Cefquinome formulations for parenteral injection for the treatment of bovine respiratory disease

Risk estimation under FDA/CVM Guidance #152 for cefquinome to evaluate potential microbiological effects on bacteria of human health concern (microbial safety)

As part of the New Animal Drug Evaluation Process for antimicrobial agents in livestock, the FDA/CVM may require a review by the Veterinary Medicine Advisory Committee. This document provides a public record of the risk estimation conducted by the sponsor for injectable cefquinome formulations for the treatment of bovine respiratory disease in accordance with FDA/CVM Guidance #152. This document has been submitted to the CVM as part of the Human Food Safety Assessment. It summarizes proprietary data and information developed and generated by the sponsor, as well as published data and information relevant to the risk estimation for the proposed use of injectable cefquinome in cattle consistent with FDA/CVM Guidance #152.

March 31, 2006

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Glossary of Terms

The following terms have been defined by the US Food & Drug Administration Center for Veterinary Medicine (FDA/CVM) in Guidance for Industry #152, entitled *"Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern"*. The definitions below are copied verbatim from FDA/CVM Guidance #152 and are the definitions used in this document.

Consequence assessment: The consequence assessment describes the relationship between specified exposures to a biological agent (the hazardous agent) and the consequences of those exposures. For the purposes of this risk assessment, FDA has decided that the potential human health consequences of exposure to the defined hazardous agent may be estimated qualitatively by considering the human medical importance of the antimicrobial drug in question.

Exposure assessment: The exposure assessment describes the likelihood of human exposure to the hazardous agent through food-borne exposure pathways. The exposure assessment should estimate qualitatively the probability of this exposure to bacteria of human health concern through food-related pathways.

Hazard: Human illness caused by antimicrobial-resistant bacteria, attributable to an animal-derived food commodity, and treated with the human antimicrobial drug of interest.

Hazardous agent: Antimicrobial-resistant food-borne bacteria of human health concern that are in or on a food-producing animal as a consequence of the proposed use of the antimicrobial new animal drug.

Hazard characterization: The process by which one may identify the hazard and the conditions that influence the occurrence of that hazard. This is based upon drug-specific

information, bacteria/resistance determinant information, and the methodology for the determination of "resistant" or "susceptible" bacteria.

Release assessment: The release assessment should describe those factors related to the antimicrobial new animal drug and its use in animals that contribute to the emergence of resistant bacteria or resistance determinants (i.e., release of the hazardous agent) in the animal. The release assessment should also estimate qualitatively the probability that release of the hazardous agent would occur. For the purposes of this assessment process, the boundaries of the release assessment span from the point the antimicrobial new animal drug is administered to the food-producing animal, to the point the animal is presented for slaughter or the animal-derived food is collected.

Risk: The probability that human food-borne illness is caused by specified antimicrobial-resistant bacteria, is attributable to a specified animal-derived food commodity, and is treated with the human antimicrobial drug of interest.

Risk estimation: The overall estimation of the risk associated with the proposed use of the drug in the target food-producing animals following the integration of the release assessment, exposure assessment and consequence assessment. The risk rankings represent the relative potential for human health to be adversely impacted by the emergence of antimicrobial resistance associated in a food-borne pathogen with the use of the drug in food-producing animals.

Abbreviations

2GC	Second-generation cephalosporins
3GC	Third-generation cephalosporins
4GC	Fourth-generation cephalosporins
ADI	Average daily intake
BRD	Bovine respiratory disease
CEESA	European Animal Health Study Center
EASSA	European Antimicrobial Susceptibility Surveillance in Animals
ELU	Extra-label use
ESBL	Extended-spectrum beta-lactamase
FDA/CVM	United States Food & Drug Administration Center of Veterinary Medicine
MIC	Minimal inhibitory concentration
NAHMS	National Animal Health Monitoring Systems
NARMS	National Antimicrobial Resistance Monitoring System
NCCLS	The National Committee for Clinical Laboratory Standards
OMP	Outer-membrane protein
PBP	Penicillin-binding protein
VMAC	Veterinary Medicine Advisory Committee

Executive Summary

Cefquinome is an extended spectrum beta-lactam and member of the fourth-generation cephalosporins. It is intended for the treatment of bovine respiratory disease (BRD). Various injectable and intramammary formulations have been approved in Europe for livestock since 1994. Cefquinome is not used in either drinking water or in feed. Formulations for the US will be available for parenteral injection, either as multiple or as single-dose products. This risk assessment has been prepared in accordance with FDA/CVM Guidance #152 to evaluate potential microbiological effects on bacteria of human health concern. For this risk assessment *Salmonella* spp. are the relevant food-borne pathogens. *Salmonella* spp. are susceptible to cefquinome whereas *Campylobacter* spp., *Enterococcus faecium*, and *E. faecalis* are not susceptible. *Escherichia coli* (*E. coli*) is susceptible to cefquinome and is therefore considered in this assessment. With the exception of *E. coli* O157:H7, *E. coli* is not normally associated with food-borne clinical infections in humans.

The overall risk estimation is based upon the following theoretical assumptions:

- The proposed use of cefquinome in cattle may cause resistance in *Salmonella* spp. (and *E. coli*) present in the bovine intestinal tract, and
- These resistant *Salmonella* spp. may contaminate the carcass at slaughter and may transfer to humans via food, and
- These resistant *Salmonella* spp. may cause infections in humans which require treatment with a fourth-generation cephalosporin (cefepime), and the effectiveness of treatment may be compromised.

Consistent with FDA/CVM Guidance #152 and the conclusions of FDA/CVM, the overall **risk estimation** is **"medium"**, thus meeting **category 2 classification**. The reasons are as follows:

- The probability that resistance in *Salmonella* spp. and *E. coli* emerges as a result of the therapeutic use of cefquinome (**Release Assessment**) is medium because:
    - 1) The conditions of use (individual treatment, parenteral use, short duration of treatment), and 2) the amount of residual cefquinome-related residues in the intestinal tract of cattle limits the actual exposure of enteric pathogens such as *Salmonella* spp. to cefquinome.
    - Cefquinome does not select for the existing beta-lactam resistance in food-producing animals.
    - The emergence of the transferable ESBL resistance mechanism in food-producing animals conferring resistance to cefquinome can not be excluded.
    - No change in susceptibility to cefquinome has been observed over time in bacterial isolates from cattle.

- According to FDA/CVM Guidance #152 the probability that humans are exposed (**Exposure Assessment**) to *Salmonella* spp. from beef is **medium**. While beef consumption represents a high exposure potential, the probability of contamination of beef with *Salmonella* spp. is low due to its low prevalence.

- Cefquinome is being developed exclusively for veterinary prescription use. Of the fourth-generation cephalosporins, only cefepime (Maxipime®, Bristol-Myers Squibb) is available for human use in the US. Appendix A of FDA/CVM Guidance #152 specifies that fourth-generation cephalosporins are **highly important** in human medicine (**Consequence Assessment**). However, 4GCs are not indicated for enteric pathogens causing food-borne disease, and are not ranked as critically important. While these drugs

are important for human medicine due to their spectrum and favorable resistance situation, treatment alternatives are available against infections caused by enteric pathogens such as *Salmonella* spp.

- This review focuses on the potential risk of compromised effectiveness of 4GCs in human medicine that may result from the use of the 4GC cefquinome in livestock. Due to the limited scope of this review, it is furthermore not the intent of this document to address public health concerns related to the use of 3GCs in human or veterinary medicine, nor to make recommendations regarding prescribing practice in human medicine.

The proposed **risk management measures** exceed those required for category 2 drugs according to FDA/CVM Guidance #152. Conditions and extent of use of cefquinome are appropriate to limit the risk of the emergence of resistance. Both cefquinome formulations currently under development for the US will be used in individual animals under prescription only. The extent of use is low in accordance with FDA/CVM Guidance #152. It is also intended to maintain cefquinome as part of the NARMS susceptibility-surveillance program. This will be an effective tool to track resistance patterns and will potentially help identify the source of antimicrobial resistance emergence.

Consistent with FDA/CVM Guidance #152, no extra-label use limitations or other measures are appropriate for cefquinome, because fourth-generation cephalosporins are not critically important and the extent of use is projected to be low.

1. Hazard Characterization

Drug-specific information

Cefquinome, an aminothiazolyl cephalosporin (sections 2.1.2 and 2.1.3), is an extended-spectrum beta-lactam classified as a fourth-generation cephalosporin (4GC) (Bryskier, 1997). Cefquinome has been used only in veterinary medicine and only for individual treatment, and is safe and well tolerated. In Europe, various formulations of cefquinome for livestock have been approved since 1994. In the US at present, cefquinome is intended for parenteral use in two different injectable formulations for the treatment of bovine respiratory disease (BRD) in individual animals. Other 4GCs (e.g. cefepime, cefpirome) are used only in humans (Bryskier et al, 1994). Cefepime (Maxipime®, Bristol-Myers Squibb) is the only 4GC approved for human use in the US.

