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GUIDELINES FOR THE CONDUCT OF PHARMACOKINETIC STUDIES IN TARGET ANIMAL SPECIES

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CONDUCT OF PHARMACOKINETIC STUDIES IN TARGET ANIMAL SPECIES

1. INTRODUCTION

This note for guidance replaces the previous guidance note (III/8089/91) in Volume 7A (1998).

Pharmacokinetic studies with veterinary medicinal products can be carried out to support studies on clinical efficacy, tolerance in the treated animal and safety for the consumer. The principal objectives are to estimate the factors involved in the absorption, distribution, metabolism and elimination (basic pharmacokinetic studies) of active substances in the product formulation.

Another objective can be to obtain pharmacokinetic parameters allowing the comparison between the bioavailability of the active substance from two or more product formulations and/or routes of administration (specific bioequivalence studies). This particular application of pharmacokinetic studies is described in another guideline (conduct of bioequivalence studies in animals).

This note is proposed to provide guidance and assistance to applicants in the design, execution and interpretation of pharmacokinetic investigations of a given active substance in the target species in relation to efficacy and safety issues, irrespective of the route of administration of the veterinary medicinal product, the pharmacological class of the active substance or the animal species in which use of the product is intended.

This note for guidance only considers general principles and all the points mentioned do not necessarily apply to each active substance and all species. Therefore, each study should be planned and designed to take into account the properties and uses of the active substance and the anatomical, physiological and behavioural peculiarities of the species in which the active substance is investigated.

Pharmacokinetic studies are essential to employ medicinal products under the best conditions of efficacy and safety, to establish effective plasma concentrations and correct therapeutic schedules or to proceed to dosage adjustments in particular cases (e.g. drugs that require dosage adjustments during repeated administrations such as antiseizure, sedative agents, chemotherapeutic agents). This particularly applies to veterinary medicinal products with a narrow therapeutic range and to those for which a close relationship between plasma concentrations and therapeutic or toxic effects may be demonstrated or expected.

When relevant in terms of efficacy or risk for consumers, pharmacokinetic parameters of main metabolites should be investigated.

When of relevant importance the problem of isomerism should be kinetically considered (see appropriate guideline Investigating chiral active substances, EMEA/CVMP/128/95). For active substances that will be administered as racemate, separate pharmacokinetic studies should be performed for both enantiomers, unless their pharmacodymamic or toxic properties are the same.

2. PHARMACOKINETIC FACTORS TO BE INVESTIGATED

Depending on the active substance and its use(s), all or some of the following items should be studied: active substance release from the dosage form (product), absorption, distribution and elimination (metabolism and excretion).

2.1 Absorption

Both the rate and extent to which the active substance or active moiety are available systemically should be determined.

Generally the rate of absorption can be determined only from plasma/blood concentration-time curve data following extravascular administration; urine data can be used only to measure the extent of absorption, in case urine is the main route of elimination.

a) active substances intended to produce systemic effects

Whatever the route of administration (e.g. oral, intramuscular, subcutaneous, transdermal, pulmonary) of the veterinary medicinal product, the rate of absorption of the active substance should be quantified.

Whenever possible, comparison with an equivalent intravenous dose should be made, as only intravenous (bolus or infusion) data permit the evaluation of the absolute systemic availability.

Preferably, a precise pharmacokinetic analysis of the entire plasma concentration profile, including absorption, distribution and elimination should be made. This refers particularly to special formulations, for which delayed release of the active substance or a prolonged duration of action is claimed. Failing this, at least data on active substance(s) and relevant metabolite(s) concentration at peak (Cmax), time to reach peak (Tmax) and area under the concentration/time curve ($AUC_{0-\infty}$ and AUC_{0-t}) should be provided.

For orally administered products pharmacokinetic studies in the target species should be performed under standardized feeding conditions taking into account the modalities for drug administration.

If there is reason to expect that some physiological status of the gastrointestinal tract (fed *versus* fasted state, presence of food), might substantially affect absorption after per oral administration separate pharmacokinetic studies in suitable groups of target animals should be performed.

b) active substances not intended to produce systemic effects

Showing absence of absorption may substantiate the prediction of no systemic effect of the active substance.

In this case systemic availability is usually determined by measurement of the concentration of the active substance (or therapeutic moiety or metabolite) in plasma as a function of time. Urinary and biliary excretion (active substance, metabolites) and acute pharmacological effects may also be considered.

