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STUDIES TO EVALUATE THE SAFETY OF RESIDUES OF VETERINARY DRUGS IN HUMAN FOOD: REPRODUCTION TESTING (REVISION 1)

Revision at Step 9

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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.

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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 effects on reproduction. The objective of this guideline is to ensure international harmonisation of reproduction testing that is appropriate for the evaluation of effects on reproduction from long-term, low-dose exposures; these effects may be encountered from the presence of veterinary drug residues in food.

1.2. Background

There was a 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 in some aspects of detail, all required a multigeneration study in at least one rodent species, dosing beginning with the parental (P) group and continuing through at least two subsequent (F_1 and F_2) generations. All three regions also required developmental toxicity (teratogenicity) studies. Developmental toxicity studies are the subject of a separate guideline (see VICH GL32) and will not be further addressed in this guideline, except to note that it is no longer recommended that a developmental toxicity phase be included as part of a reproduction toxicity study.

The VICH approach to reproduction and developmental toxicity testing of veterinary drug residues 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] The ICH guideline advocates a combination of three studies, in which dosing extends for shorter periods to cover adult fertility and early embryonic development, embryo-fetal development, and pre- and postnatal 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 lifetime exposure. For long-term, low-dose exposure, a reproduction toxicity 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 including extended one-generation reproductive toxicity study (EOGRTS) for the safety evaluation of veterinary drug residues in human food.

This guideline is one of a series of guidelines developed to facilitate the mutual acceptance, by the relevant regulatory authorities, of safety data necessary for the determination of Acceptable Daily Intakes (ADIs) for veterinary drug residues in human food. 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 GL33). It was developed after consideration of the existing ICH guideline for pharmaceuticals for human use on “Detection of Developmental and Reproductive Toxicity for Human Pharmaceuticals”[1] and the European Chemicals Agency publication on “Evaluating results from 55 extended one-generation reproductive toxicity studies under REACH: final report of the EOGRTS review project”,[2] in conjunction with the current practices for evaluating veterinary drug residues in human food in the EU, Japan, the USA, Australia, Canada, New Zealand, and the UK.

1.3. Scope of the guideline

This document provides guidance on the core requirement for a multigeneration study including EOGRTS for those veterinary drugs that leave residues in human food. However, it does not seek to limit the studies that may be performed to establish the safety of veterinary drug 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 veterinary drug residues with respect to reproduction in the target species.

1.4. General principles

The aim of a multigeneration reproduction toxicity study including EOGRTS is to detect any effects of veterinary drug residues (i.e., the drug substance and/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 the reproduction 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.

Reproduction testing intends to detect not only any effects on adult reproduction, but also 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. Effects on reproductive tract development and function in males and females following exposure to sex hormones and their analogues during this critical period are well known. 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 effects in successive generations.

The design of the study should be able to detect any effects on reproduction, the dose(s) at which they occur and the dose(s) giving rise to no adverse effects. The highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering.[3, 4]

2. GUIDELINE

2.1. Test species

A multigeneration test including EOGRTS in one animal species is normally sufficient. In practice, these studies for all classes of chemicals have been conducted in the rat, which will continue to be the species of choice for most 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 test substance.

The rat is the preferred species for testing. If other species (such as mouse) are used, justification should be given. For example, studies on test substances originally used for other purposes but later proposed for veterinary use have sometimes been conducted in mice. Also, there may be scientific reason to conduct a study in other species, such as when the mouse is a more appropriate model due to metabolism in common with the target animal species or similar metabolites formed as those predicted in humans.

2.2. Number of generations

Studies in one generation 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 from the prenatal to pubertal period. A multigeneration reproduction toxicity study including EOGRTS is therefore considered necessary for this assessment and to evaluate the reproductive effects of long-term exposures (see Section 1.4.).

A study of more than one generation will also allow confirmation of any effects in the first generation, clarify equivocal effects at any stage in the test, or give an indication of effects that are not observed in the first generation.

The minimum number of generations necessary to give clear and interpretable results in most cases is considered to be two. In some cases, an extended one-generation test protocol as described in OECD Test Guideline 443 may also be acceptable.[4] A decision on whether to assess the second (F2) generation should reflect existing knowledge of the chemical being evaluated. Criteria for internal triggers for extending the study to the second generation are described in OECD Guidance Documents 117 and 151.[5, 6]

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

2.3. Number of litters per generation

A study with one litter per dam and per generation is sufficient if the results clearly show either absence of any effects or presence of adverse effects with well-defined no-observed-adverse-effect levels (NOAELs). Under certain circumstances, however, it may be appropriate to extend the study to produce second litters. 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, due to chance, or due 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 (P) generation animals is not too large, 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 dam and per generation be conducted. It may be necessary, under certain circumstances mentioned above, to extend the study by producing second litters and it is recommended that results from the study be closely monitored to enable such a decision to be taken, if necessary.

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 reproduction of veterinary drug residues in human food. This guideline includes discussion of the selection of test animals, selection of doses, timing of commencement of treatment, timing of mating, observations, evaluation, and reporting of results, all of which are relevant for the testing of veterinary drugs for the safety evaluation of residues in human food.

If an extended one-generation study is planned, the OECD Test Guideline 443, “Extended One-Generation Reproductive Toxicity Study”,[4] is an appropriate reference method. In addition to evaluating the reproduction safety, the EOGRTS protocol allows additional investigation on the developing nervous and immune systems. However, VICH considers the males of the parental (P) generation in the pre-mating period should be dosed to cover at least one complete spermatogenic cycle, e.g., a minimum of 10 weeks in the pre-mating period rather than the two weeks for rats, as described in the EOGRTS protocol.[4] It is important to leverage existing data and knowledge and use a weight-of-evidence approach to help determine whether an EOGRTS is appropriate.

If a benchmark dose approach is intended as an alternative to the NOAEL approach, the study design, such as dose selection, number of dose groups and number of animals per group, should be considered accordingly.

3. REFERENCES

1. ICH. 2020. ICH Harmonised Tripartite Guideline S5(R3). Detection of reproductive and developmental toxicity for human pharmaceuticals. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

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6. OECD. 2013. OECD Environment, Health and Safety Publications, Series on Testing and Assessment, No. 151. Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test. Organisation for Economic Cooperation & Development.