Mechanism and type of action

Like other beta-lactams, cefquinome is bactericidal by inhibiting the cell wall synthesis of actively growing bacteria. The cefquinome molecule differs from third-generation cephalosporins (3GCs), e.g. cefotaxime, by a quarternary ammonium side chain attached to the C-3 position of the beta-lactam nucleus (section 2.1.3). As with other 4GCs, cefquinome is a zwitter-ionic compound with improved penetration into the periplasmatic space of Gram-negative bacilli, and enhanced binding to penicillin-binding proteins. As a result of their molecular structure, 4GCs show a higher stability against beta-lactamase such as the stably de-repressed Bush class I beta-lactamase enzymes (Bryskier, 1997). This is an important resistance mechanism against beta-lactam antibiotics.

Spectrum of activity

Owing to its molecular characteristics, cefquinome has a wider spectrum of activity than earlier generations of beta-lactam antibiotics. The activity of cefquinome also differs from that of earlier generations of cephalosporins (Rose et al., 2004; Thomas et al., 2004). 4GCs are generally more active *in vitro* than cefotaxime and ceftriaxone against a variety of

organisms, e.g. Enterobacteriaceae, *Proteus* spp., and *Citrobacter* spp. Cefepime has similar or better activity to ceftazidime against *Pseudomonas aeruginosa* and it is active against some ceftazidime-resistant strains. 4GCs are not, however, clinically active against enterococci and most Gram-negative anaerobes (The Sanford Guide, 2003).

According to scientific data, *Salmonella* spp. as major food-borne zoonotic pathogens relevant to human health are susceptible to cefquinome and cefepime (section 2.1.11). Also *E. coli* is susceptible to cefquinome, which is, with the exception of *E. coli* O157:H7, normally not associated with food-borne clinical infections in humans. *Campylobacter* spp., as food-borne pathogens, and *Enterococcus faecium* and *E. faecalis*, both usually not pathogenic, can be regarded as intrinsically resistant to cefquinome. 4GCs are not clinically active against *Listeria monocytogenes*, another food-borne pathogen; this organism, however, is susceptible to a range of other antimicrobials (The Sanford Guide, 2003; Bahk & Marth, 1990).

Susceptibility testing and susceptibility surveillance data

For the purpose of susceptibility surveillance, cefepime (Maxipime®) has been validated and used as a surrogate marker for cefquinome (Murphy et al., 1994). In terms of antimicrobial spectrum it is closely related to cefquinome and is the only 4GC approved for human use in the US. The majority of susceptibility testing was performed according to National Committee for Clinical Laboratory Standards (NCCLS).

High and sustained antimicrobial activity of cefquinome and cefepime was demonstrated against *Salmonella* spp. isolates obtained from cattle between 2000 and 2003. Testing was performed within NARMS, an extensive antibiotic susceptibility surveillance program conducted in the US. All *Salmonella* spp. (and *E. coli*) isolates tested were highly susceptible to cefquinome or cefepime, whereas variable resistance rates were observed for older cephalosporins routinely tested in the NARMS program. Similar results were obtained in Europe where cefquinome has been used in livestock for ten years. *Salmonella* spp. and

E. coli were susceptible to the 4GC cefpirome, which is registered in Europe for use in humans (Bywater et al., 2004; Thomas et al., 2004). Generally, *Salmonella* spp. isolated from livestock appear to be susceptible to 4GCs. Similarly, fecal *E. coli* isolates collected from US feedlot cattle were susceptible to 4GCs.

In human medicine, an international antibiotic susceptibility surveillance program that regularly includes the cefquinome surrogate marker cefepime is the SENTRY Program (University of Iowa College of Medicine). Between 1997 and 2000, cefepime was active against 96.5% to 100% of *Salmonella* spp. and *E. coli* in all regions of the world. *Salmonella* spp. and *E. coli* isolates were nearly 100% susceptible in Northern America and Europe. Susceptibility to cefepime was similar in *Enterobacter* spp. and *Klebsiella* spp. isolates collected in the SENTRY program (Gales et al., 2002; Jones et al., 2003). Importantly, susceptibility to cefepime was consistently above 98% between 1997 and 2003 in *E. coli* and *Salmonella* spp. isolates obtained from hospitals throughout the US.

Resistance information

As demonstrated by the susceptibility surveillance data, resistance to 4GCs is currently limited or nonexistent. Resistance development to 4GCs is a multi-step process with a change in outer membrane proteins with a decreased permeability and the presence of extended beta-lactamase activity (Fung-Tomc et al., 1996). Generally, resistance affecting other beta-lactams, including earlier generations of cephalosporins, has no influence on susceptibility to 4GCs because 4GCs were designed to circumvent beta-lactam resistance mechanisms.

- The 4GCs are active against Gram-negative bacteria carrying Bush class I beta-lactamases (AmpC-type beta-lactamase).

- The potential for 4GCs to induce AmpC-type mechanisms of resistance is lower than for other beta-lactams (Jones, 1998).

- The 4GCs are less likely to be hydrolyzed by extended spectrum beta-lactamases (ESBLs) than 3GCs (e.g. ceftriaxone) (Bryskier, 1997).

Relative importance of 4GCs in human medicine

All generations of cephalosporins are widely used for a variety of infections in humans. They have been readily accepted as low-toxicity drugs with few side effects and a broad spectrum of activity. However, there are alternatives to cephalosporins for treating diseases in humans (The Sanford Guide, 2003).

This review focuses on the potential risk of compromised effectiveness of 4GCs in human medicine that may result from the use of the 4GC cefquinome in livestock.

Cefepime (Maxipime®), the only 4GC approved for human use in the US, has been available since 1997 as a reconstitutable powder for intravenous or intramuscular injection in hospitalized patients for the treatment of pneumonia, empiric therapy for febrile neutropenic patients, uncomplicated and complicated urinary tract infections, skin and skin structure infections and intra-abdominal infections (Maxipime® Product Label, 1999; The Sanford Guide, 2003).

Cefepime, as with other 4GCs, is not indicated for the treatment of food-borne pathogens (e.g. *Salmonella* spp.). Alternatives are available for the treatment of Gram-negative enteric bacteria like *Salmonella* spp., which include trimethoprim/sulfonamide, fluoroquinolones, azithromycin, amoxicillin/clavulanate, 3GCs, ticarcillin, piperacillin, and carbapenems. These drugs may also be used for treatment of infections with *E. coli*; however, treatment of *E. coli* O157:H7 infections is contraindicated. Preference should be given to carbapenems if an extended-spectrum beta-lactamase (ESBL) resistance mechanism is suspected or has been demonstrated for *Enterobacter cloacae* or other Gram-negative bacilli in nosocomial infections. Cephalosporins are not recommended for *Campylobacter* infections, and therefore this group of organisms needs not be considered. Similarly, there is no need to consider

Enterococcus spp., as they are intrinsically resistant to cephalosporins (The Sanford Guide, 2003).

Appendix A of FDA/CVM Guidance #152 confirms that 4GCs are not indicated for enteric pathogens responsible for food-borne disease. Generally, 4GCs are considered as highly important in human medicine because they may be the sole approach to the treatment of neutropenic fever. In addition, they are indicated for treatment of enteric pathogens in non-food-borne disease.

Hazard identification and definitions

Although 4GCs are not indicated for treatment of infections with *Salmonella* spp., these organisms are the major focus of this risk assessment because they are important relevant food-borne pathogens and susceptible to 4GCs, including cefquinome. The hazard for the purpose of this risk assessment is therefore described as follows: The use of cefquinome in livestock may cause the development of resistance in *Salmonella* spp. because cefquinome residues may reach the intestinal tract of the target animal. Cefquinome resistant *Salmonella* spp. from the intestinal tract may contaminate the carcass at slaughter and be transferred to humans via food. If these resistant *Salmonella* spp. cause an infection in humans, which are treated with a 4GC (cefepime), treatment duration may, for example, be longer, a higher dose may be required, treatment may fail, or another antibiotic may be needed for treatment.

The qualitative risk assessment for cefquinome is based on the following definitions:

- **Hazard**: Human salmonellosis, caused by cefquinome-resistant *Salmonella,* attributable to a food commodity derived from cattle, and treated with a 4GC, e.g. cefepime.

- **Hazardous agent**: 4GC-resistant food-borne *Salmonella* that are in or on cattle as a consequence of the proposed use of cefquinome.

- **Risk**: The probability that human salmonellosis caused by a 4GC-resistant *Salmonella* strain is attributable to a food commodity derived from cattle and is treated with a 4GC.

2. Qualitative Antimicrobial Risk Assessment

2.1 Release Assessment

2.1.1 Description of the product

Cefquinome will be presented as a multi-dose and as a single dose formulation for injection.

