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Committee for Veterinary Medicinal Products (CVMP)

Guideline on injection site residues

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This guideline replaces the guideline on injection site residues (EMA/CVMP/542/03-FINAL).

Keywords	*Injection site residues, withdrawal periods*

*The current revision consists of administrative changes made in order to align the guideline to the new definitions and terminology provided by Article 4 of Regulation (EU) 2019/6. The reference to Injection Site Residues Reference Values (ISRRV) was added. The references to the legislation applicable and other scientific guidelines have also been updated.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
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Guideline on injection site residue

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Executive summary

This document, originally published in 1994 as a working document on principles concerning the assessment of injection site residues and replaced in 2004 by the guideline on injection site residue, provides detailed guidance on how to establish withdrawal periods when considering injection site residues and was developed by the CVMP in order to provide a standardised approach for derivation of withdrawal periods within the European Union. This guideline should be used in conjunction with the 'Guideline on determination of withdrawal periods for edible tissues' (EMA/CVMP/SWP/735325/2012 Rev.2) [1].

1. Introduction (background)

Consumer safety needs to be assessed for all pharmacologically active substances which are intended for use in food producing animals in accordance with Regulation (EC) No 470/2009 [2]. Pre-slaughter withdrawal periods are determined in order to ensure that the residues deplete to permissible concentrations. Whereas the maximum residue limit (MRL) or Injection Site Residues Reference Value (ISRRV) for muscle applies to the active substance itself, the withdrawal period is set individually for each veterinary product as part of the marketing authorisation process.

Apart from the pharmaceutical formulation and the dose and frequency of dosing, the length of the withdrawal period is largely dependent on the route of administration. Injectable formulations may exhibit depletion kinetics from injection sites which are significantly slower than those in other edible tissues. This may be attributed to their design as slow release or depot formulations, to the physico-chemical properties of the substance itself or of the formulation or to other factors such as whether the product is administered subcutaneously or into the muscle itself or into connective tissue between muscles. Following administration, tissue reactions such as fibrosis, encapsulation or necrosis are another potential cause for retarded release of substances from injection sites. Consequently, residues at injection sites can be comparatively high, and tend to deplete erratically so that animal to animal variation is sometimes large. Non-uniform dispersion at the site of administration of certain drug formulations may lead to non-homogeneous residue distribution patterns. Unlike other tissues, the exact location of injection site samples taken for analysis can have a considerable impact on the residues found. Furthermore, metabolism and/or degradation of substances at injection sites and, as a result, the overall composition of residues can be quite different from that in other tissues. This all shows that, from a pharmacokinetic point of view, injection site may not be directly comparable with muscle or other edible tissues. Accordingly, withdrawal periods established for muscle tissue remote from the injection site are normally not adequate to ensure that residues at injection sites have depleted to concentrations below the MRL and Acceptable Daily Intake (ADI) or other reference value. Therefore, residues at injection sites need particular consideration as regards their possible risk for consumers of treated animals.

Considering the characteristics of injection site residues, the CVMP agreed in 1994 a Working Document (III/5933/94-EN) on principles concerning the assessment of injection site residues. In 2005, this working document was replaced by the present guideline, which underwent an update in 2021 taking notice of the most recent regulation and guidance on the assessment of residues and establishment of withdrawal periods. See further section 'Assessment' for recent regulation and guidance documents.

2. Scope

This guideline addresses the assessment of potential consumer risk from veterinary drug residues remaining at intramuscular and subcutaneous injection sites and the elaboration of appropriate pre-

slaughter withdrawal periods. Risk management aspects e.g. implications of injection site residues for residue monitoring and surveillance programs are not within the scope of this guideline.

All *in vivo* studies conducted by an applicant to support the establishment of withdrawal periods should be in accordance with the requirements of Directive 2010/63/EU [3] on the protection of animals used for scientific purposes and the 3Rs principles of replacement, reduction and refinement (EMA/CHMP/CVMP/JEG-3Rs/450091/2012 [4]; EMA/CHMP/CVMP/3Rs/164002/2016 [5]).