In the case of active substances in veterinary medicinal products intended for topical use which can produce systemic effects at very low plasma levels (e.g. corticosteroids, antibiotics, ectoparasiticides) or in particular pharmaceutical dosage forms (e.g. intrauterine, intramammary formulations) passage into circulation should be studied.

2.2 Distribution

The extent of distribution will often be reflected in the volumes of distribution. If appropriate, these distribution parameters should be estimated. When relevant to the claims or safety aspects, distribution in body fluids (CSF, synovial fluid, mucus, milk, etc.) and in body tissues (lung, liver, kidney, skeletal muscle, etc.) can be considered in order to substantiate clinical efficacy of new active substances. This constitutes the distribution pattern of the active substance.

If plasma protein binding of the active substance is considered to contribute much to its distribution the percentage and, in certain cases, characteristics of binding (e.g. amount, duration) of the active substance to plasma proteins should be studied over the anticipated range of plasma concentrations obtained after administration of the proposed dose(s).

2.3 Metabolism

Normally, formation of metabolites should be investigated.

If there is an indication that pharmacologically or toxicologically active metabolites are formed and if there is reason to suspect that they contribute to the therapeutic activity and/or adverse effects, the pharmacokinetic data on such metabolites, the rate of their formation, distribution and elimination should be investigated in target animals.

2.4 Excretion

The relative contribution of the different routes of excretion of the total substance [active substance + metabolite(s)], if relevant to the claimed effect, should be quantified (e.g. expressed as a percentage of the administered dose). For example, it is useful to know the fraction of the dose subjected to renal and/or hepatic elimination in order to predict the influence of renal and/or hepatic disease on elimination of the active substance from plasma.

3. METHODOLOGY AND CONDITIONS OF STUDY

All pharmacokinetic studies should be performed according to validated and internationally accepted methods. Studies conducted following Good Laboratory Practice (GLP) indications are preferred.

3.1 Subjects

Studies should be performed in representative animals under well defined and controlled conditions. The animal species, breed, group size, age (adult, young, neonate) and gender should be specified and justified. In the particular case of population pharmacokinetic studies, data should be obtained from the field.

Basic pharmacokinetic studies should be carried out in a sufficient number of clinically healthy animals of the target population. The number of animals used should be justified.

3.2 Administration

Special attention should be given to the route and method of administration of the veterinary medicinal product, as this may affect the absorption of the active substance.

For administration of the active substance to individual animals, dose should be expressed on a body weight basis; if the dose is intended to be on a body area basis, it should be expressed both on a body weight and body area basis. The procedure used to estimate body area should be described.

In case of fixed formulations, e.g. tablet or bolus, which cannot be administered precisely, i. e. on a mg/kg body weight basis, dose data should be corrected to individual body weight.

When the product is administered via the feed or drinking water, the daily dose of the active substance in mg/kg b.w. should be calculated, preferably on an individual animal basis. If the exact dose per animal cannot be measured, the dose should be expressed using the most appropriate reference in order to give reliable values and a measure of the variance (e.g. number of groups, number of animals per group, concentration in food and quantity of ingested food for medicated feeds).

For substances or active substances intended to be administered via food or drinking water, special attention should be given to the behaviour of the animals (daily intake of food and water, delay between intake of active substance and sampling, etc.).

The nature and characteristics of the excipient(s) contained in the product under investigation should be described.

If radioactive isotopes are used, the tracer dose may be combined with a quantity of non-labelled substance/active substance to attain the proposed dose range.

3.3 Fixed combinations

For most fixed combinations, bioavailability studies should be performed on each active substance as well as the combination product to evaluate possible interactions. Exceptions may include certain topical or local treatments, but omission of data should be justified.

3.4 Specification of products

Specification of the physicochemical properties of the active substance and formulation of the veterinary medicinal product should be given.

3.5 Dosing

For a new active substance, kinetic studies using at least three different doses should be performed, the central dose being the expected recommended dose as determined by dose determination studies. Omission of the data should be justified. Appropriate statistical tests should be carried out to determine linearity.

For established active substances where dose linearity exists in the target species, single dose studies, corresponding to the highest intended therapeutic dose, are generally sufficient. Where there is no dose-linearity or a very steep dose/effect curve, studies using three different dose levels may be necessary if a range of therapeutic doses is recommended, the central dose being the median of the dose range. If the active substance is used only at one dose level, a single-dose study may be sufficient, but should be justified.