2.1.2 Proposed conditions of use

Route of administration:	Parenteral by injection.
Dosing regimen:	Consecutive daily injections for several days or single injection.
Proposed indication:	For the treatment of bovine respiratory disease (BRD) associated with proposed label pathogens, under prescription only.
Target animal species:	Cattle – including lactating and non-lactating cattle (not for pre-ruminant calves).
Withdrawal time:	A pre-slaughter withdrawal time will be assigned by FDA/CVM if necessary.

2.1.3 Drug substance description

The search for more beta-lactamase-stable, broad-spectrum cephalosporins led to the development of the new class of beta-lactams: the so-called 4GC such as cefquinome (initially coded as HR IIIV) for exclusive use in veterinary medicine, as well as similar cephems such as cefepime and cefpirome in human medicine. The principal chemical difference between cefquinome and 3GCs (e.g. cefotaxime or ceftriaxone) is the introduction of a quarternary ammonium side chain attached at C-3 of the beta-lactam nucleus (Bryskier, 1997). Cefquinome is a zwitter-ionic compound with improved penetration into the periplasmatic space of Gram-negative bacilli and enhanced binding to penicillin-binding

proteins. Cefquinome is chemically unsuitable for oral administration via feed or drinking water, due to its relative instability when exposed to moisture.

Drug class: Cephalosporins

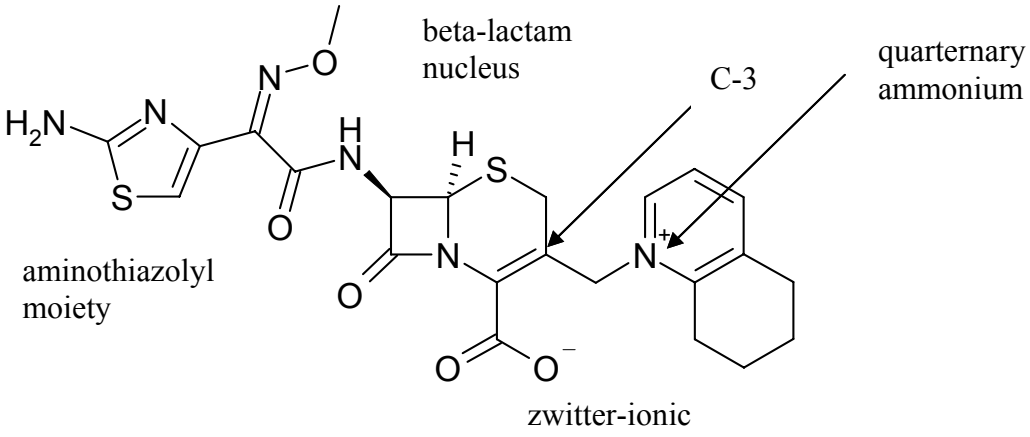
Drug subclass: Fourth-generation cephalosporin (4GC)

Drug common name: Cefquinome

Chemical formula: $C_{23}H_{24}N_6O_5S_2$

CAS Number: 118443-89-3

Structure:



2.1.4 Safety aspects specific to drug class and substance

Cephalosporins are widely accepted as low-toxicity drugs with few side effects. Cefquinome is safe and well tolerated in animals, and is used in veterinary medicine only for individual animal treatment with a veterinarian's prescription. FDA/CVM has reviewed and accepted data on toxicology and microbiology to assess any human health impact of cefquinome residues in edible tissues from target animals. The accepted average daily intake (ADI) of cefquinome emphasizes the non-toxic character of the compound. FDA/CVM has also reviewed and accepted data, and has concluded that there is no allergenic response expected in humans, if cefquinome tissue residues are ingested.

2.1.5 Mechanism and type of action

Like all other beta-lactam antibiotics, the mode of action of cefquinome is related to the binding of the beta-lactam structure to bacterial carboxypeptidases, transpeptidases, and endopeptidases inhibiting peptidoglycan formation. This interferes with cell wall synthesis, which leads to a bactericidal effect. These enzymes and other bacterial proteins, to which the beta-lactam structure binds, are called the penicillin-binding proteins (PBPs). These proteins and their ability to bind to the beta-lactam structure differ between Gram-positive and Gram-negative bacteria and in anaerobic species, giving each compound a unique spectrum of activity. Like other beta-lactam antibiotics, cephalosporins are generally considered more effective against actively growing bacteria (Plumb Veterinary Drug Handbook, 2002).

Compared with earlier generations of cephalosporins such as the 3GCs, the specific molecular structure of 4GCs including cefquinome provides the following novel characteristics with regard to its mechanism of action:

- Higher affinity to penicillin binding proteins.
- Lower affinity and higher stability to beta-lactamases.
- Improved penetration into the periplasmatic space increases the intrinsic potency.

2.1.6 Spectrum of activity

The chemical modifications of the basic cephalosporin structure to create the 4GC have produced a zwitter-ionic compound with enhanced bioavailability and potency, as well as improved spectrum of activity. Cefquinome has a broad spectrum of activity against both Gram- positive and Gram-negative bacteria including *Pseudomonas aeruginosa* and activity against Enterobacteriaceae producing AmpC-type beta-lactamase (section 2.1.8). The *in vitro* activity of cefquinome against veterinary as well as human bacterial isolates has been

extensively studied (Murphy et al., 1994; Limbert et al., 1991; Chin et al., 1992; Rose et al., 2004).

The antimicrobial activity of cefquinome can be summarized as follows:

- Enterobacteriaceae (e.g. *E. coli*, and *Salmonella* spp.).
- Fastidious respiratory tract pathogens (e.g. *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*).
- Gram-positive cocci, e.g. oxacillin-susceptible *Staphylococcus aureus*, coagulase-negative staphylococci including *S. epidermidis*, *Streptococcus pneumoniae*, and beta-hemolytic streptococci.
- *Listeria monocytogenes,* enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus* spp., and *Campylobacter* spp. may be regarded as intrinsically resistant to cefquinome.

Susceptibility surveillance data (as measured by the minimal inhibitory concentration, MIC) are presented in section 2.1.11. Data are presented for *Salmonella* spp. (the major relevant food-borne zoonotic pathogens) and for *E. coli*. With the exception of *E. coli* O157:H7, *E. coli* are not commonly associated with food-borne clinical infections in humans.

2.1.7 Pharmacokinetics

Metabolism studies using radiotracer methodology were performed in cattle with both proposed cefquinome formulations at doses equal to the proposed maximum label doses. From these data it is concluded that less than 10% of the administered dose may be excreted as cefquinome-related residues via the intestinal tract. Only less than 10% of these cefquinome-related residues are the parent compound cefquinome. Considering the dose administered, and assuming an average daily fecal output of cattle, the concentrations of

cefquinome in cattle feces are estimated to be around 0.25 µg/g (250 ppb). These concentrations fall into the MIC range of *Salmonella* spp. and *E. coli* (section 2.1.11). However, this assumes a worst case of completely microbiologically active parent cefquinome in colonic contents, and assumes that cefquinome is unbound and not rapidly degraded. Any microbiologically active parent cefquinome is unlikely to affect commensal enteric bacteria such as *Bacteroides* spp. and *Fusobacterium* spp. because the MICs for these bacteria are >100 µg/ml (Limbert et al., 1991). This calculation also presumes that microbiologically active cefquinome metabolites are absent. However, it is a cautious calculation because it has been demonstrated *in vitro* and *in vivo* that the residual cefquinome-related activity is attenuated in the intestinal tract.

With respect to the assessment of the microbial safety to cefquinome tissue residues, study data demonstrate a reduction of cefquinome parent compound activity in human feces and in rat colon:

- In human fecal samples spiked with cefquinome, a sharp decrease of cefquinome activity of >90% was observed within 4 hours using a microbiological assay with a limit of detection of 50 ppb (Data reviewed and accepted by FDA/CVM).

- No microbiological activity was detected in rat colon after oral administration of cefquinome (Data reviewed and accepted by FDA/CVM), although the chemical assay measured about 250 ppb. This concentration is identical to the anticipated concentration for cattle feces resulting from the treatment with cefquinome at the proposed maximum label dose.

Loss of microbiological activity in the gastrointestinal tract has been shown for another parenteral cephalosporin. Metabolic studies on the cephalosporin ceftiofur (Gilbertson et al., 1990) show a lack of microbiological activity in cattle feces after parenteral administration (limit of detection 0.1 µg/ml, 100 ppb).

However, microbiological effects of cefquinome-related residues on *Salmonella* spp. and *E. coli* present in the intestinal flora cannot be excluded. For example, in an exploratory study, cattle receiving cefquinome parenterally showed a transient reduction in total counts of the enteric *E. coli* populations post treatment. Total counts returned to pre-treatment level within a few days. Similarly, MIC_{90} values were elevated as a result of the cefquinome treatment but returned to pre-treatment level after a few days.

In conclusion, the anticipated concentrations of cefquinome-related residues in cattle feces resulting from therapeutic dosages of cefquinome could *potentially* have microbiological effects on *Salmonella* spp. and *E. coli* present in the enteric microflora. However, there is evidence that these cefquinome-related residues may have only limited microbiological activity owing to degradation or binding effects.