3. Legal basis

In line with article 10, 11, 14, 16 and 35 of Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC, [6] the product information for veterinary medicinal products for use in food producing species shall contain information on the withdrawal period. The dossier requirements in order to enable the determination of withdrawal periods are mentioned in part II. 3B and IIIa. 3B of the Commission Delegated Regulation (EU) 2021/805 [7]. Article 4 of Regulation (EU) 2019/6 defines the withdrawal period:

"(34) 'withdrawal period' means the minimum period between the last administration of a veterinary medicinal product to an animal and the production of foodstuffs from that animal which under normal conditions of use is necessary to ensure that such foodstuffs do not contain residues in quantities harmful to public health"

4. Assessment

In consideration of the risk assessment of a substance according to Regulation (EC) No 470/2009 [2] and the conclusions of the corresponding European public MRL assessment report (EPMAR), the assessment of residues at injection sites and the determination of withdrawal periods is to be based on the MRL, the Injection Site Residue Reference Value (ISRRV), the ADI or, if necessary, an alternative exposure limit. The assessment of injection site residues should follow the general principles set out in

- Regulation (EU) 2018/782 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009 [8], which replaced the former Volume 8 [9].
- The CVMP Guideline on determination of withdrawal periods for edible tissue (EMA/CVMP/SWP/735325/2012) Rev. 2 [1], which replaced the former Note for Guidance: Approach towards Harmonisation of Withdrawal Periods (EMEA/CVMP/036/95) [10]
- and the VICH guidelines: VICH GL 48 on marker residue depletion studies to establish withdrawal periods [11], VICH GL 49 on validation of analytical methods used in residue depletion studies [12] and VICH GL 57 on marker residue depletion studies to establish withdrawal periods in aquatic species [13].

It should be noted that the withdrawal period at the injection site obtained according to this guideline is not necessarily the final withdrawal period for the product. The withdrawal period at the injection site is to be compared with the withdrawal periods based on the depletion of residues in the other edible tissues and the longest of these withdrawal periods will be considered as the regulatory withdrawal period for the veterinary product under consideration.

As a default approach establishment of the withdrawal period is at the time point where the concentrations of residues in all tissues for all animals are at or below the respective MRLs as are laid down in Commission Regulation (EU) No 37/2010 [14] or below the ISRRV if applicable. If no MRLs or ISRRV are available other reference values may be used, such as the ADI or alternative exposure limit).

See also figure 1 and the Guideline on determination of withdrawal periods for edible tissue (EMA/CVMP/SWP/735325/2012 Rev. 2) [1].

4.1. MRL based approach

For substances where there is an MRL for muscle, the injection site is usually treated as muscle tissue and the assessment of the residues should take into consideration the MRL and the marker residue in muscle. The withdrawal period should ensure that the concentration of the marker residue has depleted below the muscle MRL at the injection site[1,2.] Withdrawal periods should be set according to the Guideline on determination of withdrawal periods for edible tissue (EMA/CVMP/SWP/735325/2012 Rev. 2) [1].

Experience shows that the MRL based approach, in most cases, leads to adequate and safe withdrawal periods at the injection site. When this approach is applied, it should however be ascertained that the marker residue in muscle is valid for predicting the residues of concern at injection sites as well, for example, a marker residue may not be considered appropriate if it is not a component of the residue at the injection site (e.g. a metabolite in muscle not present at injection sites)[3]. In other words, in certain circumstances, the MRL based withdrawal period does not necessarily ensure that residue intake in the standard food basket including the injection site is below the ADI. If there is any indication that the MRL based approach might be inconsistent with the ADI, an ADI based estimate needs to be performed to confirm the appropriateness of the calculated withdrawal period (for ADI based approach see below).

4.2. ISRRV based approach

As depicted in Regulation (EU) 2018/782 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009 [8], an Injection Site Residue Reference Value ('ISRRV') may have been established for those injectable substances for which depletion of injection site residues would result in extended (prohibitive) withdrawal periods when compared to the muscle MRL. The ISRRV is set at a level that ensures that, at the likely withdrawal period, a standard food basket including 300g of injection site muscle would contain residues below the ADI. The ISRRV is not published in the Annex to Commission Regulation (EU) No 37/2010 [14]; however, the value is available in the European Public MRL Assessment Report ('EPMAR').

The derivation of the withdrawal period using the ISRRV based approach is in principle analogous to the approach described for MRL based approach. The withdrawal period is to be estimated by comparing residue data with the ISRRV.

4.3. ADI based approach

For substances where there is no MRL for muscle (usually substances with a "no MRL required" entry in table 1 of the Annex to Commission Regulation (EU) No 37/2010 [14]) or ISRRV, the reference value for the assessment of injection site residues is usually the ADI.

An ADI based assessment of residues at injection sites should cover all relevant ADI endpoints of a substance (i.e., the pharmacological, toxicological and microbiological ADI, if necessary). Depending on

[1] For lipophilic substances it might be necessary to investigate and assess residues in fat at the injection site, where the product is administered by subcutaneous injection and residues are present in the fat layer over the site of injection. In such cases, the residues in that fat layer should be compared to the MRL for fat.

[2] The standard edible portion of 300 g muscle is assumed to consist entirely of injection site tissue.

