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GUIDELINE ON

INJECTION SITE RESIDUES

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GUIDELINE ON INJECTION SITE RESIDUES

Introduction

1. Consumer safety needs to be assessed for all pharmacologically active substances which are intended for use in food producing animals in accordance with Council Regulation (EEC) No 2377/90. Pre-slaughter withdrawal periods are determined in order to ensure that the residues deplete to permissible concentrations. Whereas the maximum residue limit (MRL)[1] 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.

2. 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). Therefore, residues at injection sites need particular consideration as regards their possible risk for consumers of treated animals.

3. 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. Since this period the basic guidance on the establishment of MRLs given in Volume 8 of the Rules Governing Medicinal Products in the European Union [1] has undergone changes and new guidelines on the assessment of residues and establishment of withdrawal periods have been issued, e.g. the Note for Guidance: Approach towards Harmonisation of Withdrawal Periods (EMEA/CVMP/036/95) [2]). In view of these modifications of the scientific and regulatory context, the CVMP has reviewed the current Working Document on injection site residues. Once adopted in its final version this guideline will replace the CVMP document “Working document III/5933/94-EN" on injection site residues.

[1] MRL and marker residue as published on the amendments of the Annexes of Council Regulation (EEC) No 2377/90 and in the corresponding CVMP Summary Report for the substance.

Scope

4. 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.

Assessment

5. In consideration of the risk assessment of a substance according to Council Regulation (EEC) No 2377/90 and the conclusions of the corresponding EMEA/CVMP Summary Report, the assessment of residues at injection sites and the determination of withdrawal periods is to be based on the MRL, the ADI or, if necessary, an alternative exposure limit. The assessment of injection site residues should follow the general principles set out in Volume 8 [1] and the CVMP Note for Guidance on the approach towards Harmonisation of Withdrawal periods (EMEA/CVMP/036/95) [2].

   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.

MRL based approach

6. 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[2,3]. Withdrawal periods should be set according to the Note for Guidance: Approach towards Harmonisation of Withdrawal Periods (EMEA/CVMP/036/95) [2].

7. 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)[4]. 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 assessments see below).

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2 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.

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

4 According to Council Regulation (EEC) No 2377/90, 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.

ADI based approach

8. For substances where there is no MRL for muscle (usually Annex II compounds), 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 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.

9. 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:

   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)[5];
   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;
   iv. Estimate the withdrawal period based on the guideline on harmonisation of withdrawal periods EMEA/CVMP/036/95 [2].

Approach based on alternative exposure limit

10. This applies in principle to substances in Annex II of Council Regulation (EEC) No 2377/90 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.

11. The derivation of this withdrawal period is in principles analogous to the approach described for MRL or ADI based estimates. 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 approach) or an amount of residues (i.e. analogous to the ADI approach).

[5] 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 paragraphs 21-23 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)

Residue Studies

General Principles

12. 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 Volume 8 [1].

13. 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) or, where appropriate, in residue depletion studies aiming at characterisation of the pharmacologically, toxicologically or microbiologically active residue components.

14. 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 paragraph 15). Supporting data to estimate the residue of concern (e.g. on the basis of ratios) may then be obtained from published literature. Small differences in product composition may have significant effects on injection site depletion and therefore residue studies must be provided (in addition to bioequivalence studies) in order to assess depletion of residues from the injection site.

15. For substances having a MRL for muscle, and for which therefore depletion of the marker residue in muscle below this MRL 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.

Study Design and Sampling

16. 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.

17. 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[6].

[6] 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.

18. 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.

19. 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.

    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 the approximate dimensions of which should be[7]:

    - 10 cm diameter and 6 cm depth for intramuscular injections.
    - 15 cm diameter and 2.5 cm depth for subcutaneous injections.

    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.

20. 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 can not always be achieved, in particular with neck 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.

21. In practice, collection of samples of an exact weight can not 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 ± 20 %.

22. 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.

[7] 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.

23 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[8].

24. In addition to general data reporting according to Volume 8 [1], 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.

Literature

[1] Volume 8: The Rules Governing Medicinal Products in the European Union, Notice to Applicants and Note for guidance on "Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin", European Commission, DG Enterprise, June 2003.

[2] EMEA/CVMP/036/95: Note for Guidance: Approach towards Harmonisation of Withdrawal Periods (CVMP adopted April 96).

[8] 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.

Glossary

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 injections 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 paragraph 19). 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: Acceptable Daily Intake: The estimate of the residue, expressed in terms of weight units per kilogram of bodyweight, that can be ingested daily over a lifetime without any appreciable health risk.

MRL: Maximum Residue Limit: The maximum concentration of a marker residue in an animal tissue (e.g. liver, kidney, muscle or fat) or animal product resulting from the use of a veterinary medicinal product (expressed in mg/kg or µg/kg on a fresh weight basis) that is legally permitted on or in a food within the EU.

Withdrawal period*:* The interval between the last administration of a veterinary medicinal product to animals under normal conditions of use and the production of foodstuff from such animals to ensure that such foodstuffs do not contain residues in quantities in excess of the maximum residue limits laid down.

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. 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 paragraph 9 and Figure 2).

Marker residue: An analyte that is suitable to test for the presence of residues in a 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.

