RESIDUE GUIDELINE No.25

RESIDUES IN MILK – ANTIMICROBIALS

INTRODUCTION

This guideline provides advice on conducting milk residue trials for registration of antimicrobial products that will be administered to dairy animals. Although the guideline refers to cows per se, it applies also to other dairy animals. The guideline applies to all routes of administration (intramuscular, intravenous, oral, intrauterine, topical, and intramammary) for both dry and lactating cows. In this guideline, the term “antimicrobials” refers to antibiotics and synthetically and semi-synthetically derived antimicrobials. These drugs are used in dairy animals as chemotherapeutic agents and as growth promotants.

Antimicrobial drugs are generally used in dairy animals in one of three ways:

1. Whole herd treatments, where there is “blanket” treatment of the majority of animals eg antibiotic feed additives and (in some herds) intramammary dry cow treatments;

2. Partial herd treatments, where a minority of the herd is treated, eg intramammary dry cow treatments; and

3. Individual cow treatments, where only individual or a few animals are treated at any time, eg lactating cow intramammary treatments and injections for bacterial disease control.

The use of most antibiotics in lactating dairy cattle will result in residues in milk. MRLs for antimicrobial agents in milk are based on individual cow data, which should cover the biological variability that can occur from farm to farm and from breed to breed. The actual residues in milk in the market place may be considerably lower than the maximum residue limits (MRLs) because of the bulking and blending of milk from treated animals with milk from untreated animals. Despite the possibility of residue concentrations being substantially reduced between the farm and the factory, a “bulking factor” is not applied when determining MRLs of antimicrobial agents. Any reduction in milk residues as a result of bulking and blending is a trade benefit to the dairy industry.

A number of guidelines already in the Vet Requirement Series provide advice on residues in tissues eg. Residue Guidelines No. 11 “Reporting of residue data”; No. 16 “Injectable veterinary products”; and No. 23 “Data requirements for animal tissue residue trials”. The information contained in those guidelines is also applicable to antimicrobial agents.

Applications for extensions of use and for new antimicrobials WILL BE SUBMITTED, by the NRA, to the Department of Health and Aged Care’s Working Party on Antibiotics (WPoA), for advice on risk of resistance, as part of the registration process. The WPoA’s data requirements are set out in Part 10, Special Data Requirements of the NRA’s Guidelines for Registering Veterinary Chemicals.

It is also a requirement for registration of intramammary products in Australia that an approved blue dye be incorporated pursuant to Regulation 10 of the Agricultural and
Veterinary Chemicals Code Regulations. Procedures for incorporation of the dye in lactating cow products are outlined in “Guideline for Registration of Intramammary Preparations for Treatment of Bovine Mastitis”, pages 18-21 and available from the Veterinary Evaluation Section of the NRA on request. There is also a similar requirement for dry cow products, and a guideline is under consideration.

OBJECTIVES

The objective of this guideline is to provide guidance in planning residue trials in dairy animals to determine residues in milk, to allow the establishment of MRLs and withholding periods (WHPs).

In addition to the requirement for milk residue data, tissue residues in cows and calves must be addressed. Guidance for tissue residue trials can be found in Guideline No. 23. Further details on the establishment of MRLs and WHPs can be obtained from Guidelines Nos. 11 and 23.

GENERAL REQUIREMENTS

Many of the requirements described in Residue Guideline No. 22 “Residues in milk - non-antimicrobials” apply also to the systemic administration of antimicrobial agents and applicants are referred to this guideline.

Before residue trials can be undertaken for antimicrobial drugs, a residue definition must be determined based on the findings of metabolism studies and analytical methodology. The route of administration (whether intramuscular, intravenous, oral, intrauterine, topical, or intramammary) must also be considered before proceeding with any metabolism studies. Oral metabolism studies alone may not suffice for intramammary, intramuscular or intravenous administration. With respect to new veterinary drugs intended for intramammary infusion, the findings of previous metabolism studies involving systemic administration may be applicable if no metabolism of the active constituent occurs. However, a full metabolism study via intramammary infusion will be required where there is significant metabolism following systemic administration.