[3] According to Regulation (EC) No 470/2009 and associated guidelines, selection of the MRL and marker residue in muscle is to be based solely on the residue pattern observed in non-injection site muscle. Hence, this marker and its ratio marker/total residues are not automatically predictive for the injection site residues. If the muscle marker residue is not present/"under-represented" at the injection site, the withdrawal period according to the MRL approach alone may be too short to ensure that residues of concern at the injection site and in a food basket including the injection site have reached levels below the ADI.

the type of ADI, residues of concern may be either the total drug related residues or the toxicologically, pharmacologically and/or microbiologically active fraction of the total residues.

Estimates of dietary exposure to residues are to be based on the standard food basket which should include the injection site. The injection site is treated as muscle tissue and the 300 g food basket portion of muscle tissue should represent the residues at the injection site.

The procedure for the calculation of the withdrawal period according to the ADI based approach is as follows (see also fig. 2):

i. Determine the amount of residue of concern in the 300 g injection site portion for each animal on each time-point as well as the amount of residues of concern in the other edible tissues (taking account of ratios marker/residue of concern, if necessary)[4];

ii. For each animal on each time-point, determine the sum of the residues in the standard food basket, where the residue amount in muscle is replaced by the amount at the injection site as derived under (i);

iii. Identify the appropriate ADI;

Estimate the withdrawal period based on the Guideline on determination of withdrawal periods for edible tissue (EMA/CVMP/SWP/735325/2012 Rev.2) [1].

4.4. Approach based on alternative exposure limit

This applies in principle to substances with a "no MRL required" entry in table 1 of the Annex to Commission Regulation (EU) No 37/2010 [14] and for which no ADI has been established but when used in specific injectable formulations, have the potential to leave relatively high amounts of residues at injection sites that may still possess biological activity. Examples for appropriate exposure limits may be the recommended upper dietary allowance (e.g. vitamins), tolerable upper intake levels (e.g. minerals/trace elements), naturally occurring base levels for compounds which also occur endogenously or any other appropriate limit. The appropriateness of the chosen exposure limit and assessment approach needs to be scientifically justified.

The derivation of this withdrawal period is in principles analogous to the approach described for MRL, ISRRV or ADI based approach. The withdrawal period is to be estimated by comparing residue data with the alternative limit which usually refers to a certain concentration (i.e. analogous to the MRL or ISRRV approach) or an amount of residues (i.e. analogous to the ADI approach).

5. Residue Studies

5.1. General Principles

Residue studies at the injection site, including description and validation of the analytical methods used, should be in conformity with the general principles and requirements described in Regulation (EU) 2018/782 establishing the methodological principles for the risk assessment and risk management recommendations referred to in Regulation (EC) No 470/2009 [8], and should be conducted in accordance with VICH GLs 48 [11] and 49 [12] and VICH GL 57 for aquatic species [13].

[4] The standard edible portion of 300 g muscle is assumed to consist entirely of injection site tissue. This 300 g consumption figure must not be confused with the ca 500 g target weight of the test samples to be collected for analysis in residues depletion studies (see section 'Study design and Sampling' and Figure 2). The amount of residues in the 300 g food basket portion is to be derived from the concentration in this 500 g test sample (for details see example in figure 2).

For an injectable product, the residues of concern remaining at injection sites need to be known. For products containing new active substances, this normally implies appropriate experimental characterisation of the drug-related residues, including metabolites and degradation/conversion products of possible biological significance. This information is generally obtained in radiometric residue depletion studies (i.e., total residues; general guidance on conducting radiometric residue studies can be found in VICH GL 46 [15]) or, where appropriate, in residue depletion studies aiming at characterisation of the pharmacologically, toxicologically or microbiologically active residue components.

For products containing known substances with known composition of the residues (of the active ingredient) at the injection site, radiometric residue depletion studies are normally not necessary and it is sufficient to measure parent compound or any other relevant residue component at the injection site (e.g. the marker residue where appropriate, see above paragraph). Supporting data to estimate the residue of concern (e.g. on the basis of ratios) may then be obtained from published literature.

In the case of generic products administered subcutaneously or intramuscularly, small differences in composition may have significant effects on injection site depletion which may not be detected in the standard blood level bioequivalence studies. Therefore, for such formulations, in addition to the demonstration of bioequivalence, equivalent depletion of residues from the injection site should be demonstrated, in order that the withdrawal period established for the reference product can be adopted. However, if it is demonstrated that the depletion is slower at the injection site, resulting in a longer withdrawal period than that established for the reference product, this longer withdrawal period should be taken as the overall withdrawal period. See also 'Bioequivalence GL, section 4.4' [16]. If it is demonstrated that the depletion of the generic product is faster at the injection site when compared to the reference product, then the established withdrawal period for the reference product will still be adopted. This is because it is not known whether the other edible tissues may be withdrawal determining.

