ZUPREVO™ (tildipirosin) You're in control

# TECHNICAL BULLETIN

## Zuprevo™: Proven speed of action in dairy calf ranches in California, feedyards in Nebraska and starter yards in Alabama

### Introduction

As opposed to an antimicrobial product used to control a disease outbreak, the success of a drug for its use as an initial treatment of BRD depends on several factors:

- Progression of the disease at the time of detection
- Accuracy of the diagnosis of BRD
- Characteristics of the drug itself
- Selection and application of preventive strategies—vaccination, prior antibiotic use, commingling cattle

The hallmark of a highly effective antimicrobial product used to treat bacterial respiratory disease is its speed of action. This is true because of the first two points above. Many times, when a calf is pulled, we don't know whether it is early, in the middle or late in the BRD process. Is it even BRD or is the calf showing signs that look like BRD when it may have something else going wrong? We do know that the principal pathogens that cause BRD—*Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*—grow rapidly once they infect lung tissue, roughly doubling their numbers every 20 minutes. So, the supporting hallmark of a highly effective antimicrobial is bactericidal activity. Using a drug that is slow to eliminate pathogenic bacterial populations decreases the likelihood of a successful outcome. Four BRD antimicrobials provide the critical combination of speed and cidal activity to help ensure treatment successes. When asking "What antibiotic should I use?" consider these simple answers determined by your specific needs.

| Deciding Features | Answer |
|---|---|
| Speed + Cidal + Control of Pyrexia | Resflor Gold® (florfenicol and flunixin meglumine) |
| Speed + Cidal + Best in Class Duration | Zuprevo (tildipirosin) |
| Speed + Dose Volume + Syringeability | Zuprevo (tildipirosin) |
| Speed + Cidal + Excellent Susceptibility Profile | Nuflor® (florfenicol)<br>Nuflor Gold® (florfenicol)<br>Resflor Gold® (florfenicol and flunixin meglumine) |

How is this speed generated? For fast speed of action, the drug is expected to have:

- Rapid peak plasma concentration
- Little binding in plasma
- Rapid distribution into tissues
- Potent bacteria killing ability at low concentrations
- Minimal adverse effects

Zuprevo 18% possesses each of those characteristics (Figure 1). It is proving itself to be a highly effective drug to treat BRD in all types of cattle operations, from dairy calf ranches in California, to feedyards in Nebraska and starter yards in Alabama.



MERCK Animal Health

**Figure 1:**
Summary of product features of Zuprevo 18% (tildipirosin)

**Tildipirosin**

| Metric | Numbers to Remember | Importance |
|---|---|---|
| Time to peak plasma concentration | 45 minutes | Zuprevo is rapidly absorbed from injection |
| Concentration in lung at 4 hours | 9.3 µg/gram | Zuprevo is rapidly absorbed in lung tissue above MIC for all BRD organisms |
| Concentration in lung at 24 hours | 14.8 µg/gram | Zuprevo has very high lung concentrations in 1 day |
| Volume of distribution (Vd) | 50 L/kg | Zuprevo is widely distributed to tissues |
| Terminal half-life in plasma | 9 days | Zuprevo is slowly eliminated |
| Time lung concentration greater than 2 µg/gram | 28 days[1] | Zuprevo stays in the lungs for extended time |

## Key Points on Zuprevo™ (tildipirosin) Pharmacokinetics

- Tildipirosin has a high absolute bioavailability (78.9%).
- Tildipirosin is rapidly absorbed from the subcutaneous injection site, with a $T_{max}$ for plasma of 45 minutes.
- Tildipirosin has a limited plasma protein binding (approximately 30%), allowing for a high volume of distribution (approximately 50 L/kg).
- Tildipirosin has a long mean terminal half life (approximately 9 days), implicating a long persistence in plasma and target tissues.
- Tildipirosin accumulates at the site of respiratory tract infection, demonstrated by high and sustained concentrations in lung and bronchial fluid. These far exceed those in blood plasma.
- The mean total excretion of a single dose is within 14 days, with 24% of the drug excreted in urine and 40% in the feces.