2.1.8 Resistance mechanisms and genetics

The 4GCs were designed to circumvent beta-lactam resistance mechanisms occurring in Gram-negative bacteria. 4GCs are active against bacteria carrying Bush class I beta-lactamases (AmpC-type beta-lactamase). The potential for 4GCs to induce AmpC-type mechanisms of resistance is lower than for other beta-lactams (Jones, 1998). Also, 4GCs are less likely to be hydrolyzed by extended spectrum beta-lactamases (ESBLs) than 3GCs (e.g. ceftriaxone) (Bryskier, 1997). Resistance development to 4GCs is a multi-step process with a change in outer membrane proteins (decreased permeability) and the presence of extended beta-lactamase activity. The relevant mechanisms are described in sections 2.1.8.1, 2.1.8.2, and 2.1.8.3.

2.1.8.1 AmpC-type beta-lactamase

The main mechanism of resistance to beta-lactam antibiotics is the destruction or deactivation of the antimicrobial by beta-lactamases (cephalosporinases) produced by the

target bacteria. Hyperproduction of beta-lactamases is linked with the de-repression ('unlocking') of the chromosomal AmpC gene found in Enterobacteriaceae, including some isolates of *Salmonella* spp. and *E. coli*. De-repression and the resulting hyperinduction of AmpC is an important mechanism of resistance against beta-lactam antibiotics in food-producing animal Enterobacteriaceae (Bradford et al, 1999). In some bacteria, the expression of beta-lactamases is induced in the presence of an antimicrobial. The extent of AmpC induction varies among different antimicrobials. Carbapenems and cephamycins (2GCs) for example are potent inducers, whereas 4GCs have a lower risk of inducing AmpC type beta-lactamase (Jones et al., 1997; Jones, 1998).

The 4GCs combine high cellular penetration and high stability against beta-lactamases. They therefore exert no selection pressure for AmpC-mediated resistance among AmpC-producing Enterobacteriaceae. The use of 4GCs may decrease the frequency of AmpC-hyperproducing strains and may offer improved therapeutic efficacy against human isolates of *Enterobacter*, *Citrobacter* and *Serratia* spp..

Recently, plasmid-mediated AmpC beta-lactamases such as CMY-2 have been identified in both human and animal isolates of Enterobacteriaceae. Plasmid transfer occurs between different strains and species, including *E. coli* and *Salmonella*. Such strains are resistant to 3GCs, but remain susceptible to both 4GCs and carbapenems. It appears that there is no cross-resistance between 4GCs and 3GCs for the AmpC resistance mechanism.

The difference of *in vitro* activity between 4GCs (cefepime, cefpirome, and cefquinome) and 3GCs (cefotaxime, ceftazidime) was shown in genetically modified *E. coli* strains expressing representative plasmid-encoded AmpC cephalosporinases isolated from other enterobacterial species (Rose et al., 2004). The activity of 3GCs was reduced (cefotaxime) or reached resistance level (ceftazidime) (Table 1).

Table 1: *In vitro* activity of 3GCs and 4GCs in genetically modified *E. coli* strains expressing AmpC plasmid-mediated cephalosporinases[a] (minimum inhibitory concentrations [µg/ml]) (Rose, et al., 2004)

Strains	3GCs		4GCs		
(cephalosporinase)	Ceftazidime[a]	Cefotaxime[a]	Cefepime[a]	Cefpirome[a]	Cefquinome[a]
E. coli DH10B (control)	0.12	0.06	0.03	0.03	0.06
E. coli DH10B (MIR-1)	8	16	0.12	0.25	0.12
E. coli DH10B (DHA-2)	32	2	<0.06	0.06	<0.06
E. coli DH10B (ACC-1)	32	8	0.5	4	1
E. coli JM109 (CMY-2)	32	16	0.5	1	0.5
E. coli JM109 (FOX-3)	32	4	<0.06	< 0.06	<0.06

[a] Resistance (R) breakpoints: ceftazidime ≥32 µg/ml, cefotaxime ≥32 µg/ml, cefepime ≥32 µg/ml, cefpirome ≥32 µg/ml, cefquinome ≥8 µg/ml (Luhofer et al., 2004).

Given the potency and low induction potential of cefquinome against both chromosomal and plasmid-mediated AmpC-resistance-determinant in Gram-negative bacilli, therapeutic use may potentially decrease prevalence of AmpC-producing strains.

2.1.8.2 Extended-spectrum beta-lactamases (ESBLs)

The term "Extended Spectrum Beta-Lactamases" (ESBLs) was coined in response to the development of a new group of beta-lactamase enzymes capable of hydrolyzing extended spectrum antimicrobials such as the 3GCs. By definition, the hydrolysis is inhibited by 2 µg/ml of clavulanate. ESBLs are plasmid-mediated and highly mobile derivatives of the TEM enzymes, for example. ESBLs may hydrolyze cefquinome to various degrees as opposed to conventional beta-lactamase. Cefquinome may retain partial activity against bacteria carrying the ESBL-resistant determinants (Table 2).

Table 2: *In vitro* activity of cefquinome in *E. coli* carrying different types of plasmid resistance determinants (Data reviewed and accepted by FDA/CVM)

Enzyme	Resistance determinant	Cefquinome	
		% hydrolysis	MIC (μg/ml)
TEM-1	Beta-lactamase	<0.0	0.06
TEM-2	Beta-lactamase	<0.1	0.12
TEM-3	ESBL	5	8
TEM-7	ESBL	–	4
TEM-9	ESBL	45	64
OXA-1	Beta-lactamase	–	2
OXA-2	Beta-lactamase	<0.1	–
SHV-1	Beta-lactamase	<0.1	0.25

In humans, the occurrence of ESBLs in the most monitored species, *E. coli* and *K. pneumoniae*, varies widely within the US and the world. In contrast to elevated rates in the world, North America appears to have the lowest overall rates of ESBL isolates. ESBL plasmids have been reported in *Salmonella* spp. isolates from humans. The so-called CTX-M-type ESBL has been associated with human *E. coli* and *Salmonella* Typhimurium isolates, originally in South America, Japan, and Taiwan**.**

In contrast, bacterial strains with ESBL phenotypes are extremely rare in animal isolates. Molecular analysis of *Salmonella* spp. isolates with suspected ESBLs has revealed that the underlying mechanism is AmpC-related (section 2.1.11.1). The genetic analysis of bovine *Salmonella* spp. from the NARMS program (2000) with reduced susceptibility to cephalosporins carried AmpC resistance mechanisms; however, no ESBL were detected. These isolates were susceptible to cefquinome and cefepime. It appears that no true ESBL-producing *Salmonella* spp. or *E. coli* isolates obtained from animals have been reported in the US. For example, published data indicate that cephalosporin resistance among veterinary

isolates of *E. coli* and *Salmonella enterica* is associated with CMY-2 (AmpC) and not with ESBLs (Bradford et al., 1999; Gray et al., 2004).

2.1.8.3 Outer-membrane proteins

A change in outer membrane proteins (OMP) is a different mechanism of resistance in comparison to enzymatic hydrolysis via beta-lactamases, such as AmpC and ESBLs. Membrane protein changes can result in decreased permeability of the antimicrobial or increased efflux preventing the compound interacting with intracellular target molecules (PBPs). OMP resistance is chromosomally encoded and based on the occurrence of a mutational event, and therefore not among the transferable mechanisms. Multi-passage studies with *Enterobacter cloacae* variants showed that resistant strains selected by 4GCs differ from those selected by 3GCs. Eighty percent (vs. 10% for 3GCs) of strains selected by 4GCs lacked or had diminished levels of a 39 to 40 kDa major porin protein known to be involved in the permeation of cephalosporins (Fung-Tomc et al., 1996).

2.1.8.4 Conclusions

The 4GCs, such as cefquinome, combine high cellular penetration and beta-lactamase stability with low AmpC-induction potential; thus they are unlikely to select for highly resistant mutants among AmpC-producing Enterobacteriaceae. ESBLs in *Salmonella* spp. and *E. coli* isolates from livestock have not been reported, and are not a focus of this risk assessment. Emerging resistance among Enterobacteriaceae selected by 4GC therapy appears to be very rare. High-level resistance to 4GCs appears to require the synergistic activity of two mutations: enhanced beta-lactamase hyperproduction and hydrolysis, and decreased membrane permeability (Fung-Tomc et al., 1996; Tzouvelekis et al., 1998).

2.1.9 Occurrence and rate of transfer of resistance determinants

Plasmid-mediated resistance might be transferred by conjugation or by transposon integration among bacteria. As shown in section 2.1.8, plasmid-mediated beta-lactam resistance is unlikely to be associated with cefquinome in food-producing animals. Alterations of the outer-membrane proteins are chromosomally encoded. The emergence of the transferable ESBL resistance mechanism in food-producing animals conferring resistance to cefquinome can not be excluded.