If the metabolism studies for intramammary or systemic formulations administered at label rates or greater show non-detectable tissue residues, tissue residue trials may not be necessary. Otherwise, tissue residue data, in accordance with the Residue Guideline No. 23, will be required.

When milk residue trials are necessary, at least one residue trial conducted in accordance with the proposed maximum treatment regimen should be undertaken. If trials are conducted in either the USA or the EU, they should fulfil the requirements for Australian registration. For any trials conducted in Australia, milk from treated cows must not be used for human consumption or fed to bobby calves or supplied for processing if the milk or meat products do not meet current MRLs as listed in the Food Standards Code. Furthermore, a permit from the

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1 Vet Requirements Series Part 4 – Metabolism and Kinetics
NRA may be required in certain circumstances. Applicants should contact the NRA/State Coordinator for further information. For those veterinary drugs whose administration to cows may result in exposure of calves in utero or via milk, tissue residues in calves must be addressed (this is the subject of another guideline to be developed).

When milk residue trials are undertaken, they should be conducted under conditions as similar as possible to those likely to be encountered when the product is used commercially in Australia. Important factors to be considered in the design of trials include how the drug partitions in milk and what influence the stage of lactation might have on the magnitude of residues. When establishing MRLs and WHPs, the NRA considers the worst case scenario.

**SPECIFIC REQUIREMENTS**

Guidance with the planning of residue trials with injectables can be found in Residue Guideline No.16, “Injectable Veterinary Products”.

Seven aspects that are unique to dry cow intramammary therapy need to be addressed:

1. Cows may calve earlier than expected after dry cow intramammary therapy;
2. Cows may be accidentally administered antimicrobial drugs late in the dry period and within the withholding period;
3. The possibility of a treated dry cow rejoining the milking herd prematurely, and her milk entering the farm milk supply;
4. The additional possibility of a lactating cow being inadvertently treated with dry cow intramammary therapy;
5. The occurrence of residues in calves as a result of access to milk from treated cows, either from suckling during the first few days post partum or being fed discarded milk;
6. The occurrence of in utero drug transfer resulting in tissue residues in calves; and
7. The effects of timing of antibiotic administration with respect to involution of the mammary gland, as per farm guidelines for mastitis control.

In these scenarios, the milk may contain residues thereby rendering it unsuitable for human consumption or processing, and/or leading to the condemnation of calves that have suckled their dams early post partum. Without the establishment of a meat WHP for the bobby calves, the presence of residues in the milk may also render it unsuitable for consumption by calves removed from their mothers. Therefore, the residue trials must establish the pre-calving interval during which dry cow therapy is permitted. Pre-calving intervals that approximate the period between drying off and calving increase the risk of violative milk residues, should cows calve early. Long pre-calving intervals are therefore unacceptable.

The trials must also establish any period post partum when milk, destined for consumption by either humans or by calves likely to be slaughtered for human consumption, could contain residues greater than the MRL. As most calves destined for human consumption are allowed
to suckle their dams or consume milk taken from treated cows, tissue residues in calves must be addressed, either by data and/or argument. In particular cases it may be possible to establish an appropriate meat WHP for calves consuming milk from antibiotic treated cows on the basis of analysis of available data and/or argument.

In milk residue trials of dry cow intramammary formulations, milk collection is expected to commence at the time of calving rather than at 72-96 hours post-calving when milk first becomes suitable for human consumption. Milk residue data emanating from the trials must be submitted to support the proposed milk MRL and milk WHP. The presence of antimicrobial residues in colostrum would be expected to preclude its use for human consumption.

**ANIMALS**

For milk residues, generally one trial with a minimum of twenty (20) cows should be conducted, taking into account relevant factors such as the fat content of the milk and the volume of production. Generally, the trial should include both low and high volume production cows, with a minimum of 5 animals at each production level. The production levels should be representative of those reported in Australia. For dry cow treatments, the product is administered at drying off and the trial animals are selected at that time.