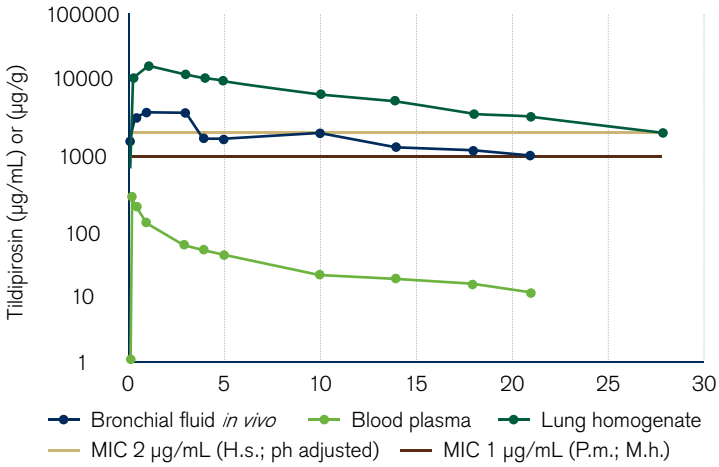
## Tildipirosin Pharmacokinetic Properties

Tildipirosin is rapidly and extensively distributed to the respiratory tract followed by slow elimination. The pharmacokinetics of tildipirosin (Zuprevo 18% [180 mg/mL] solution) in cattle were investigated in studies collecting blood plasma, lung tissue, and *in vivo* samples of bronchial fluid (Table 1). After a single subcutaneous injection at 4 mg/kg body weight, maximum plasma concentration ($C_{max}$) were 0.7 µg/ml. $T_{max}$ was 45 minutes based on 14 calves in US label studies. Mean residence time (MRT) from the time of dosing to the time of last measurable concentration ( $MRT_{last}$) and terminal half-life ($T_{1/2}$) was 6 and 9 days, respectively. A strong dose-response relationship with no significant sex effect was shown for both $C_{max}$ and area under the plasma concentration-time curve from time 0 to the last sampling time with a quantifiable drug concentration ($AUC_{last}$) over the range of doses up to 6 mg/kg. Absolute bioavailability was 78.9%. The volume distribution based on the terminal phase (Vz) was 49.4 L/kg, and the plasma clearance was 144 mL/h/kg. The time concentration profile of tildipirosin in bronchial fluid and lung far exceeded those in plasma (Figure 2). In lung, tildipirosin concentrations reached 9.2 µg/g at 4 h, peaked at 14.8 µg/g at day 1, and slowly declined to 2.0 µg/g at day 28. In bronchial fluid, the concentration of tildipirosin reached 1.5 and 3.0 µg/g at 4 and 10 h, maintained a plateau of about 3.5 µg/g between day 1 and 3, and slowly declined to 1.0 at day 21. T1/2 in lung and bronchial fluid was approximately 10 and 11 days.[2] The pharmacokinetic profile in pre-ruminant and ruminant calves was similar, with more rapid absorption (earlier $T_{max}$) and slightly lower exposure (lower AUC) in pre-ruminants.

**Table 1:**
Plasma pharmacokinetic properties of Zuprevo 18% for the US label following subcutaneous injection in the neck at 4 mg/kg body weight.

| Parameter | Average |
|---|---|
| $C_{max}$ (µg/mL) | 0.767 |
| $T_{max}$ (min) | 45 |
| AUC 0-last (h*µg/mL) | 21.0 |
| AUC 0-inf (h*µg/mL) | 24.9 |
| MRT0-last (h) | 152 |
| $t_{1/2}$ (hr) | 210 (approx. 9 days) |

**Figure 2:**
Mean tildipirosin concentrations in plasma, bronchial fluid (collected *in vivo* from non-anesthetized cattle) and lung in relationship to MIC values.




## Partitioning of Tildipirosin Into Tissues and Body Compartments

It is generally recognized that low macrolide plasma concentrations are not predictive for clinical efficacy. Therefore, the basis of the PK/PD model for tildipirosin is the relationship between concentrations over time at the site of respiratory infection in lung tissue and, more specifically, bronchial fluid, and the $MIC_{90}$ values of the three major BRD bacterial pathogens. As evidenced in Table 2, mean bronchial fluid concentrations exceed the $MIC_{90}$ values of *M. haemolytica* and *P. multocida* up to 21 days and for about 3 days for *H. somni* (4 µg/mL). Concentrations in lung tissues at or above $MIC_{90}$ were observed for over 21 days for *M. haemolytica* and *P. multocida* and for over 14 days for *H. somni*. The bronchial fluid to plasma ratio (B/P ratio) illustrates high bronchial fluid concentrations relative to plasma values.