2.1.10 Resistance selection pressures

Resistance to 3GCs in Enterobacteriaceae of animal origin is often associated with plasmid-mediated AmpC-based enzymes, and is not related to ESBLs. However, these isolates are usually susceptible to 4GCs, such as cefquinome. Therefore, the selection pressure of cefquinome is lower in comparison to earlier generations of beta-lactams.

Resistance development for 4GCs is slower than for 3GCs, which has been shown in an *in vitro* multi-passage study, involving *Enterobacter cloacae* as the model bacterial species. Using cefepime as the proposed "surrogate marker" for cefquinome, only two of ten strains had a reduced (intermediate) susceptibility after multiple passages. This reduced susceptibility was associated with changes in outer-membrane structure. In contrast, the 3GCs ceftriaxone and ceftazidime exhibited resistance in nine of ten and eight of ten strains, respectively. Resistance to 4GCs required both a diminished amount of porin protein and hyperproduction of beta-lactamase. Therefore, two events are required for resistance to develop to the 4GC compounds; with the implication that resistance development is much slower than with the 3GCs (Fung-Tomc et al., 1996; Tzouvelekis et al., 1998). This limits the potential for resistance development in 4GCs.

The conditions of use limit the exposure of enteric pathogens to cefquinome-related residues, which further reduces the selection pressure. Cefquinome will be labeled for the treatment of

respiratory disease in cattle. Use of cefquinome will be solely therapeutic and of short duration. Cefquinome will be administered parenterally at therapeutic doses to individual animals. Only a small percentage of the total number of animals in a feedlot or dairy will be treated. The pre-slaughter selection pressure should be minimal because treatment will generally occur at least two months (about 60 days) before expected slaughter.

Overall, the anticipated selection pressure resulting from the use of the two cefquinome formulations is expected to be low. The reasons are as follows:

- As a 4GC, cefquinome does not select for the current most prevalent beta-lactam resistance mechanisms (AmpC).
- No change in susceptibility to cefquinome has been observed over time in bacterial isolates from cattle.
- The conditions of use include individual treatment, parenteral use, short treatment duration, and prescription-only status. This results in only limited exposure of enteric pathogens (e.g. *Salmonella* spp.) to cefquinome-related residues, thus limiting selection pressure.

2.1.11 Baseline prevalence of resistance

This section summarizes susceptibility surveillance data as measured by the minimal inhibitory concentration (MIC) for animal isolates and human isolates. FDA/CVM has reviewed and accepted data demonstrating that the use of the human 4GC cefepime as susceptibility-surveillance marker for cefquinome is appropriate for Enterobacteriaceae and Gram-positive bacteria. The MICs for cefepime are highly predictive of those for cefquinome. According to NCCLS, the equivalent MIC breakpoints established for cefepime are ≥32 μg/ml and ≤8 μg/ml for resistant and susceptible strains, respectively. These

breakpoints are being further refined. Cefquinome-specific breakpoints will be developed based on the susceptibility of target pathogens, pharmacology, and conditions of use.

2.1.11.1 Animal data

Extensive susceptibility surveillance programs have been performed by the sponsor. Although cefquinome has been marketed in Europe for more than 10 years, post-approval data demonstrate that the susceptibility of target pathogens, food-borne, and commensal bacterial species to cefquinome has remained unaffected. For the US (pre-approval), bovine *Salmonella* spp. isolates have been shown to be highly susceptible to cefquinome.

2.1.11.1.1 European susceptibility data of bovine *Salmonella* spp. and *E. coli*

Non-diagnostic *Salmonella* spp., *E. coli*, and *Campylobacter* spp. were collected at slaughter from healthy-food-producing animals between 1999 and 2001 within the scope of the European Antimicrobial Susceptibility Surveillance in Animals (EASSA), a program coordinated by the European Animal Health Study Center (CEESA) (Bywater et al., 2004). The isolates were used as sentinel organisms in order to monitor antimicrobial resistance in zoonotic bacteria from poultry, pork, and cattle in eight European countries. Susceptibility testing to a panel of antimicrobials commonly used in human medicine was conducted at a central laboratory according to NCCLS standards. Newer antibiotic compounds included cefepime, cefotaxime, and ciprofloxacin.

This study provides cefquinome-related post-marketing data for the 4GC class represented by cefepime. There is a high level of susceptibility with no resistance in European *E. coli* and *Salmonella* spp. veterinary isolates for cefepime even after years of cefquinome use in livestock (Table 3).

Table 3: *In-vitro* activity of cefepime in *E. coli* and *Salmonella* spp. isolates collected between 1999 and 2001 in various European countries (Bywater et al., 2004)

Country	Species	*E. coli*			Salmonella		
		n	MIC_{90} μg/ml	% Resistant[a]	n	MIC_{90} μg/ml	% Resistant
France	Chicken	199	0.063	0.0	75	0.125	0.0
Netherlands	Chicken	204	0.063	0.0	–	–	–
Sweden	Chicken	199	0.063	0.0	–	–	–
UK	Chicken	200	0.063	0.0	43	0.125	0.0
France	Cattle	21	0.032	0.0	–	–	–
Germany	Cattle	355	0.032	0.0	–	–	–
Italy	Cattle	189	0.032	0.0	–	–	–
UK	Cattle	99	0.032	0.0	–	–	–
Denmark	Pig	200	0.032	0.0	100	0.125	0.0
Netherlands	Pig	200	0.063	0.0	31	0.125	0.0
Spain	Pig	48	0.016	0.0	15	0.063	0.0
Sweden	Pig	204	0.063	0.0	–	–	–

[a] % resistant; resistance breakpoint ≥32 μg/mL (NCCLS for Enterobacteriaceae). Note that *Salmonella* for cattle have not been reported due to very low prevalence.

High and sustained level of susceptibility in bovine *Salmonella* spp. to 4GCs was shown for amoxicillin-resistant *Salmonella* Typhimurium isolates (Table 4) collected in the United Kingdom between 1993 and 2001 (Thomas et al., 2004). None of the isolates were resistant to 3GCs and 4GCs. Some resistance to older cephalosporins was observed from 1993 to 1999, but this was not observed in 2000 and 2001.

Table 4: *In vitro* activity of seven cephalosporins in amoxycillin-resistant *Salmonella* Typhimurium isolated from cattle in the UK between 1993 and 2001 (n = number of tested isolates)

Antibiotic (resistance breakpoint)		1993 (n=23)	1994 (n=26)	1995 (n=26)	1996 (n=26)	1997 (n=29)	1998 (n=30)	1999 (n=29)	2000 (n=23)	2001 (n=20)
Cephapirin	MIC_{50}	8	8	8	8	8	4	8	8	8
(>16 μg/ml)	MIC_{90}	64	16	16	8	8	8	8	8	8
	% R	30	3.8	7.7	0	3.4	3.3	3.3	0	0
Cepha-	MIC_{50}	8	4	4	4	4	4	4	4	4
lothin	MIC_{90}	32	8	8	8	8	4	4	4	8
(>16 μg/ml)	% R	30	0	3.8	0	3.4	0	0	0	0
Cefoxitin	MIC_{50}	4	2	2	2	4	2	2	2	2
(>16 μg/ml)	MIC_{90}	16	4	4	4	4	4	4	4	4
	% R	0	0	3.8	0	3.4	0	0	0	0
Ceftiofur	MIC_{50}	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
(>4 μg/ml)	MIC_{90}	2	1	1	1	1	1	1	1	1
	% R	0	0	3.8	0	0	0	0	0	0
Ceftа-	MIC_{50}	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
zidime	MIC_{90}	0.5	0.25	0.5	0.5	0.25	0.25	0.25	0.25	0.25
(>16 μg/ml)	% R	0	0	0	0	0	0	0	0	0
Cef-	MIC_{50}	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
quinome	MIC_{90}	0.25	0.125	0.125	0.25	0.25	0.125	0.25	0.125	0.125
(≥8 μg/ml)[a]	% R	0	0	0	0	0	0	0	0	0
Cefepime	MIC_{50}	0.25	0.125	0.25	0.25	0.125	0.125	0.25	0.125	0.25
(>16 μg/ml)	MIC_{90}	0.25	0.25	0.5	0.5	0.5	0.25	0.25	0.25	0.25
	% R	0	0	0	0	0	0	0	0	0

[a] Luhofer et al., 2004.

Similar findings were reported for amoxicillin-resistant *Salmonella* Typhimurium isolates obtained from France in 2001 and Germany in 2003. This stable situation within and across cephalosporin generations was observed even after the first cefquinome marketing authorization in Europe for bovine respiratory disease in 1994. The underlying amoxicillin resistance did not confer resistance to 4GCs and the 3GC ceftazidime, nor did it cause any decrease of cephalosporin activity or cross-resistance against *Salmonella* Typhimurium in the long term (Thomas et al., 2004).