The use of diseased animals in residue trials for intramammary lactating cow and intrauterine treatments is presently under consideration. Although there is no requirement to trial diseased animals at this time, the applicant is encouraged to also use some diseased cows in milk residue trials should the opportunity present itself during efficacy trials.

After treatment, all animals should be kept together according to normal husbandry practices, including feeding regimen and feed type. The report of the trial should include details of the facilities and the feeding practices. Details of the experimental animals to be included are:

- species, breed, weight, age, pregnancy status, stage of lactation, milk production, fat content of milk, number of animals treated, date and time of treatment(s), dates of calving, animal health during the study.

**ADMINISTRATION**

The test material should be the commercial formulation. If code names/numbers are used, the relationship to the product proposed for registration needs to be stated. Where it is considered relevant, the accuracy of any applicator should be determined at the beginning and end of administration.

Each animal is treated according to the label or proposed label use pattern. It is important that the residue studies address the maximum treatment regimen ie. the maximum dose rate, the maximum number of treatments and the minimum retreatment interval. If the dosage is based on weight ranges, the maximum treatment regimen which applies to the lightest lactating animal in the lowest weight bracket on the label needs to be addressed.
In residue trials of intramammary formulations, **all quarters should be treated** at the maximum recommended rate. This represents the maximum use situation and is expected to lead to the maximum residue levels likely to be observed following commercial use of the product. Treatment of all quarters allows pooling of milk from the different quarters of an individual cow for a given milking for the purpose of assay.

For dry cow treatment, the product should be infused into the teat canal of each quarter immediately after the last milking of the lactation period. For lactating cow treatment, the product should be infused into the teat canals of all quarters in accordance with the proposed use pattern. For trial purposes, all quarters are required to be infused with the trial product, to represent the worst case scenario and to account for potential diffusion.

When treatment is other than by intramammary infusion and where daily or continuous exposure is involved, it is necessary to demonstrate that milk residues have reached a plateau concentration. An adequate number of time points at the plateau concentration is required (3 or 4 are generally regarded as acceptable). This allows the maximum concentration in milk to be established prior to the commencement of residue depletion.

**SAMPLE COLLECTION**

With regard to sample collection, Guideline No. 22 should be followed in the case of systemic and oral dosage forms.

When sampling the udder quarters, they should be milked out, the whole sample mixed thoroughly, and a sub-sample taken. Appropriate records must be maintained to verify the validity of the sampling programme. In addition, the fat content of milk samples should be determined for all samples taken to demonstrate the validity of samples.

**For intramammary treatments**, additional information on sample collection follows.

- With dry cow treatments, the milk sample collection times must be reported and any udder cleansing procedures described. Milk samples should be collected twice daily, after calving, at sufficient sampling points to characterise the decline of the residue to the limit of quantification (LOQ).

- Milk samples from lactating animals treated with intramammary infusions should be collected prior to the first treatment, from the first milking after the last treatment, and from sufficient milkings twice daily thereafter to characterise the residue decline to the LOQ. Udder cleansing procedures must be described.

The following information pertains to both dry and lactating cow intramammary infusions. Milk residue analyses should be conducted on samples from individual cows, with the cow having been milked out from all quarters, and the milk pooled, mixed and sub-sampled. It is essential that milk samples are thoroughly mixed prior to sub-sampling for analyses. Blending of milk from different milkings is not acceptable. In addition to the labelling suggestions described in Guideline No. 22, milk samples should be labelled with the trial’s identification number, farm identity and date sample collected.
STORAGE CONDITIONS AFTER COLLECTION

Milk samples are generally maintained at 4°C and sent to the analytical laboratory within 24 hours of collection. In situations where longer periods of storage at either 4°C or 0°C are necessary, storage stability studies will need to be conducted (refer to Residue Guideline No. 8 “Stability of residues during storage”). The storage temperature must be reported.