For substances having an MRL for muscle or ISRRV, and for which therefore depletion of the marker residue in muscle below this MRL or ISRRV has to be demonstrated at the injection site, a marker residue depletion study has to be supplied. Where the injectable product contains a specifically formulated derivative of the marker residue (e.g. ester derivative of parent compound), the approved regulatory analytical method for muscle may need some specific modification (e.g. clean-up, cleavage/hydrolysis steps etc) to determine the actual concentration of marker residue at the injection site.

5.2. Study Design and Sampling

In residue studies, the veterinary product should be administered at the site that would be used as injection site in veterinary practice, also reflecting meat industry guidance to avoid injection site tissue damage/rejection of prime cuts of meat. It is essential that data be generated to demonstrate maximum possible residues. Therefore, the formulated veterinary product should be administered in full compliance with the intended label instructions. Residues should be examined following application of the maximum possible dose and, where the intended use requires multiple treatments, the product should be used accordingly and for the maximum number of treatments. Animals should be representative of the age/weight group of the target animal population for which the product is intended and normal conditions of animal husbandry should be used throughout the study. See also VICH GL 48 for guidance on study design [11].

As residues at injection sites may not only be correlated with the (relative) dose but also with the (absolute) amount of the drug injected, residue depletion studies should also include maximum possible injection volumes. If a product is intended for animals of all ages/weight classes but residue depletion studies are only available in small/low weight animals and for comparatively small injection volumes, it is normally not possible to extrapolate the expected residues following the maximum possible dose

volume[5]. In the case of generic products, where known, it is recommended that the maximum injection volume administered per injection site should be the same as that approved for the reference product. In all cases, the practicality of the injection volume, animal welfare and local tolerance should be taken into account.

For the purpose of residue studies, the site of injection should be permanently marked so that it can be clearly located and identified at slaughter. The veterinary product should then be administered at the centre of the underlying tissue. The injection site should be sampled immediately after slaughter.

The method of tissue sampling at the injection site can affect the apparent concentration of residues and so it is desirable that the sampling method is as optimal as far as reasonably practicable: slaughter and sampling procedures should be designed to ensure removal of a representative tissue sample from the region where maximum residue concentrations occur (see also fig. 2).

As a standard approach, it is recommended to collect a primary sample (core sample) of the injection site as described below.

Where the size of the animal allows it, an approximately 500 g piece of tissue is removed for homogenisation. This sample should be centred on the point of injection and take the form of a cylinder with approximate dimensions as given in the table below:[6]:

	Cattle / Sheep	Pigs	Poultry
Injection Site Muscle	Core of muscle tissue ~500 g 10 cm diameter x 6 cm deep for IM; 15 cm diameter x 2.5 cm deep for SC	Core of muscle tissue ~500 g 10 cm diameter x 6 cm deep for IM; 15 cm diameter x 2.5 cm deep for SC	Collect sample from entire site of injection, e.g., chicken whole neck, whole breast or whole leg. Larger birds, not to exceed 500 g

Care must be taken to ensure that, whenever possible, the needle track, the area of drug release and any area of tissue reaction are included.

In case of multiple treatments/injection sites, sampling should include the site of the last injection and, at least, an injection site from the site where most of the injections were given. The study design should be such that the last injection site will occur on the side of the animal receiving the higher number of injections.

In order to provide assurance that the sampling method as described above has been adequate to represent the relevant concentration of residues, it is recommended to collect and analyse, where possible, at each injection site a second concentric ring-shaped control sample of approximately 300 g from the region immediately surrounding the excised core sample (surrounding sample). It is recognised that sampling of an extra 300 g amount of tissue cannot always be achieved, in particular with neck

[5] In this case, a restriction on use as regards a maximum weight of the target animals/maximum injection volume may be considered. Alternatively, where appropriate, small differences between the actual and the maximum possible injection volumes can be compensated by adding an additional safety span to the calculated withdrawal period.

[6] This does not apply to small animals where the size/anatomy of the animals do not allow sampling of 500 g. Here, a general recommendation cannot be made and the optimum sampling strategy needs to be designed on a case- by-case basis. The chosen sampling technique and target weight should be adequately justified. Also in this case, a surrounding sample around the excised core injection site should be collected to confirm the reliability of the approach, where possible. The core and the surrounding sample should be roughly proportional to the recommendation made in this guideline. The calculation procedure should be the same as outlined in the guideline. In case of samples from small animals of less than the recommended sample size, the concentration of the residue in the sample as measured, without any allowance for dilution, should be used for the calculations.

injections. If the experimental situation requires it, the surrounding sample weight may be reduced as is necessary. It is essential, however, that the material collected is enough to perform an analysis.