In addition, the use of cefquinome in food-producing animals in Europe has not led to a decrease of susceptibility in a broad range of pathogens, including *E. coli* that were isolated from cases of clinical mastitis in dairy cows. *E. coli* isolates collected in 1994 (n=76) and 1999 (n=79) consistently exhibited a MIC_{90} of 0.125 μg/ml.

In Europe, cefquinome formulations have been developed and approved for the following species and indications:

- Cattle for the treatment of respiratory disease (1994).
- Dairy cattle for the treatment of clinical mastitis (1996).
- Cattle for the treatment of *E. coli* mastitis, *E. coli* sepsis, footrot (1997).
- Pigs/piglets for the treatment of respiratory disease and other diseases (2001/03).
- Use in dry cows (2004).

Despite the use of cefquinome for these indications, it can be concluded from the susceptibility surveillance data that the use of cefquinome in food-producing animals has not led to any observable decrease in susceptibility or the occurrence of resistance in *Salmonella* spp. and *E. coli* or in target pathogens (Tables 3 and 4) (Bywater et al., 2004, Thomas et al., 2004).

2.1.11.1.2 US susceptibility data of bovine *Salmonella* spp.

Since pre-approval data are important to assess changes in bacterial susceptibility post approval, the sponsor initiated the co-testing of cefquinome and cefepime in *Salmonella* spp. isolates from cattle within the NARMS program in 2000. A summary of the testing results for cefquinome from 2000 until 2003 is provided in Table 5. Non-diagnostic isolates were

obtained from raw products collected from federally inspected slaughter and processing plants and represent either carcass swabs or ground product.

Salmonella isolates from 2000 showed high susceptibility to cefquinome with a MIC_{90} of 0.5 µg/ml (range of ≤0.015 to 1.0 µg/ml) for diagnostic isolates, and a MIC_{90} of 0.25 µg/ml (range ≤0.015 to 4.0 µg/ml) for non-diagnostic isolates, respectively. From 2001 to 2003, all *Salmonella* spp. isolates remained highly susceptible to cefquinome. Changes in MIC_{90} and in the MIC range of one dilution step are considered inherent to MIC testing procedures (NCCLS, 2002). It is noteworthy that the 2003 MICs of non-diagnostic isolates matched those from 2001. The MICs for cefepime were comparable to those from cefquinome thus confirming the "surrogate marker" status of cefepime for cefquinome. Overall, the data indicate that the susceptibility situation is stable with no upward trend.

Table 5: *In vitro* activity of cefquinome in bovine *Salmonella* spp. isolates collected from the NARMS program between 2000 and 2003

Year	2000	2001	2002	2003
Number of isolates tested (n)	1,936	1,183	901	671
Diagnostic isolates (n)	216	286	199	–
MIC_{50} (µg/ml)	0.03	0.12	0.12	–
MIC_{90} (µg/ml)	0.5	1.0	1.0	–
Non-diagnostic isolates (n)	1,720	897	702	671
MIC_{50} (µg/ml)	0.06	0.06	0.12	0.06
MIC_{90} (µg/ml)	0.25	0.5	1.0	0.5

For example, 74 different serotypes of *Salmonella* spp. isolates were identified and ranked during the NARMS study in 2001. Among the non-diagnostic isolates the top-ranking serotypes were *S.* Montevideo, *S.* Newport, *S.* Typhimurium, and *S.* Anatum, which had prevalence rates between 8 and 15%. *S.* Newport and *S.* Typhimurium were the most frequent serotypes among the diagnostic isolates.

In addition, the genetic background of the *Salmonella* spp. NARMS isolates (year 2000) with reduced susceptibility or resistance to older cephalosporins was investigated. The majority of the isolates (n=43) were represented by the serotypes *S.* Newport and *S.* Typhimurium. Susceptibility testing showed resistance to a broad range of beta-lactam antibiotics except for 4GCs (cefepime, cefpirome and cefquinome) and carbapenems (imipenem). All isolates had additional multi-drug resistance to at least chloramphenicol, sulfonamides, streptomycin, and tetracycline. In all isolates the CMY-2 beta-lactamase gene (AmpC-related) was identified (section 2.1.8). None of the isolates carried the ESBL-resistance mechanism thus confirming the absence of ESBLs in *Salmonella* spp. from animal origin.

2.1.11.1.3 US susceptibility data of *Salmonella* spp. from dairy cattle

The susceptibility of 1052 *Salmonella* spp. isolates obtained in 1997 from US dairy farms and in 2002 in the NAHMS Dairy Cattle Study were tested against cefquinome and cefepime. All isolates were non-diagnostic fecal isolates (Table 6).

Table 6: *In vitro* activity of cefquinome and cefepime in *Salmonella* spp. collected from feces of dairy cattle in the US

Year	MIC_{50}		MIC_{90}		MIC range	
Number of	(μg/ml)		(μg/ml)		(μg/ml)	
Isolates	Cefquinome	Cefepime	Cefquinome	Cefepime	Cefquinome	Cefepime
1997 (n = 758)	0.06	0.06	0.12	0.06	<0.015 – 0.5	<0.015 – 0.25
2002 (n = 294)	0.06	0.06	0.12	0.12	0.03 – 1	0.03 – 1

In 1997, all isolates were highly susceptible to cefquinome, with little change five years later. Changes within one dilution step are considered inherent to MIC testing procedures (NCCLS, 2002). In terms of the prevalence of serotypes, *S.* Newport and *S.* Typhimurium have become more frequent in 2002 compared with 1997.

2.1.11.1.4 US susceptibility data of non-diagnostic bovine *E. coli*

Susceptibility data are also available for *E. coli*, which, with the exception of *E. coli* O157:H7, are normally not associated with food-borne clinical infections in humans. *E. coli* were isolated from fecal samples of feedlot cattle in four different geographical regions of the US. The susceptibility of these isolates was tested against cefquinome, cefepime, ceftriaxone, and ceftazidime (Table 7). Similar to the results with bovine *Salmonella* spp., these data represent baseline susceptibility. MICs were comparable to those from Europe (section 2.1.11.1.1). There was no resistance to any of the cephalosporins tested.

Table 7: *In vitro* activity of cephalosporins in non-diagnostic *E. coli* isolates collected in 2002 from feces of feedlot cattle in the US (n=189)

	Cefquinome	Cefepime	Ceftriaxone	Ceftazidime
MIC_{90} (μg/ml)	0.125	0.063	0.125	0.25

2.1.11.1.5 Conclusions

A high level of suceptibility to cefquinome (or its surrogate marker cefepime) has been demonstrated in *Salmonella* spp., *E. coli*, and veterinary pathogens in Europe post approval, and for bovine *Salmonella* spp. and bovine *E. coli*. pre-approval in the US.

2.1.11.2 Human data

In human medicine, an international susceptibility surveillance program that regularly includes the cefquinome surrogate marker cefepime is the SENTRY program (University of Iowa College of Medicine). Between 1997 and 1999, cefepime was active against 96.5% to 100% of *Salmonella* spp. and *E. coli* in all regions of the world. *Salmonella* spp. and *E. coli* isolates were nearly 100% susceptible to cefepime in Northern America and Europe (Table 8). Susceptibility to cefepime was similar in *Enterobacter* spp. and *Klebsiella* spp. isolates collected in the SENTRY program (Gales et al., 2002; Jones et al., 2003).

Table 8: *In vitro* activity of cefepime in *E. coli* and *Salmonella* spp. isolates collected from the SENTRY surveillance program (1997-1999)

Source of isolates	Surveillance program	*E. coli* MIC_{90}	*E. coli* Number of isolates	*E. coli* % suscept-ible	*Salmonella* spp. MIC_{90}	*Salmonella* spp. Number of isolates	*Salmonella* spp. % suscept-ible
All regions	SENTRY	≤0.12	13,205	98.9	≤0.12	433	99.5
North America	SENTRY	≤0.12	6,253	99.8	≤0.12	90	100
Europe	SENTRY	≤0.12	3,816	99.1	0.25	128	100
Latin America	SENTRY	0.5	2,033	96.5	≤0.12	127	98.4
Asia-Pacific	SENTRY	0.25	1,103	97.6	≤0.12	88	100

For *E. coli* and *Salmonella* spp. isolates obtained from hospitals throughout the USA from 1997 until 2003, resistance rates for cefepime were between 0.1% and 1.4%, with no upward trend observed (Table 9 and Table 10). This susceptibility surveillance network is of particular interest because it provides timely susceptibility information on key enteric species such as *E. coli* and *Salmonella* spp.. It is concluded from the data that: 1) modern beta-lactams appear to have maintained high levels of activity, and 2) a range of therapeutic options are apparently available to treat infections associated with *Salmonella* spp. and *E. coli* infections.