If the posology requires repeated treatment or if therapeutic use of the substance/active substance relies on steady-state conditions, multiple dose and steady-state experiments should be performed.

Multiple dose studies and steady-state experiments should be conducted with the recommended dosage regimens (dose, dosing interval, number of administrations); multiple dose studies should give insight into questions such as accumulation kinetics, steady-state levels, linearity and induced effects (e.g. altered metabolism rate and altered disposition). Comparison of plasma concentration profiles after administration of the first and last dose is highly desirable.

Multiple dosing followed by examination of the washout period may elucidate the existence of a slow elimination phase which might not be detected following a single dose.

3.6 Sampling

Suitable biological fluids (blood, plasma, serum, urine, etc.) and tissues should be selected for pharmacokinetic investigation. Plasma is generally considered to be the most useful biological fluid for such studies.

a) Blood sampling

Attention should be given to the site of blood collection, sampling procedure, material used for sampling, blood collecting tubes, anticoagulant and conditions of centrifugation to obtain plasma. The stability of the substance during sampling and under conditions of storage pending analysis should be assessed.
The number of blood samples and the timing of sampling should be appropriate to allow an adequate determination of absorption, distribution and elimination.

Blood samples in the post-absorption phase should be obtained over as long a period as is necessary for the purpose of the investigation.

b) Other biological fluids and tissues

In some cases, the collection of other biological fluids and tissues may be considered appropriate for the determination of general pharmacokinetic parameters (e.g. if analytical constraints limit the usefulness of blood samples, urine samples may be used to determine the terminal disposition slope, if this is the main route of elimination) or of particular interest (e.g. local distribution to support a claim).

Collection of some of these fluids requires special attention (e.g. immediate pH measurement of urine, conditions of storage, etc.).

For tissues, repeated biopsies using local anaesthesia may be considered for scientific reasons as this conserves animals and allows an individual-based analysis. However, special attention should be paid to ascertain absence of pain and discomfort when using a biopsy method.

3.7 Analytical procedure

Active substance concentration should be determined using appropriate analytical methods. The use of a chemical assay method is generally preferable (e.g. HPLC methods).

The method should be accurately described in an internationally accepted standard and the following items should be reported:

- purpose and scope
- reagents and apparatus
- collection and storage of samples
- preparation (and eventual cleanup) of samples
- procedure for the measurement of plasma or serum concentrations
- calculation of results (e.g. method of standardization, mathematical model, calibration curves)
- statistical analysis
- quality control (internal)

The method chosen should be adequately validated and the following characteristics should be given:

- specificity (if applicable)
- accuracy
- precision (repeatability, i.e. intra and inter assay variation)
- limit of detection
- limit of quantitation
- susceptibility to interference where appropriate [known experimental conditions that may be subjected to fluctuation (e.g. stability of reagents or samples, pH, temperature) affecting the analytical results should be indicated]

3.8 Pharmacokinetic calculations and interpretation

Appropriate mathematical methods should be used to generate basic parameters (compartmental and/or "non-compartmental" analysis).

Appropriate pharmacokinetic computer programs should be used under specified conditions (regression methods, weighting factor, etc.).

Pharmacokinetic parameters should be calculated using time concentration data from individual animals and these parameters should be expressed with the indication of the mean and variation values. The individual animal data should be provided

However, when using population kinetic approach (data obtained from the field) or when sparse data are obtained (for example from fish or poultry) a pooled data analysis should be performed (e.g. non linear mixed model) using appropriate software.

Standard equations or equivalent calculations should be used to calculate pharmacokinetic parameters and interpretation provided of the values obtained.

Dosage regimen determination (to include the size of dose, dosing interval and route of administration) should take into account the range of therapeutic plasma concentrations, systemic availability of active substance from the dosage form (veterinary medicinal product) administered and values of pharmacokinetic parameters in the target species.

Special approaches (simultaneous modelling of pharmacokinetics and pharmacodynamics, population kinetics, deconvolution for the study of controlled-released formulations, etc.) are encouraged if applicable, especially where appropriate to the efficacy of the active substance (e.g. AUC/MIC and Cmax/MIC ratios for antimicrobial agents).