参考資料Ｎｏ．２

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

VICH GL22 (SAFETY: REPRODUCTION)
June 2001
For implementation at Step 7

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

Recommended for Implementation
at Step 7 of the VICH Process
on June 2001
by the VICH Steering Committee

THIS GUIDELINE 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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STUDIES TO EVALUATE THE SAFETY OF RESIDUES OF VETERINARY DRUGS IN HUMAN FOOD: REPRODUCTION TESTING

1. INTRODUCTION

1.1. Objective of the guideline

In order to establish the safety of veterinary drug residues in human food, a number of toxicological evaluations are required, including the assessment of any risks to reproduction. The objective of this guideline is to ensure international harmonisation of reproduction testing, which is appropriate for the evaluation of risks to reproduction from long-term, low-dose exposures, such as may be encountered from the presence of veterinary drug residues in food.

1.2. Background

There has been considerable overlap in the reproduction and developmental toxicity testing requirements of the EU, Japan and the USA, for establishing the safety of veterinary drug residues in human food. Although each region differed on some aspects of detail, all required a multigeneration study in at least one rodent species, dosing beginning with the first parental (P0) group and continuing through at least two subsequent (F1 and F2) generations. All three regions also required developmental toxicity (teratology) studies. Developmental toxicity studies are the subject of a separate guideline (see VICH GL -) and will not be further addressed here, except to note that it is no longer recommended that a developmental toxicity phase be included as part of a multigeneration study.

This approach to reproduction and developmental toxicity testing of veterinary products differs in some respects from that adopted by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).1,2 The ICH guideline advocates a combination of three studies, in which dosing extends for shorter periods to cover adult fertility and early embryonic development, pre- and postnatal development and embryo-fetal development. While such an approach is considered appropriate for most human medicines, exposure to veterinary drug residues in human food may be long-term, including exposure throughout life. For long-term, low-dose exposure, a multigeneration study, in which dosing extends through more than one generation is considered more appropriate. This guideline provides harmonised guidance on the core requirement for a multigeneration study for the safety evaluation of veterinary drug residues in human food.

The current guideline is one of a series of guidelines developed to facilitate the mutual acceptance of safety data necessary for the determination of Acceptable Daily Intakes (ADIs) for veterinary drug residues in human food by the relevant regulatory authorities. This guideline should be read in conjunction with the guideline on the overall strategy for the safety evaluation of veterinary residues in human food (see VICH GL -). It was developed after consideration of the existing ICH guideline for pharmaceuticals for human use on "Detection of Toxicity to Reproduction for Medicinal Products"1 and its Addendum, "Toxicity to Male Fertility"2, in conjunction with the current practices for evaluating veterinary drug residues in human food in the EU, Japan, the USA, Australia and New Zealand.

1.3. Scope of the guideline

This document provides guidance on the core requirement for a multigeneration study for those veterinary medicinal products that leave residues in human food. However, it does not seek to limit the studies that may be carried out to establish the safety of residues in human food with respect to reproductive function. Neither does it preclude the possibility of alternative approaches that may offer an equivalent assurance of safety, including scientifically-based reasons as to why such data may not need to be provided. This guideline is not intended to cover the information that may be required to establish the safety of a veterinary product with respect to reproduction in the target species.

1.4. General Principles

The aim of a multigeneration reproduction toxicity study is to detect any effect of the parent substance or its metabolites on mammalian reproduction. These include effects on male and female fertility, mating, conception, implantation, ability to maintain pregnancy to term, parturition, lactation, survival, growth and development of the offspring from birth through to weaning, sexual maturation and the subsequent reproductive function of the offspring as adults. While multigeneration studies are not specifically designed to detect developmental abnormalities because malformed offspring may be destroyed by the dams at birth, such studies may provide an indication of developmental toxicity if litter size at birth, birth weight or survival in the first few days after birth are reduced.

The study of more than one generation allows detection not only of any effects on adult reproduction, but also any effects on subsequent generations due to exposure in utero and early postnatally. Critical aspects of development, which affect adult reproductive capacity, take place prenatally and early postnatally. Adverse effects of sex hormones and their analogues administered during this critical period on reproductive tract development and function in males and females are well known. More recently, studies of other chemicals with endocrine disrupting potential have illustrated the critical role of exposure during the early developmental period on subsequent reproductive function in adult life. This can result in much greater effects on the reproductive capacity of subsequent generations compared with the original parental generation. Studies of more than one generation may also allow detection of reproductive effects due to bioaccumulation of the test substance. Interference with the developing reproductive tract or bioaccumulation may manifest themselves via increasing degree or severity of adverse effects in successive generations.

The design of the study should be such that where any effects on reproduction are detected, the dose(s) at which they occur and the dose(s) giving rise to no adverse effects are clearly identified. Some observations may require further studies to fully characterise the nature of the response or of the dose-response relationship.

2. GUIDELINE

2.1. Number of species

A multigeneration test in one species is normally sufficient. In practice, the majority of multigeneration studies for all classes of chemical have been conducted in the rat and the rat will undoubtedly continue to be the species of choice for most future studies. Provided strains with good fecundity are used, rats generally give more consistent reproductive performance than mice. There is also a much larger historical database available for rats. Reference can also be made, if necessary, to the results of other kinetic, metabolic and toxicity tests on rats within the overall test battery for the compound. However, studies on compounds originally used for other purposes but later proposed for veterinary use have sometimes been conducted in mice for historical reasons. Or there may be good scientific reason to conduct a study in mice (e.g. if there is known metabolic similarity to humans). Provided reproductive performance is satisfactory, there is no general reason why the mouse should not also be an acceptable test species.