Table 9: Percentage of resistance to beta-lactam antibiotics including beta-lactamase inhibitor combination in *E. coli* isolates collected from US hospitals between 1997 and 2003

Antimicrobial		1997	1998	1999	2000	2001	2002	2003
Cefepime	Total n	1,395	19,644	41,842	72,642	94,939	110,903	80,952
	Res[a] %	0.1	0.5	0.7	0.4	0.3	0.5	0.5
Ceftazidime	Total n	42,361	67,713	96,617	128,984	150,839	163,485	108,118
	Res[a] %	0.8	1.2	1.2	1	1.1	1.2	1.3
Ceftriaxone	Total n	60,412	90,867	123,020	171,156	188,120	201,300	141,573
	Res[a] %	0.3	0.3	0.4	0.4	0.5	0.7	0.7
Imipenem	Total n	40,302	58,806	88,990	136,756	149,984	162,193	105,902
	Res[a] %	0	0	0	0	0	0	0
Piperacillin/	Total n	13,016	26,976	54,885	112,152	138,348	152,322	104,661
tazobactam	Res[a] %	1.6	1.6	1.6	1.4	1.2	1.1	1

[a] Resistance.

Table 10: Percentage of resistance to beta-lactam antibiotics including beta-lactamase inhibitor combination in *Salmonella* spp. isolates collected from US hospitals between 1997 and 2003

Antimicrobial		1997	1998	1999	2000	2001	2002	2003
Cefepime	Total n	10	143	295	466	562	615	358
	Res[a] %	0	1.4	0.7	0.2	0.9	1	0.6
Ceftazidime	Total n	378	606	731	776	869	926	480
	Res[a] %	0.3	0.3	0.5	2.2	2.6	2.8	2.7
Ceftriaxone	Total n	439	708	916	1,038	1,063	1,179	674
	Res[a] %	0	0.1	0.2	1	1.2	2.2	2.1
Imipenem	Total n	314	481	595	733	781	845	420
	Res[a] %	0	0	0	0	0	0	0
Piperacillin/	Total n	108	240	268	538	735	766	446
tazobactam	Res[a] %	2.8	1.7	1.5	2.8	2.3	2.1	2

[a] Resistance

2.1.12 Conclusions

As shown in Table 11, the probability of the emergence of cefquinome-resistant food-borne bacteria is low. With the exception of spectrum of activity, all variables are considered

having a low or medium probability for resistance development, and thus diminish the concerns about a potential hazard for human health.

Table 11: Summary of relevant aspects to assess the probability of the emergence of cefquinome-resistant food-borne bacteria of potential human health concern

Relevant variables	Summary of relevant aspects and extent to which factors may favor the emergence of resistance
Mechanism of action:	Binding to bacterial penicillin-binding proteins thus inhibiting peptidoglycan cell wall formation. Bactericidal. Probability: **LOW**
Spectrum of activity:	High affinity to penicillin-binding proteins, and low affinity and high stability to beta-lactamases, and fast penetration into periplasmatic space provides an extremely broad spectrum of activity, including activity against *Pseudomonas*". Probability: **HIGH**
Pharmacokinetics:	Microbiological effects of cefquinome residues on *Salmonella* spp. and *E. coli* in the enteric microflora of cattle not completely excluded. Probability: **LOW**
Pharmacodynamics:	Time-dependent bactericidal activity, post antibiotic effect comparable to other beta-lactams. Probability: **LOW**
Resistance Mechanisms:	No selection for the existing beta-lactam resistance mechanisms: cefquinome is effective against bacteria producing the beta-lactams - most relevant mechanism (AmpC), and no evidence of ESBLs in food-producing animals. Cefquinome resistance requires either changes in outer-membrane proteins (reduced permeability) plus enhanced beta-lactamase activity or ESBLs that confer resistance to 4GC. Probability: **MEDIUM**
Resistance transfer:	Relevant outer-membrane modification is chromosomally encoded and therefore not transferable. Plasmid related ß-lactam resistance is not relevant for cefquinome: cefquinome does not select for AmpC, and ESBLs are not prevalent in food-producing animals. Since the emergence of the ESBL resistance mechanism in food-producing animals can not be excluded and due to the transferable nature of this mechanism this variable is ranked medium. Probability: **MEDIUM**
Selection pressure:	Expected to be low because: - Cefquinome should not select for the existing beta-lactam resistance mechanisms (AmpC). - Conditions of use with individual treatment, parenteral use, and short duration of treatment result in limited exposure of enteric pathogens (e.g. *Salmonella*) to cefquinome-related residues. Probability: **LOW**

Table 11: Summary of relevant aspects to assess the probability of the emergence of cefquinome-resistant food-borne bacteria of potential human health concern

Relevant variables	Summary of relevant aspects and extent to which factors may favor the emergence of resistance
Baseline prevalence of resistance:	A high level of susceptibility to cefquinome (or cefepime) was observed post approval (Europe) in *Salmonella* and *E. coli* and in veterinary pathogens, and pre-approval (US) in bovine *Salmonella* and in bovine *E. coli*. Probability: **LOW**
Other factors:	Respiratory disease requiring treatment generally occurs during early stage of production, long before animals are ready for entry into the food chain. Probability: **LOW**

Salmonella spp. are the focus of this risk assessment because *Salmonella* spp. are the major food-borne zoonotic pathogens of concern. Both *Salmonella* spp. and *E. coli* are susceptible to cefquinome. Other relevant bacteria of potential concern, such as *Campylobacter* spp., *Enterococcus faecium, E. faecalis* and *Listeria monocytogenes,* are not susceptible to cefquinome.

The probability that resistance in *Salmonella* spp. (and *E. coli*) emerges as a result of the treatment of BRD with cefquinome is medium because:

- 1) The conditions of use (individual treatment, parenteral use, short treatment duration), and 2) the amount of residual cefquinome-related residues in the intestinal tract of cattle limits the exposure of enteric pathogens such as *Salmonella* spp. to cefquinome.
- Cefquinome does not select for the existing beta-lactam resistance mechanisms occurring in food-producing animals.

- The emergence of the transferable ESBL resistance mechanism in food-producing animals conferring resistance to cefquinome can not be excluded.

- No change in susceptibility to cefquinome has been observed over time in bacterial isolates from cattle.

2.2 Exposure Assessment

FDA/CVM Guidance #152 defines the process for qualitatively ranking the probability of human exposure to a given bacteria in food commodities based on national surveys of food commodity consumption in the US and the food commodity contamination rate data.

As previously described, bovine *Salmonella* spp. are the major focus of this risk assessment. It is the most relevant food-borne pathogen that may cause human disease, and reflects a worst case scenario. *E. coli*, with the exception of *E. coli* O157:H7, are normally not associated with food-borne clinical infections in humans. However, there is zero-tolerance for the presence of *E. coli* O157:H7 in food. For this reason, and because treatment of disease associated with *E. coli* O157:H7 is controversial, *E. coli* is not considered relevant to this risk assessment.

The potential for human exposure to resistant *Salmonella* spp. as a result of use of cefquinome is primarily via consumption of contaminated beef. Cefquinome is also intended for treatment of dairy cattle, however the likelihood of exposure from milk is low as >99% of milk consumed in the US is pasteurized. For this reason, milk is not a major pathway of human exposure, and has not been considered in this risk assessment.

USDA Economic Research Service estimates annual US beef consumption for 2001 to be 62.9 lb per capita. While beef consumption represents a high exposure potential, the

probability of food contamination with *Salmonella* spp. is low because prevalence of these organisms in beef commodities is very low as illustrated in Table 12.

Table 12: *Salmonella* spp. prevalence and its ranking in beef commodities in 2001 according to FDA/CVM Guidance #152

Commodity	% prevalence *Salmonella*	Qualitative ranking
Cows / bulls	2.4	Low
Steers / heifers	0.6	Low
Ground beef	2.8	Low

These default values provided in FDA/CVM Guidance #152 for beef result in the overall exposure assessment for beef as **medium** (Table 13)

Table 13: Probability of human exposure to *Salmonella* spp. from beef is *medium* (H=high, M=medium; L=low)

Amount of food	Amount of food commodity being consumed		
Commodity contamination	High	Medium	Low
High	H	H	M
Medium	H	M	L
Low	**M**	L	L

2.3 Consequence Assessment

Relative importance to human medicine

Cefquinome is being developed exclusively for veterinary medicine and will be used under veterinary prescription only. Cefepime (Maxipime®) is the only 4GC that is available for humans in the US (Maxipime® Product Label, 1999). Cefepime is an established and generally well-tolerated parenteral drug with a broad spectrum of antibacterial activity. Similar to cefquinome, cefepime is stable against many of the common plasmid- and

chromosomal- mediated beta-lactamases and is a poor inducer of AmpC beta-lactamases. Cefepime retains activity against Enterobacteriaceae that are resistant to earlier beta-lactams. As with cefquinome, cefepime may be hydrolyzed by some ESBLs produced by some members of the Enterobacteriaceae, but to a lesser extent than 3GCs.