In practice, collection of samples of an exact weight cannot be achieved and the weight of the samples varies around the target values. Test samples for the core injection site in the range of 400 g to 600 g (500 ± 20 %) are normally considered acceptable. Test samples weighing far less than this may not include a relevant portion of injection site tissue and are generally considered as unrepresentative (in the absence of clear and convincing evidence to the contrary). The weight for the surrounding injection site sample should normally not be outside the range of 240 g to 360 g (300± 20 %).

Alternative quality control sampling procedures (others to using the "surrounding sample" approach) to ensure that the collected tissue actually encompasses the injection site are possible according to VICH GL 48 [11]: The approaches should be justified on a case-by-case basis, taking into account the data available and the formulation characteristics. The following methodologies, alternatively to using the "surrounding sample" may be considered, however, this list is not to be considered comprehensive.

- Collection of an elliptical (or other appropriate shape) sample along the injection track and/or the site of irritation. The Sponsor should provide evidence that this method correctly targets the injection site residues, such as with accompanying photographs of the site(s) of sampling.
- Provide data on the migration potential of injection site residues based on information obtained from the total radiolabeled residue (TRR) study. For example, a circular core (or elliptical) sample would be taken along the injection track and/or site of irritation as well as several adjacent samples for TRR comparisons. If this protocol demonstrates an appropriate sample collection technique, only the primary sample should be collected during the marker residue depletion study. It might be constructive to include an additional time point (*i.e.,* at a longer withdrawal time) in this study.
- Provide data on the migration potential of injection site residues based on information obtained from a target animal safety study (*i.e.* pathological examinations of the physical injection site).
- Conduct one of the above study designs using the candidate product mixed with a coloured dye to provide a visual assessment of the migration potential of injection site residues.

Use of a concept of multiple injection sites per animal is possible in accordance with VICH GL 48 [11]: Where the withdrawal period will clearly be determined by residue depletion at the site of injection, there is an option of collecting data from two injection sites per animal (and using the data from both sites in a determination of the withdrawal time). This practice can have a positive impact on study design with respect to animal welfare by reducing animal numbers. An example of where this approach is applicable is as follows: For a product that utilizes only a single injection, treatment can be given on the right side of the neck on day 0 and then on the left side of the neck on day 4. Euthanasia on day 7 following the final treatment would provide depletion data at 7 days (left injection site) and 11 days (right injection site) withdrawal. In this case, however, collection and assay of the other tissues would not be warranted since the product was administered contrary to the label (two injections vs. one injection) and residues could be excessively elevated. Such a dosing regimen is designed specifically for determination of injection site residue depletion.

Following removal, the entire samples for the core and surrounding injection sites, as collected, should each be homogenised thoroughly prior to sub-sampling for residue determinations, in order to avoid analysis of potentially non-homogeneous material.

For the assessment of withdrawal periods, the results from both the analysis of core and surrounding injection site samples should be considered. If for an animal the residues concentrations for the surrounding sample are higher than the core sample, unless an acceptable justification is provided, the

point should not be included in the statistical calculations as indicated in this guideline, an alternative approach might also be followed[7].

In addition to general data reporting according to Regulation (EU) 2018/782 [8], residue studies at the injection site should be accompanied by a complete and detailed description of the study design/experimental conditions in relation to selection of the anatomical site(s) of drug injection, the injection technique and equipment used, depth of injection (intramuscular), measures taken to allow precise location and identification of the injection site at slaughter, relevant technical details on sample collection procedures and sample preparation techniques. Expert judgement on the appropriateness of the chosen sampling approach to detect relevant residue concentrations should be provided.

[7] Further experience will be needed to find the optimum approach on how to include these results in the overall calculations. Once this experience is gained this guideline will be revised to include more detailed indications.

Definitions

Injection site definition

An injection site is the tissue area on the animal's body where a veterinary product has been injected. Test samples collected from injection sites for the purpose of residue studies should be representative of what is likely to be encountered as edible tissue following normal slaughterhouse procedures. The test sample collected should therefore include muscle tissue as well as connective tissue and subcutaneous fat in natural proportions (i.e., extra trimming of samples to remove the connective tissue and adhering fat from the underlying muscle is considered artificial as it does not mimic the real situation). The injection site should not include a portion of skin overlying the injection site because this is not required in this analysis of the injection site residues.

