

# **National Registration Authority**

## **INTERIM GUIDELINES FOR DATA TO SUPPORT EFFICACY AND SAFETY OF TEAT SANITISERS**

**June 2002 VERSION**

# **CONTENTS**

## **1. INTRODUCTION**

## **2. TESTING TEAT SANITISERS FOR IRRITANCY**

### 2.1 Testing Safety of Teat Sanitisers Under Normal Conditions

- Selection of herds, cows and quarters.
- Experimental design
- Trial treatment periods
- Data collection
- Evaluation of skin reactions
- Evaluation of milk quality

### 2.2 Testing Safety of Teat Sanitisers Under Adverse Conditions

## **3. DETERMINING THE EFFICACY OF TEAT SANITISERS IN REDUCING THE INCIDENCE OF NATURALLY OCCURRING NEW INTRAMAMMARY INFECTIONS**

### 3.1 Antiseptic determination

### 3.2 Clinical Studies (field trials)

- General requirements
- Experimental design
  - Selection of experimental herds, cows, and quarters
  - Duration
  - Culturing
  - Criteria of diagnosing infections
  - Presentation of data
  - Statistical analysis

## **1. INTRODUCTION**

Data is required by the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) to support efficacy and safety of new teat sanitisers. In assessing data, the NRA needs to be satisfied that before registering a teat sanitiser, the product will be:

- safe for the animal, and
- effective in reducing the incidence of infectious and environmental mastitis in the herd.

These guidelines pertain to pre- and post-milking teat sanitisers (dips, sprays, udder washes, and related entities).

These guidelines are based on principles recognised by the scientific community as appropriate and necessary for collecting scientific data. They are by no means complete. They deal with the product-specific issues listed below. The key areas are irritancy to teat and udder skin and bactericidal efficacy of the teat sanitiser.

These guidelines should be read in conjunction with Part 8 of the Vet. Requirement Series - Guidelines for Registering Veterinary Chemicals. All trial protocols must be approved by an Animal Care and Ethics Committee before any animal experimentation.

Submitted labels must be in accordance with the *Code of Practice for the Labelling Code for Veterinary Chemical Products*.

These guidelines do not include public health (including milk residues) or environment safety considerations.

## **2. TESTING TEAT SANITISERS FOR IRRITANCY**

This section should be read in conjunction with Part 8-5 of the Veterinary Requirements Series.

The applicant should demonstrate that the proposed product is safe to the target animal under “normal Australian climatic conditions”. This does not necessarily mean that the product will improve the skin condition of teats or maintain them in good condition during periods of adverse weather. pH of the final solution should be between 4 and 6. Where the applicant wishes to make a label statement for inclusion of extra emollient during periods of adverse weather, efficacy data must be provided for use of the product with this extra emollient.

### **2.1 Safety of Teat Sanitisers Under Normal Conditions**

#### **Selection of herds, teat, cows and quarters:**

Test the product for at least 6 weeks in at least two herds using a minimum of 10 normal multiparous (multiple pregnancies) cows and 10 normal primiparous (single pregnancy) cows in each herd. Tests should be carried out on lactating cows with mainly light-coloured teats. Half

of each group should be at early lactation and half at late lactation. Cows with grossly deformed teats should not be included in the trial.

A description of each test animal should be submitted stating the animal's number, breed, age, lactation stage, and daily milk production. Cows should be under constant supervision during trial periods. Any adverse reaction and consequential treatments between these times must be recorded.

## Experimental design

1. Teats should be examined for skin reaction one week before and one day before beginning of the trial.
2. All four quarters are to be treated at the same time according to the manufacturer's instructions.
3. Examination of the teats must be carried out by a veterinarian or other competent investigator familiar with the anatomy and normal and abnormal appearance of cows' teat. Examinations of the teats must be carried out prior to milking by the same person.
4. Teats should be examined for skin reactions every 48 hours during the treatment and post-treatment periods (days 8 through 42).

## Evaluation of skin reactions

At each examination prior to milking, the condition of each teat should be recorded as below:

### i) Erythema (redness of skin)

- Skin visibly normal 0
- Very slight erythema (barely noticeable) 1
- Well-defined erythema (clearly visible) 2
- Moderate to severe erythema 3
- Severe erythema (beetroot redness) 4

### ii) Oedema Formation

- Skin visibly normal 0
- Very slight oedema (barely noticeable) 1
- Slight oedema (edges or area well defined by definite swelling) 2
- Moderate oedema (swelling raised approximately 1 mm) 3
- Severe oedema (swelling raised more than 1 mm and extending beyond the area of product exposure) 4

### iii) The occurrence and severity of any of the following lesions should also be recorded:

- Drying, roughening and/or scaling of the teat skin.
- Lesions on the teat barrel. A lesion is defined as a fissure or other abnormalities (eg. ulceration, blister) in which there is a break in the epidermis.
- Lesions of the teat end. These include ulceration or eversion of the teat orifice as well as skin lesions occurring within 1 cm of the teat orifice.
- iv) Any degree of skin tenderness on touch.