It is therefore recommended that a study in a single rodent species, preferably the rat, be conducted.

2.2. Number of generations

Studies in one generation only have been the normal testing requirement for pharmaceuticals for human use, where the main concerns are exposure during short-term dosing periods. However, multigeneration studies of two or three generations have long been the usual requirement for food additives and food contaminants such as pesticides and veterinary drug residues. One-generation studies, in which treatment is terminated when the first generation of offspring is

weaned, do not permit assessment of the reproductive performance of animals that have been exposed to the test substance prenatally through to puberty. A study of more than one generation is therefore considered necessary (see 1.4.).

A study of more than one generation will also allow confirmation of any effects seen in the first generation or clarification of equivocal effects seen at any stage in the test. It may also give an indication effects due to bioaccumulation.

The minimum number of generations necessary to give clear and interpretable results in most cases is considered to be two. While early multigeneration test protocols for some chemical classes required a third generation in certain cases, it is now generally considered that effects which are clear in the third generation can also be adequately detected in the second generation.

It is therefore recommended that a study of two generations be conducted.

2.3. Number of litters per generation

A study with one litter per generation may be sufficient if the results clearly show either absence of any effects or presence of adverse effects with a well-defined no-adverse-effect level. Under certain circumstances however, it may be appropriate to extend the study to produce second litters and it is recommended that results from the study be closely monitored to enable such a decision to be taken, if necessary. The value of second litters is that they may help to clarify the significance of any apparently dose-related or equivocal effects in first litters, which may be either the result of treatment, or due to chance, or to poor reproductive performance unrelated to treatment. Poor reproductive performance in controls can be minimised by avoidance of nutritional problems and other disturbances, ensuring the weight variation of the parental (P0) generation animals is not too great, and by not mating animals when they are too young or too old.

It is therefore recommended that in general a study with one litter per generation be conducted. It may be necessary, under certain circumstances mentioned above, to extend the study by producing second litters.

2.4. Recommended study protocol

The OECD Test Guideline 416 "Two-Generation Reproduction Toxicity Study" 3 is an appropriate reference method for a multigeneration study to establish the safety of any veterinary residues in human food. This OECD Test Guideline, established in 1983, includes discussion of the selection of test animals, selection of doses, timing of commencement of treatment, timing of mating, observations and reporting of results, all of which are relevant for the testing of veterinary products for the safety evaluation of residues in human food. It is noted that this Test Guideline is currently being updated. The Revised Draft Guideline 416 (1999 et seq.),4 in addition to the usual observations included in a multigeneration study conducted according to the 1983 Test Guideline 416,3 also includes evaluation of adult sperm parameters, sexual maturation of offspring and provision for functional investigations of offspring, if such investigations are not included in other studies. The inclusion of these additional parameters is considered appropriate for the testing of veterinary products to modern standards.

3. REFERENCES

1. ICH. 1993. ICH Harmonised Tripartite Guideline S5A. Detection of Toxicity to Reproduction for Medicinal Products. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

2. ICH. 1995. ICH Harmonised Tripartite Guideline S5B. Toxicity to Male Fertility: An Addendum to the ICH Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal Products. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

3. OECD. 1987. Test Guideline 416. In: Guidelines for the Testing of Chemicals. Two-Generation Reproduction Toxicity Study. Paris, Organisation for Economic Cooperation & Development.

4. OECD. 1999. Test Guideline 416. Two-Generation Reproduction Toxicity Study. Revised Draft Guideline 416, August 1999. Paris, Organisation for Economic Cooperation & Development.

参考資料Ｎｏ．３

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

VICH GL23 (SAFETY: GENOTOXICITY)
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STUDIES TO EVALUATE THE SAFETY OF RESIDUES OF VETERINARY DRUGS IN HUMAN FOOD: GENOTOXICITY TESTING

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

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 genotoxins 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 Guideline on General Testing Approach). 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 and New Zealand.

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, eg. 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.

An *in vitro* test for chromosomal effects in mammalian cells.

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 & TA 100. 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 (eg. inorganic arsenic compounds).

The second test should evaluate the potential of a chemical to produce chromosomal effects. In the EU, the *in vitro* cytogenetic test using metaphase analysis is preferred, which detects both clastogenicity and aneugenicity. In the USA, the mouse lymphoma test is preferred, which, with modification, can detect both gene mutation and chromosomal damage. Either test is acceptable in Japan.

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 (eg. 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 according to the protocol set out in OECD Test Guideline 471.

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

Chromosome aberration tests should be performed according to OECD Test Guideline 473. These cytogenetic tests should detect clastogenicity and may also detect heteroploidy. To detect induction of polyploidy, longer (eg. 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 (eg 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.

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 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.

4.4. *In Vivo* Test for Chromosomal Effects

Either a mammalian erythrocyte micronucleus test (OECD Test Guideline 474) or a mammalian bone marrow chromosome aberration test (OECD Test Guideline 475) may be performed as part of the initial battery of genotoxicity tests. The mammalian erythrocyte micronucleus 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.

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

OECD. 1997. Test Guideline 473. *In Vitro* Mammalian Chromosome Aberration Test. In: OECD Guideline for Testing of Chemicals. Paris, Organization for Economic Cooperation & Development.

OECD. 1997. Test Guideline 474. Mammalian Erythrocyte Micronucleus Test. In: OECD Guideline for Testing of Chemicals. Paris, Organization for Economic Cooperation & Development.

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

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.

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.