The target weight of the standard injection site sample taken on the centre of the injection site is 500 g (designated as "core injection site"). It should take the form of a cylinder the approximate dimensions of which should be: 10 cm diameter and 6 cm depth for intramuscular injections and 15 cm diameter and 2.5 cm depth for subcutaneous injections (see section 'Study Design and Sampling). In addition to the core sample, it is recommended to submit for residue analysis a second concentric ring-shaped sample of approximately 300 g from the region immediately surrounding the excised primary sample (designated as "surrounding injection site"). Analysis of the surrounding sample can provide assurance that the standard sampling method has been adequate to represent the relevant concentration of residues at the injection site at each slaughter day.

General terms

ADI: The Acceptable daily intake (ADI) of a chemical is the daily intake which, during an entire lifetime, appears to be without appreciable risk to the health of the consumer. The ADI most often will be set on the basis of the drug's toxicological, microbiological or pharmacological properties. It is usually expressed in micrograms or milligrams of the chemical per kilogram of body weight.

MRL: The Maximum residue limit (MRL) is the maximum concentration of a veterinary drug residue that is legally permitted or recognized as acceptable in or on a food as set by a national or regional regulatory authority.

Withdrawal period*:* The minimum period between the last administration of a veterinary medicinal product to an animal and the production of foodstuffs from that animal which under normal conditions of use is necessary to ensure that such foodstuffs do not contain residues in quantities harmful to public health.

Specific exposure and residue related terms

Standard Food basket: An estimate for the total amount of food of animal origin which is consumed on a daily basis by a 60 kg adult. The standard food basket uses arbitrary consumption figures which are assumed to represent upper percentiles of the daily intake of animal derived food. The standard consumption figures are for mammals 300 g muscle, 50 g fat or fat and skin, 100 g liver and 50 g kidney; for poultry 300 g muscle, 90 g fat and skin, 100 g liver and 10 g kidney; for fish 300 g muscle and skin in natural proportions; plus 1.5 l milk, 100 g eggs and 20 g honey. See also Regulation (EU) 2018/782 [8]. In a risk assessment, the food basket residues of a compound are usually compared with the ADI.

Edible portion (Injection Site): In case of an injectable product, the edible portion of injection site contained in the food basket is 300 g. This portion substitutes the normal 300 g muscle portion (see section 'ADI based approach' and Figure 2).

Marker residue: A residue whose concentration is in a known relationship to the concentration of total residue in an edible tissue. The marker residue can be the parent drug or any of its metabolites/degradation products or a combination of any of these. The marker can also be a chemical derivative of one or several of the residue components. The relationship of the marker residue to the concentration of the residues of concern in the standard edible tissues must be known (ratio marker residues/residue of concern). The MRL reflects the upper concentration of marker residue in the target tissues which is permitted.

Total residue: The Total residue of a drug in edible tissues is the sum of the veterinary drug (parent) and all metabolites as determined in radiolabelled studies or other equivalent studies**.** The term refers to the total drug related residues. The total residues normally include all drug-related residues (parent drug together with metabolites). The total residues are usually determined in radiometric residue depletion studies. Measurement of total residues in standard edible tissue is normally not required for generic products where the metabolic profile and the ratios of parent compound/marker substance to the total residues are well established. In these cases, total residues in the edible tissues and in the food basket can be estimated by use of this information. The same principles also apply where the drug related residues with a specific biological activity (toxicologically, microbiologically or pharmacologically active residues) are to be estimated.