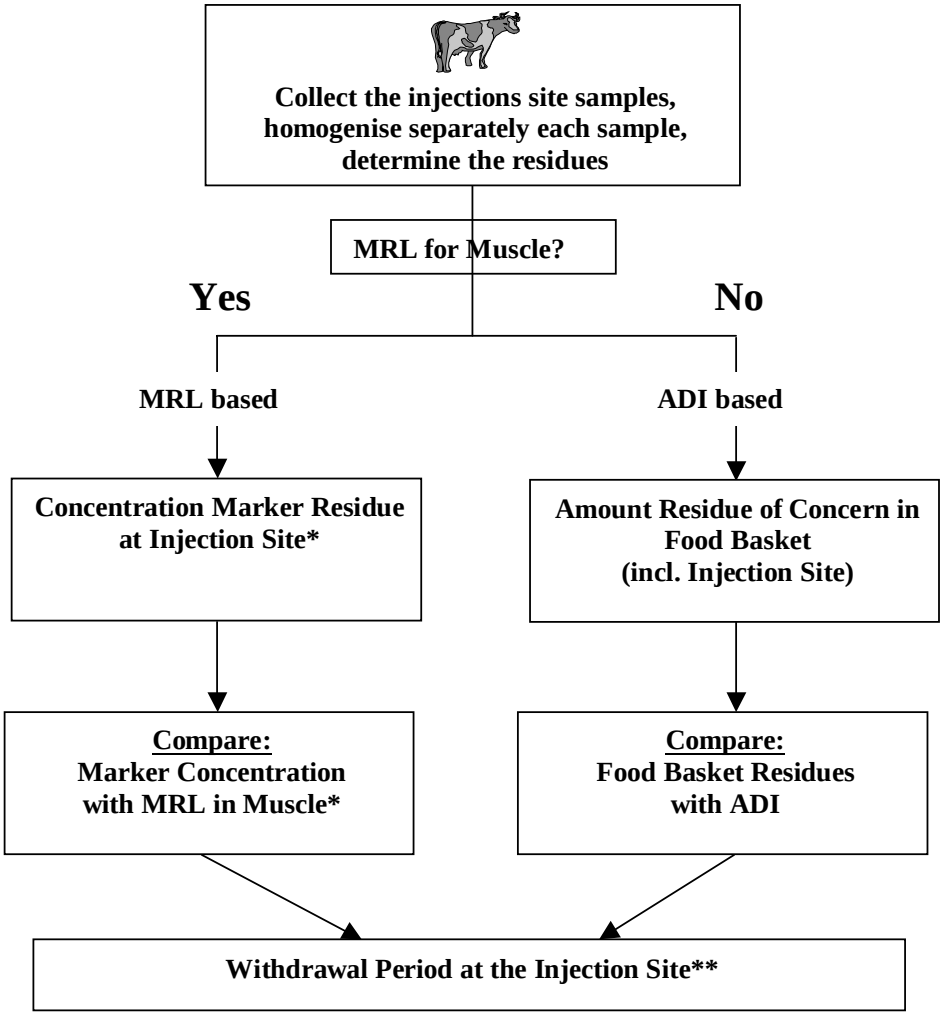
Total residue: The term refers to the total drug related residues. The total residues normally include all drug-related residues (parent drug together with metabolites). The total residues are usually determined in radiometric residue depletion studies. Measurement of total residues in standard edible 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: 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.

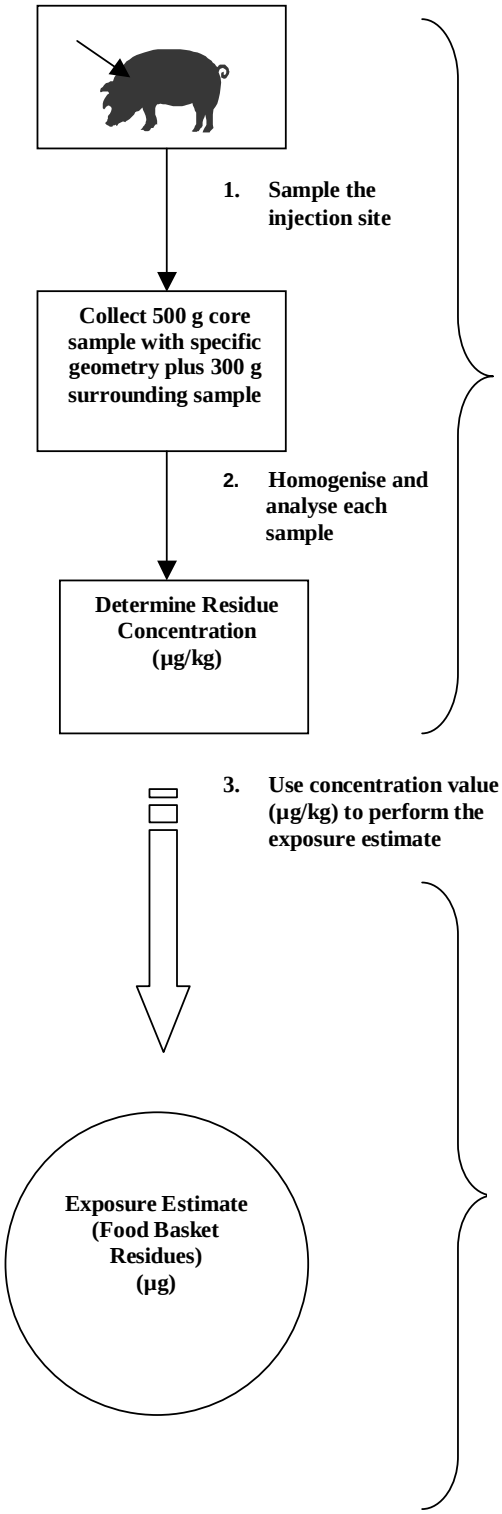
Figure 1: Sampling and analysis of injection sites and estimation of MRL and ADI based withdrawal periods



* 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 paragraph 7).

**To be calculated based on the guideline on harmonisation of withdrawal periods EMEA/CVMP/036/95 [2]. 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 paragraph 9 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).