## **Evaluation of milk quality**

Individual somatic cell counts should be determined by quantitative count methods for both treated and untreated quarters. A Fossomatic cell counter or "Direct Microscopic Somatic Cell Count in Milk" reported in *Journal of Milk and Food Technology*, Vol. 31. No. 11 November 1968 and "Design of Eyepiece Reticules for Use in the Direct Microscopic Somatic Cell Count Method" reported in the same volume (Appendix 3), are appropriate for estimation of individual cow cell count. Electronic cell counting may also be used. Somatic cell counts should be consistently determined in aliquot samples taken from the total milk production of each quarter. The method of collection of the sample should be stated. A bucket milker or similar device are recommended. Milk samples should be collected for quality evaluation every 48 hours during the treatment and post-treatment periods (days 8 through 42). Animal data sheets should include a copy of the laboratory report with the technician's signature and the dates of the analyses.

## **Trial treatment periods**

- The pre-treatment period includes one week (days 1-7) before product is administered. Baseline observations are made to confirm that all test animals are normal. During this period, two milk samples, 24-hours apart should be taken for culture and cell count from all four quarters. This procedure will help to distinguish udder irritation due to a pathogenic organism(s) from that caused by the product.
- The treatment period begins at the second week (day 8) and lasts for four weeks (day 35).
- The post-treatment period begins at the eighth week (day 36) and lasts for one week (day 42).

## **Data collection**

Information to be documented for the pre-treatment (one week), treatment (4 weeks), and post-treatment periods (one week) should include: teat condition, milk cell count, animal identification, farm site, trial number, date, test day and hour (am/pm), milk production, body temperature (where appropriate) and any other obvious clinical condition. Whenever possible, the documentation should include photographs of a representative selection of both groups at the start, during and end of the trial.

Any transient deterioration in teat condition during the immediate assessment period should be noted for use in subsequent technical information.

Any observed adverse reaction resulting from application of the suggested product in the clinical studies should be reported.

The submitted data should be statistically analysed.

## 2.2 Safety of teat sanitisers under adverse conditions

If no irritancy has been recorded for the product under normal conditions and the label refers to specific instructions of use under adverse conditions, a repeat of the above trial must be carried out under such adverse weather conditions.

Any failure of this testing under adverse conditions does not affect registration of the product for use under normal conditions. If failure occurs, claims for use of the product under adverse conditions will not be allowed.

## 3. DETERMINING THE EFFICACY OF A TEAT SANITISER IN REDUCING THE INCIDENCE OF NATURALLY OCCURRING NEW INTRAMAMMARY INFECTIONS.

This section should be read in conjunction with Part 8-4 of the Vet. Requirements series.

Specific data requirements will vary depending on the method of product application. For new actives, applicants should submit the results of properly designed and conducted laboratory and field-scale scientific studies. *In vitro* studies cannot be used as the sole basis for claims of efficacy.

### 3.1 Antiseptic determination

[See Part 8-4.2 of the Vet. Requirements series]:

The proposed product formulation (final solution) should qualify as an antiseptic by *in vitro* testing. One or more of the following tests are recommended:

- (i) The Phenol Coefficient Method for phenolic compounds.
- (ii) The Use-Dilution Methods for disinfectants miscible with water to determine the maximum dilution that kills test organisms.
- (iii) Available Chlorine Germicidal Equivalent Concentration Method for water-miscible chlorine disinfectants.

These three methods are complete end-point methods that require, within the experimental error, 100% kill of the test organism. Each of the above methods are fully described in the Official Methods of Analysis of the Association of Official Analytical Chemists 15<sup>th</sup> Edition, 1995.

The common mastitis causing pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Pseudomonas aeruginosa*.