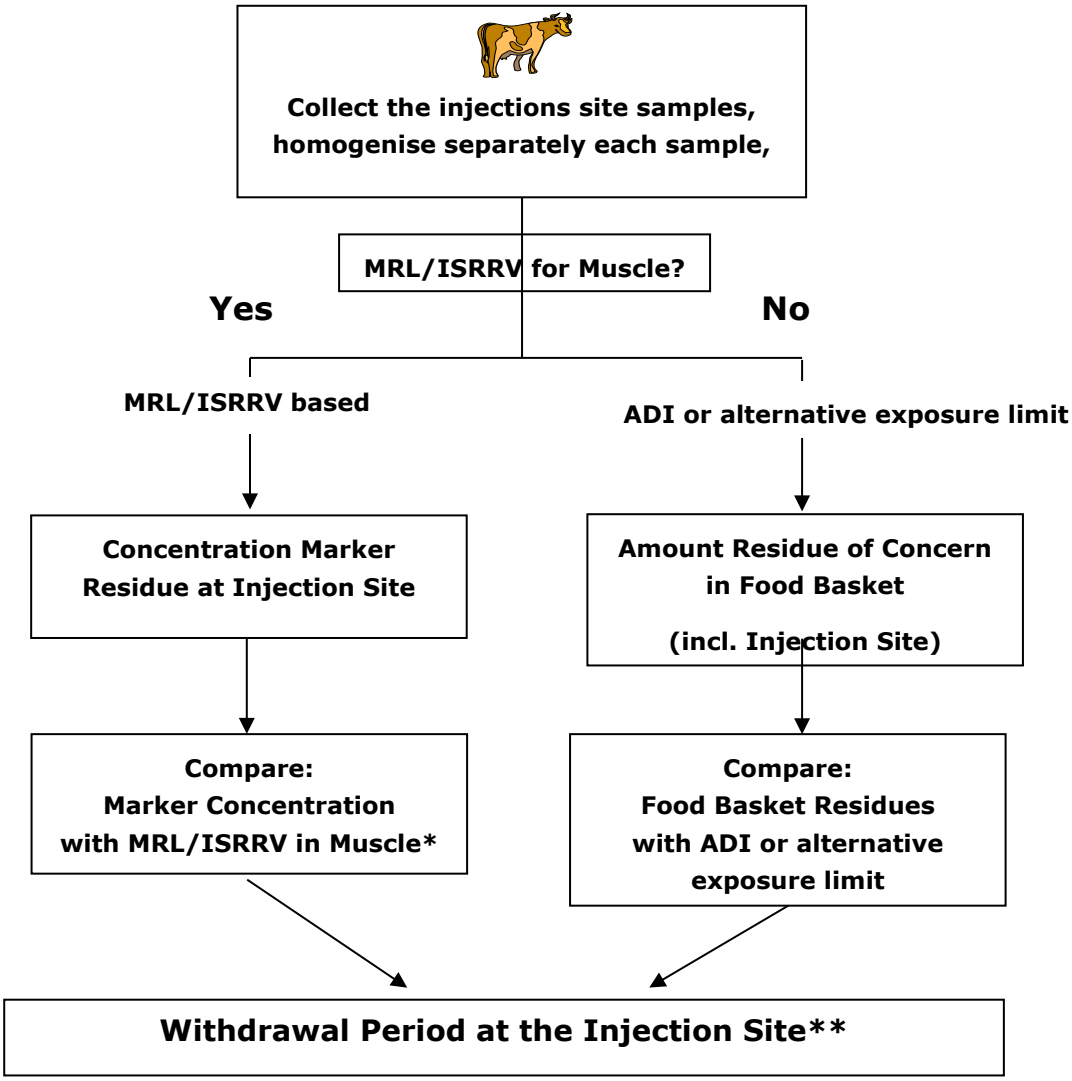
Residues of toxicological concern: Residue of toxicological concern refers to the total amount of residues that have relevance to the toxicological ADI established for the veterinary drug.

For an exposure estimate based on a toxicological ADI, the relevant residue is the residue of toxicological concern. It normally includes all drug-related compounds (parent drug together with metabolites) and, in most cases, it is identical to the total residues as measured in radiometric studies. However, if an individual residue component or fraction of the total residues has been demonstrated as being toxicologically inactive, it is possible to discount it from the total residues (e.g. bound residues or any other fraction of residues that is not orally bioavailable, or known toxicologically inactive metabolites).

Residues of pharmacological concern: For an exposure estimate based on a pharmacological ADI, the relevant residue is the residue of pharmacological concern. It usually refers to the parent compound plus other pharmacologically active residue components (if there are any). In the absence of data on the pharmacological activity of individual residue components, it is assumed that the total residue is pharmacologically active and that the pharmacological activity of the total residues, i.e. any metabolites/degradation product equals that of parent compound.

Residues of microbiological concern: For an exposure estimate based on a microbiological ADI, the relevant residue is the sum of residues with microbiological activity. In most cases it is identical to the residues as measured in microbiological assays. In the absence of such data, the total residues may be used or, alternatively, the sum of the individual residue components which are known to possess antimicrobial activity. Hereby it is assumed that microbiological potential of the total residues or the metabolites/ degradation products, respectively, equals that of parent compound.

