythrocyte micronucleus assay (OECD Guidance 474) or a mammalian bone marrow chromosome aberration assay (OECD Guidance 475) may be performed as part of the initial battery of genotoxicity tests. Although for most genotoxicity endpoints, including gene mutations and clastogenicity, it is not possible to identify a No Observable Effect Level (NOEL), if an eugenicity is the only genotoxic effect seen in vivo, it might then be possible to identify a threshold concentration.

4.5. Reproducibility of results

Reproducibility of experimental results is an essential component of research involving novel methods or unexpected findings; however, the routine testing of chemicals with standard, widely used genotoxicity tests need not always be completely replicated. These tests are sufficiently well characterized and have sufficient internal controls that repetition can usually be avoided by use of protocols with built-in confirmatory elements, such as those outlined below.

For both bacterial and mammalian cell gene mutation tests, the results of a range-finding test should be used to guide the selection of concentrations to be used in the definitive mutagenicity test. A range-finding test may supply sufficient data to provide reassurance that the reported result is the correct one. In bacterial mutagenicity tests, preliminary range-finding tests performed on all bacterial strains, with and without metabolic activation, with appropriate positive and negative controls, and with quantification of mutants, may be considered a sufficient replication of a subsequent complete test. Similarly, a well-reported range-finding test may also be a satisfactory substitute for a complete repeat of a mammalian cell gene mutation test other than the mouse lymphoma tk assay. The range-finding test should be performed with and without metabolic activation, with appropriate positive and negative controls, and with quantification of mutants.

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 well-conducted well-reported validated assays covering all three endpoints in the basic battery of tests will usually be taken as sufficient evidence of an absence of genotoxicity.

In the case of positive or equivocal results the need for further tests should be decided on a case – by-case basis. The decision will be influenced by various factors. These may include: which test(s) gave positive or equivocal results, whether the positive/equivocal results occurred in the presence

or absence of metabolic activation, the type of genotoxic effects seen, and the toxicological characteristics of the test substance and of related substances within the same class of chemicals. If further tests are necessary, then the nature of these tests should be decided on a case-by-case basis.

6. GLOSSARY

Aneugenicity:	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 (this being termed polyploidy).
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 evaluation:	Chromosome analysis of cells, normally performed on dividing cells when chromosomes are condensed and visible with a light microscope after staining.
Gene mutation:	Detectable permanent change within a single gene or its regulating sequences. The change may be point mutations, insertions, deletions.
Genotoxicity:	A broad term that refers to any deleterious change in the genetic material regardless of the mechanism by which the change is induced.
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).
Ploidy:	The number of complete sets of chromosome complements in a cell. (Examples are haploidy, diploidy, triploidy, polyploidy, etc).