## 3.2 Clinical Studies ( field trials )

[See Part 8-4.3 (c) of the Vet. Requirements Series. Note: Parts (a) and (b) are not required]:

### General requirements

- (i) The efficacy of the product must be investigated in the target species.
- (ii) Product formulation and use patterns used in the clinical studies must be identical to those being proposed for registration. Where the applicant wishes to make a label statement for inclusion of extra emollient, data must be provided for use of the product with this extra emollient.
- (iii) Trials would need to be repeated if a new method of product application is recommended for example teat dip, teat spray or automatic spray systems.
- (iv) Trials should be repeated if variable levels or types of teat emollients are recommended, as levels of active constituents ingredients may be modified by such changes.
- (v) If the product is diluted with water, the quality (pH and hardness and alkalinity) of water used in the trial should be determined and clearly stated on the product label. Extra experiments may need to be carried out if a range of parameters of water quality is to be recommended.
- (vi) Efficacy data must be collected for each pathogen for which a claim is made. The crucial parameter for the effectiveness of the product are the results of bacteriological cultures.
- (vii) “For the reduction in the herd incidence of infectious mastitis caused by....” is an acceptable label claim.
- (viii) Any concurrent antibiotic or non-antibiotic therapy administered to a study animal must be fully described.
- (ix) The following baseline data is required for each herd: herd size, number of animals currently lactating, percentage of lactating animals affected by mastitis, age structure of herd, stage of lactation, and mastitis vaccination history.

### Experimental design:

- A split herd design is recommended. Under this design, all teats of half of the cows are treated before or after milking (depending upon label claim) with the final teat sanitiser formulation according to label instructions. The remaining half serving as untreated controls.
- Determining the number of new intramammary infections that occur in all quarters of all cows both treated and control (untreated) quarters. The experimental unit is the individual teat and its associated quarter.

## **Selection of experimental herds, cows and quarters:**

- Data should be collected in a minimum of six herds in at least two geographic locations with at least two independent investigators who supervise the trial.
- Conduct trials in herds in which are expected to have 10-20% of the animals infected in at least one quarter with the target organisms.
- Only include quarters that are free of infection with major mastitis pathogens.
- Exclude cows with teats that are damaged before and during the study.
- Remove cows diagnosed as infected during the trial and subject to appropriate treatment. Their records should be included in the results.

## **Duration:**

The length of an efficacy study will depend on the number of uninfected quarters available initially, on the rate of new infection in the control group, and on the percentage reduction in infections in the treated group. However, trial duration should be a minimum of one year.

## **Culturing:**

- A pre-study culture/somatic cell count survey should be undertaken. It should include milk samples from all lactating quarters on all cows in the herd in order to establish the baseline incidence of clinical and subclinical mastitis in the herd. The survey should take place within the 2-week period prior to initiation of the treatment.
- Culture single quarter milk samples monthly during the entire trial.
- Culture all quarter for the common infectious mastitis pathogens.
- Appropriate bacteriologic culture methods of isolation are described in the *Microbiological Procedures for the Diagnosis of Bovine Mastitis*, National Mastitis Council (NMC), 1990.
- Milk must be grossly examined at each time of sampling for signs of clinical mastitis and the type of clinical mastitis encountered must be recorded.

## **Criteria for diagnosing infections:**

A new intramammary infection is diagnosed in previously uninfected quarter when:

- The same bacterial species is isolated from two consecutive samples taken during the trial.
- A single bacterial species is isolated from a single sample from a quarter with clinical mastitis.
- Clinical infections should be accompanied by the cardinal signs of inflammation, a high somatic cell count and an attempted milk culture.
- A bacteriologically negative clinical case occurs when consecutive samples do not agree.

## **Presentation of data:**

The following information must be included in the final trial report:

- Duration of the trial;
- Numbers of quarters in the study pre-treatment and each subsequent sampling;
- The number of infected and non-infected quarters in the initial survey
- The number of new or recurrent infections detected monthly or bi-monthly with somatic cell counts in the control and treated group.
- The percentage differences in new infections from each pathogen species and the type of mastitis that occurred in the treated and control groups.
- The overall reduction in the incidence of infectious mastitis types (clinical vs subclinical) should be reported for each experimental herd.
- For each pathogen for which a claim is to be made, the incidence of infectious mastitis caused by these individual pathogens should be sorted, summarised, and submitted by herd for each investigator.

## **Statistical analysis:**

- The efficacy of a teat sanitiser is measured by its ability to reduce new intramammary infections.
- Obtain biometrical advice prior the trial to ensure that statistically valid numbers and methods are used.
- The submitted data should be statistically analysed.
- Where different herds/investigators are used, statistical analysis must be conducted using methods for discrete data that accounts for differences due to herds and

investigators. The Mantel Haenszel test is an acceptable method to analyse such differences.