Cefepime was approved in the US for use in humans in 1997 as a reconstitutable powder for intravenous or intramuscular injection. Use is limited to infections in hospitalized patients and includes treatment of pneumonia, empiric therapy for febrile neutropenic patients, uncomplicated and complicated urinary tract infections, skin and skin structure infections, and intra-abdominal infections. Recommended doses range from 0.5 to 2 g every 8 to 12 hours for 7 to 10 days. Susceptible pathogens include S. *pneumoniae, P. aeruginosa, K. pneumoniae, E. coli, Staph. aureus* (methicillin-susceptible strains only*)*, and *S. pyogenes*. Cefepime has neither been labeled nor used for the treatment of food-borne illness.

Appendix A of FDA/CVM Guidance #152 confirms that cefepime is not indicated for the treatment of enteric pathogens causing food-borne disease. Generally, 4GCs are considered highly important in human medicine because they may be the sole option for the treatment of neutropenic fever. The 4GCs are also indicated for treatment of enteric pathogens in non-food-borne disease (e.g. *E. coli* associated with urinary tract infections).

Table 14 summarizes criteria to categorize antimicrobials according to their importance in human medicine, and the respective conclusions for 4GCs.

Table 14: Importance of 4GCs in human medicine as defined in Appendix A of FDA/CVM Guidance #152

Criteria	4GCs
Antimicrobial used to treat enteric pathogens that cause food-borne disease	No
Sole therapy or one of few alternatives to treat serious human disease or drug is essential component among many antimicrobials in treatment of human disease.	Yes (***only*** for neutropenic fever associated with nosocomial infection)
Antimicrobials used to treat enteric pathogens in non-food borne disease.	Yes
No cross resistance within drug class and absence of linked resistance with other drug classes.	No
Limited risk in transmitting resistance elements within or across genera and species of organisms	No

Although cefepime is an important drug for use in humans, it is not the exclusive drug used against any of the above pathogens. Alternatives to cefepime do exist and are often preferred, as indicated in The Sanford Guide 2003 to Antimicrobial Therapy (The Sanford Guide, 2003). Specifically, there is a wide range of antimicrobials that can be used for indications involving *E. coli* or *Salmonella* spp. (Table 15), including febrile neutropenia, septicemia, urinary tract infections, and intra-abdominal infections. Low intracellular penetration is one reason why cephalosporins are not generally recommended for use in enteric *Salmonella* infections.

Table 15: Choices of antimicrobials for treating *Salmonella* spp. and *E. coli*[a]

Salmonella spp.	*E. coli*
Fluoroquinolones	Beta-lactams combined with beta-lactamase inhibitors
Ceftriaxone	Fluoroquinolones
Chloramphenicol	Trimethoprim/sulfonamide combination
Trimethoprim / sulfonamide	Nitrofurantoin
Azithromycin	Carbapenems

[a] The Sanford Guide, 2003

The Sanford Guide (2003) lists the antimicrobial drugs of choice for the diseases with potential association of *Salmonella* spp. and *E. coli*.

- *Empiric therapy of gastroenteritis*: infants are given trimethoprim/sulfonamide, and individuals with severe diarrhea are given either fluoroquinolones or trimethoprim/sulfonamide.

- *Specific treatment of gastroenteritis caused by Salmonella*: accomplished with fluoroquinolones, azithromycin, trimethoprim/sulfonamide, or 3GC. Treatment of *E. coli* O157:H7-assoicated gastroenteritis is controversial.

- *Treatment of typhoid fever*: generally treated with ciprofloxacin or a 3GC, azithromycin, or chloramphenicol.

- *Acute urinary tract infections*: can be treated with fluoroquinolones, trimethoprim/sulfonamide, oral cephalosporins, nitrofurantoin, doxycycline, amoxicillin/clavulanate or azithromycin.

- *Urinary tract infections in hospitalized patients*: can be treated with fluoroquinolones, ampicillin and gentamicin, 3GC, ticarcillin/clavulanate, piperacillin/tazobactam, imipenem or meropenem.

- *Sepsis*: generally treated with ampicillin and 3GC, ampicillin and antipseudomonal aminoglycoside, ticarcillin/clavulanate, piperacillin/tazobactam, imipenem or meropenem, and fluoroquinolones.

- *Sepsis in neutropenic patients*: treated with ceftazidime, imipenem, ticarcillin/clavulanate, or piperacillin/tazobactam.

Although 4GCs are important for human medicine due to their spectrum and favorable resistance situation, there is a range of treatment alternatives for infections associated with the food-borne bacteria *Salmonella* spp. and *E. coli.*

3. Overall Qualitative Risk Estimation

The integration of results from the release assessment, exposure assessment, and consequence assessment estimates the risk that:

- The use of cefquinome in livestock may cause resistance of *Salmonella* spp., and
- These resistant *Salmonella* spp. from the intestinal tract may contaminate the carcass at slaughter and transfer to humans via food, and
- These resistant *Salmonella* spp. may cause infections in humans which require treatment with a fourth-generation cephalosporin (cefepime), and effectiveness of treatment may be compromised.

Release assessment

The release assessment is ranked as **medium** because the probability that cefquinome-resistant *Salmonella* spp. will emerge or be selected as consequence of the proposed clinical use of cefquinome is medium.

Exposure assessment

The probability that humans will be exposed to *Salmonella* as a result of exposure to food products derived from cattle is **medium**.

Consequence assessment summary

The 4GCs are considered as **highly important** in human medicine because they may be the sole approach to the treatment of neutropenic fever, and they are indicated for treatment of enteric pathogens in non-food-borne disease

Overall qualitative risk estimation

- Consistent with Table 6 of FDA/CVM Guidance #152 the overall risk estimation is **medium**. The **medium** classification is equal to a **category 2 classification**.

4. Risk Management

The conditions and extent of use for the two formulations of cefquinome are appropriate to limit the risk of the emergence of resistance. Both products are intended to be used in individual animals under prescription only. In accordance with FDA/CVM Guidance #152, the extent of use is **low**. The low ranking for extent of use, together with a prescription only marketing status, will help to maintain cefquinome as a viable treatment in cattle, and help to prevent the emergence and spread of antimicrobial resistance. It is intended that cefquinome continues to be included in the NARMS surveillance program. These risk management measures including the Veterinary Medicine Advisory Committee review exceed the risk management measures that are required for category 2 drugs according to FDA/CVM Guidance #152 (Table 16).

Table 16: Examples of potential risk management steps associated with the approval of antimicrobial new animal drugs in food-producing animals based on the level of risk (high, medium, or low)

Approval conditions	Category 1 (High)	Category 2 (Medium)	Category 3 (Low)
Marketing status[a]	Rx	Rx/VFD	Rx/VFD/OTC
Extra-label use (ELU)	ELU Restrictions	Restricted in some cases	ELU permitted
Extent of use[b]	Low	Low, medium	Low, medium, high
Post-approval monitoring (e.g. NARMS)	Yes	Yes	In certain cases
Advisory committee review considered	Yes	In certain cases	No

[a] Prescription (Rx), Veterinary Feed Directive (VFD), over-the-counter (OTC).
[b] These risk management steps may be appropriate for certain Category 2 drugs that were ranked critically important for consequence assessment and ranked "high" for release or exposure assessment.

The sponsor confirms that risk analysis is a dynamic process designed to adjust risk management strategies with evolving information. The sponsor considers the NARMS

program as an effective tool to track resistance patterns in food-borne bacteria of human health concern and to help in identifying the source of antimicrobial resistance emergence.

Consistent with FDA/CVM Guidance #152, no extra-label use limitations or other measures are deemed appropriate for cefquinome, because 4GCs are ranked highly important (rather than critically important), and because cefquinome use in animals is not ranked high for either the release or the exposure assessment. If indicated, for example by new information following approval, these risk management measures may be enhanced. Mass medication formulations of cefquinome for oral administration via feed or drinking water will not be developed, as cefquinome is chemically unsuitable for this purpose. If new information indicates a significant reduction of risk, the appropriate risk management strategies should be revised to reflect this change.

5. Conclusions

The risk that food-borne bacteria of human health concern, for example *Salmonella* spp., may be adversely impacted by the therapeutic parenteral use of cefquinome for the treatment of bovine respiratory disease (BRD) has been assessed using the procedure as laid down in FDA/CVM Guidance #152. The overall risk is **medium**. The proposed risk management measures are appropriate to minimize this risk and are consistent with prudent use guidelines. They include prescription only status, inherent low extent of use due to parenteral administration, and Veterinary Medicine Advisory Committee review. Importantly, the ongoing susceptibility monitoring of *Salmonella* spp. and *E. coli* isolates from the NARMS program allows detection of trends of increased or decreased susceptibility, and facilitates taking any necessary mitigating steps.

Considering these risk management measures, there is reasonable certainty of no harm to public health with regard to microbial food safety for the proposed veterinary therapeutic use of cefquinome.

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