The information relating to the storage of samples which needs to be submitted includes the date of sample collection, the date of dispatch to the analytical laboratory, the date of receipt by the laboratory, storage conditions while awaiting analysis and the date of analysis.

RESIDUE DEFINITION

The defining of residues for setting an MRL is documented in Residue Guideline No. 6 “Definition of residues for the purpose of setting an MRL”. If a bioassay only is used in the assessment of samples, the residue definition can be “Inhibitory substance identified as X” where the identity of the antimicrobial, X, can be confirmed by an instrumental method. It must also be demonstrated that the bioassays used are capable of detecting, at the LOQ, not only parent compound but also metabolites that are part of the residue as defined (and which are toxicologically significant and exceed 5% of the total residue). When instrumental methods are used, the residue definition should be based on the moiety measured ie. the parent compound and/or one or more active metabolites.

RESIDUE ANALYTICAL METHOD

Residue Guideline No. 19 “Residue analytical method” should be consulted. The analytical method for milk residue trials must address the residue definition and be validated. The choice between bioassays (eg. microbiological plate assays, Delvotest SP), ELISA and other validated immunological tests and instrumental methods will depend on the suitability of the respective methodologies. Each method type may be applicable, depending on circumstances. The NRA’s preference is for an instrumental method.

Analytical Methods for antibiotics can be as follows:

- **Broad-spectrum bioassays** are used extensively throughout Australia to routinely screen urine and milk for the presence of antimicrobial agents. They continue to be important in the screening of large numbers of samples for the presence of inhibitory substances\(^2\). Methods must quantify the residue definition (expressed as parent equivalents or active metabolite as the case may be);

- **A specific bioassay or immunoassay test** may be developed, but the sensitivity of this assay must be able to accommodate the proposed MRLs and the residue definition ie. the LOQs must not exceed the respective MRLs; or

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\(^2\) It is highly desirable that the applicant evaluates current testing bioassays to facilitate the screening process of milk in Australia.
• **An instrumental method** such as GLC or HPLC should be provided for confirmation of the identity of residues and is the preferred method type for residue analyses carried out in any trial data submitted. Instrumental quantitation of new antimicrobials is preferred when it provides adequate specificity and sensitivity compared to quantitation by bioassay or immunoassay. In the case of prodrugs that yield active metabolites, the instrumental method must quantify the residue as defined (expressed as parent equivalents or active metabolite as the case may be).

Correlation in residue concentrations between bioassay or immunological test and the instrumental method must be demonstrated on samples from the residue trials.

**FATE OF RESIDUES DURING PROCESSING**

The applicant is referred to Guideline No. 22 for information regarding the partitioning of antimicrobial substances between the aqueous phase and milk fat of whole milk. A partitioning study, involving a minimum of two samples, needs to be submitted. The degree of partitioning will determine whether the MRL is expressed on a whole milk or milk (in the fat) basis.

**The influence of residues on milk processing** (including the activity of starter cultures) must be addressed. As different starter cultures are used for the various dairy products, a range of starter culture organisms would need to be tested using milk with naturally incurred residues. Procedures for the testing of starter cultures are outlined in an EMEA guidance document\(^3\). If cultures are affected, the concentration of drug residue at which there is no effect should be determined.

**EXTRAPOLATION OF FINDINGS BETWEEN ANIMAL SPECIES**

A general milk MRL will be considered when adequate residue data are provided for cattle plus sheep, or cattle plus goats.

**RELEVANT RESIDUE GUIDELINES CITED**

- Residue Guideline No. 6: Definition of residues for the purpose of setting an MRL
- Residue Guideline No. 8: Stability of residues during storage
- Residue Guideline No. 11: Reporting of residue trials
- Residue Guideline No. 16: Injectable veterinary products
- Residue Guideline No. 19: Residue analytical method
- Residue Guideline No. 22: Residues in milk – non-antimicrobials
- Residue Guideline No. 23: Data requirements for animal tissue residue trials

\(^3\) Note for Guidance for the assessment of the effect of antimicrobial substances on dairy starter cultures. EMEA document CVMP/276/99.