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## HEALTH AND MASTITIS

# INFLUENCE OF MILKING FREQUENCIES OF DAIRY COWS ON EXCRETION CHARACTERISTICS OF ANTIBIOTIC RESIDUES IN MILK

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### Introduction

In Europe, regular milking intervals twice a day are the basis for determining withdrawal periods for milk after treatment of cows with antibiotics. The determination is based on the time necessary to keep below maximum residue limits (MRLs) in the composite milk of treated cows. MRLs are fixed according to Regulation 2377/90 EEC ff. for reasons of health protection of consumers. Residue limits exceeding the MRL may also disturb the manufacturing process of milk products.

The use of automatic milking systems aims at milking cows more frequently than twice daily. However in practice, also prolonged and irregular milking intervals occur. The aim of the study was to determine the excretion of antibiotics in cows milked with different milking frequencies. For the first study a cephalosporine was selected as one of the most important drugs used for mastitis therapy during lactation.

### Material and Methods

- Dairy cows: German Holstein, groups of 4-6 cows; lactation no. 1 to 6; days in lactation: 76-299; somatic cell count (SCC) in composite milk of individual cows below 100 000/ml; average milk yield per day: 22 to 38 kg, comparable milk yield between groups
- Antibiotic drug: Cobactan<sup>®</sup> LC, intramammary device containing 88.8 mg cefquinome-sulfate equivalent to 75 mg cefquinome (Hoechst Roussel Vet, Unterschleissheim, DE), recommended storage temperature below 30 °C
- MRL in milk: 20 µg cefquinome/kg; Indicated withholding time for milk: 5 days
- Treatment: 4 quarters per cow (worst case), one injector per quarter, 3 treatments within 24 hours
- Milking frequencies (milking interval):
  - 2 x per day (14/10 hours, reference);
  - 3 x per day (8 hours);
  - 1.5 x per day (16 hours)
- Qualitative detection of cefquinome by screening tests (microbiological inhibitor tests, receptor assay)

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- Quantitative determination of cefquinome by HPLC method:
  - Internal standard: sulfamethizole
  - Sample preparation: acid preparation, centrifugation, filtration
    - Solid phase extraction: Cation exchange column, elution via potassium acetate/ acetonitrile, concentration 4:1
  - LC-analysis: Reversed phase column, gradient of acetonitrile/phosphate buffer, detection at 270 nm
  - Recovery:  $\geq 80\%$ ; Limit of detection/determination:  $<5/<10 \mu\text{g}/\text{kg}$

### Results and Discussion

The experiment with milking cows twice a day had to be repeated due to improper storage temperature ( $> 30 \text{ }^\circ\text{C}$ ) of drugs used for the respective treatment trial. Large variation in excretion time of cefquinome in milk was observed between individual cows (Fig. 1).

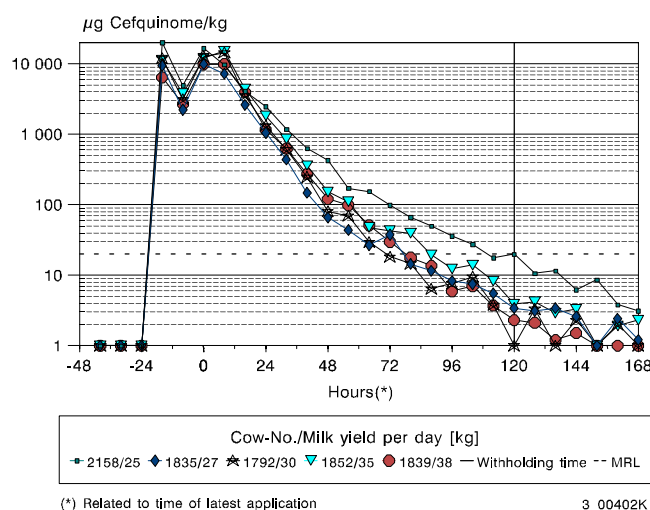


Figure 1: Excretion of cefquinome in milk – milking interval 3 x per day

The results of the excretion studies are summarised in figure 2. The shortest excretion time was observed in cows milked three times per day, the longest in cows milked twice per day.

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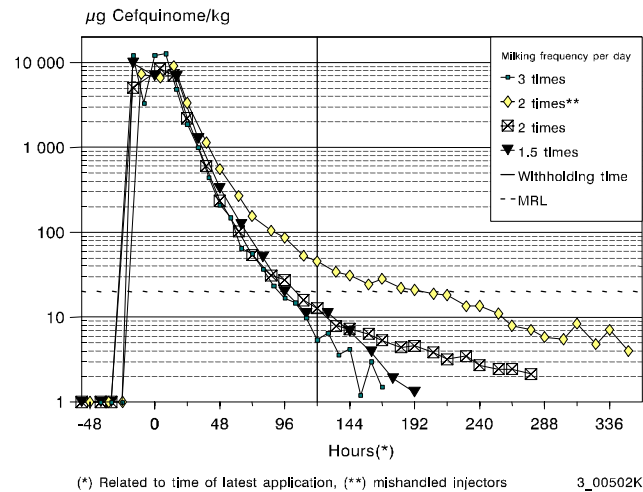


Figure 2: Excretion of cefquinome in milk dependent on milking interval, average of 4-6 cows per group

The results indicate that the influence of individual cows on the excretion time is more pronounced than the influence of the milking frequency. Due to improper storage of the drugs the excretion time in milk was almost doubled compared to the group in the respective experiment. These findings are important for use of antibiotics in dairy cows, independent on type of milking system.

Application of the Time to safe concentration (TTSC)-method (EMEA, 2000) to the current data would lead to an extended withdrawal period of 127 hours for the experimental group milked twice a day (Fig. 3).

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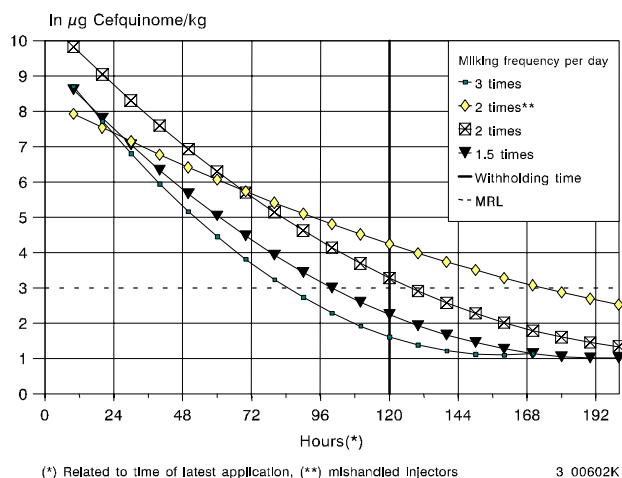


Figure 3: Determination of withholding period according to TTSC-method: Intersection of quadratic regression curve with MRL-concentration

When the 95/95 tolerance limit is calculated the withdrawal period had to be extended to more than 120 hours for all experimental groups. Interpreting these data it has to be taken into account that the tolerance limit factor applied is rather high due to the number of experimental cows which is much lower (4-6 per group) than demanded by EMEA (>19 cows) for the determination of withholding time.

Investigations on effects of different milking frequencies on excretion characteristics of different antibiotic drugs as well as studies in cows with clinical mastitis are in preparation.

### References

Council Regulation (EEC) No. 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, Official Journal of the European Communities No. L 224/1, 18/08/90  
 European agency for the evaluation of medicinal products, EMEA (2000), Committee for veterinary medicinal products: Note for guidance for the determination of withdrawal periods for milk. EMEA/CVMP/473/98-final (2000)

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