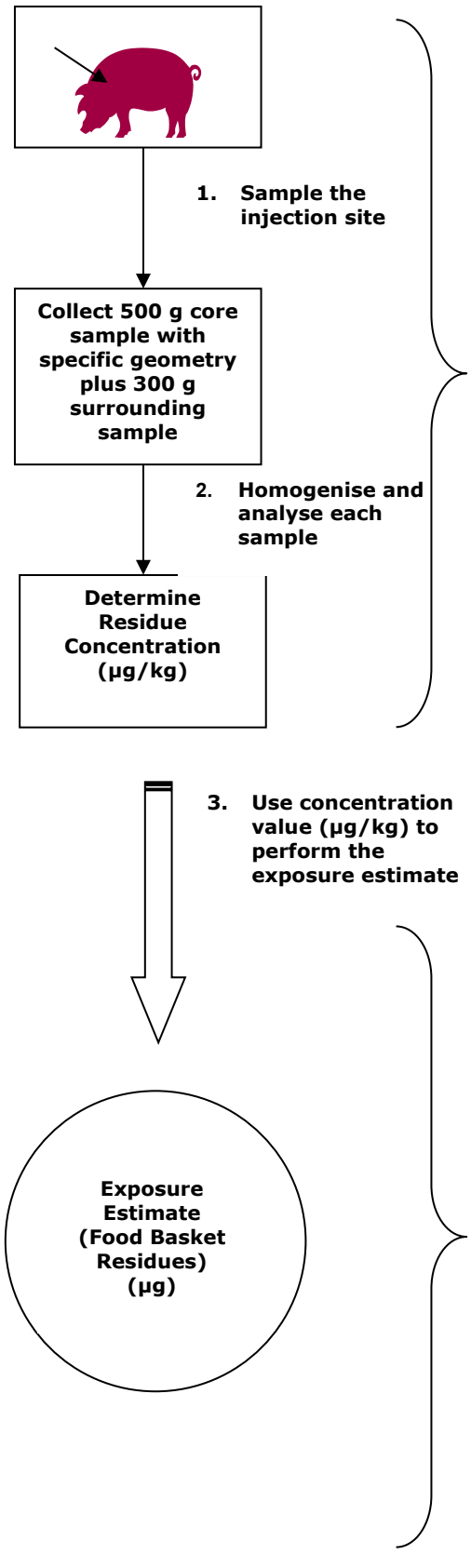
Figure 1: Sampling and analysis of injection sites and estimation of MRL and ADI based withdrawal periods



* In certain cases, an ADI based assessment may need to be performed in parallel to check the appropriateness of the MRL based withdrawal period to ensure that residues in the food basket are below the ADI (see section 'MRL based approach').

** To be calculated based on the Guideline on determination of withdrawal periods for edible tissues (EMA/CVMP/SWP/735325/2012 Rev. 2) [1]. In addition, withdrawal periods based on MRLs for other tissues have to be calculated as well. The longest withdrawal period will be considered as the most appropriate regulatory withdrawal period for the veterinary medicinal product.

Figure 2: **Sampling and analysis of injection sites and estimation of food basket exposure**



Sampling at the injection site

1. Sampling at injection sites is different from that in other edible tissues: In "normal" tissues such as liver, kidney etc, it may be assumed that residues are evenly distributed. Therefore, the location of the sample taken for analysis has no or only minimal impact on the measured residue concentration.
2. At the injection site, homogeneous distribution of residues cannot automatically be assumed. Due to dispersion/diffusion there may be a concentration gradient within the area of drug release with (normally) the highest levels around the centre of the region where the drug was injected. As a result, the precise location of sampling and the size/geometry of the sample collected can have a considerable impact on the determined concentration of residues.
3. The method of sampling should ensure that the area with the highest residues is collected. It is recommended to take from the injection site an approximately 500 g core sample centred on the point of injection. This sample should take the form of a cylinder the (10 cm diameter x 6 cm depth for intramuscular and 15 cm diameter x 2.5 cm depth for subcutaneous) and is assumed to represent the primary injection site. To provide assurance that the core sample actually includes the maximum residue, a second sample of approximately 300 g from the area surrounding the excised tissue is collected and submitted for analysis.

Exposure estimate for ADI/food basket based assessments

4. The contribution of the injection site to the food basket residues is obtained by multiplying the residue concentration with the correspondent consumption figure. The standard consumption figure is 0.3 kg, the same as for "normal" muscle (µg residue/kg injection site x 0.3 kg, see section 'ADI based approach' for further details).
5. exposure to residues in the food basket is estimated as follows: Exposure (µg) = $(Res.Con_L \times Cf_L) + (Res.Con_K \times Cf_K) + (Res.Con_F \times Cf_F) + (Res.Con_{IS} \times Cf_{IS})$ etc.

Res.Con. = Residue of concern (µg/kg), e.g. calculated from marker/total residue ratios. Cf = standard consumption figure = 0.3 kg injection site muscle; 0.1 kg liver, 0.05 kg kidney, 0.05 kg fat, etc. Indices: L, K, F, IS = liver, kidney, fat, muscle, injection site (muscle).

References

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4. Guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches (EMA/CHMP/CVMP/JEG-3Rs/450091/2012).
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16. CVMP, Guideline on the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/2000-Rev.4, July 2021).