**Table 2:**
Mean tildipirosin concentrations in plasma, bronchial fluid (collected *in vivo* from non-anesthetized cattle) and lung.

| Time (d) | Time (h) | Bronchial Fluid (µg/g) Average | Plasma (µg/mL) Average | Bronchial Fluid to Plasma Ratio | Lung Tissue (µg/g) Average |
|---|---|---|---|---|---|
| | 4 | 1.543 | 297 | 5.2 | 9.237 |
| | 10 | 2.975 | 242 | 12.3 | 9.253 |
| 1 | 24 | 3.448 | 136 | 25.4 | 14.768 |
| 3 | 72 | 3.489 | 71 | 49.3 | 10.996 |
| 4 | 96 | 1.644 | 60 | 27.3 | 10.155 |
| 5 | 120 | 1.619 | 52 | 30.9 | 9.313 |
| 10 | 240 | 1.937 | 27 | 71.5 | 6.007 |
| 14 | 336 | 1.225 | 26 | 47.0 | 4.978 |
| 21 | 504 | 935 | 17 | 55.6 | 3.036 |

# TECHNICAL BULLETIN

## Comparative Pharmacokinetics of Tildipirosin

How do the pharmacokinetics compare to the PK values for tulathromycin (Draxxin,® Zoetis) and gamithromycin (Zactran,® Merial)? Figures 3 and 4 were developed based on published research of PK values for tulathromycin[3] and gamithromycin[4] in comparison to the pharmacokinetic properties of tildipirosin[2] for plasma and lung tissue concentrations. As evidenced by these illustrations, tildipirosin demonstrates a long persistence in both plasma and lung tissue compared to current long-duration macrolides in the US market place.

**Figure 3:**
Comparisons over time of plasma concentrations of tildipirosin, tulathromycin and gamithromycin.

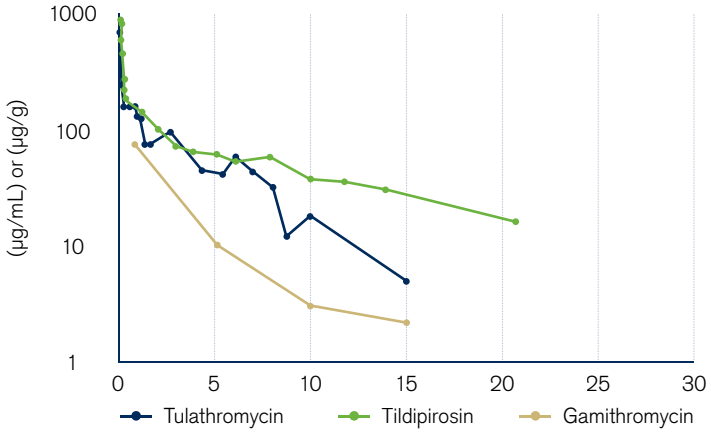
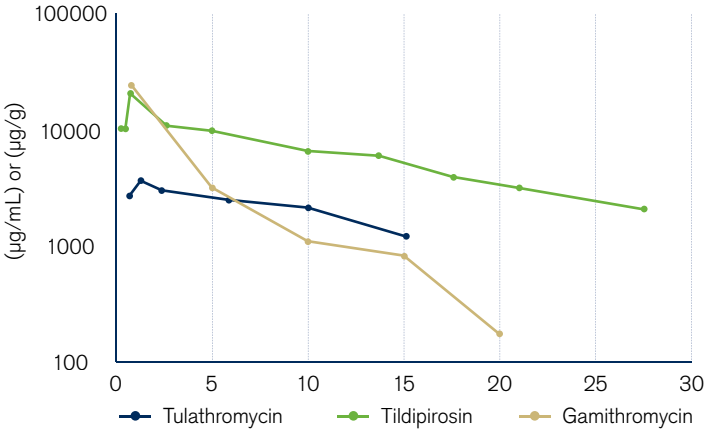



**Figure 4:**
Comparison over time of lung concentrations of tildipirosin, tulathromycin and gamithromycin.




## SUMMARY

Zuprevo 18% is extensively partitioned into lung tissues, as demonstrated by the high concentrations of tildipirosin in bronchial fluids collected *in vivo*. After Zuprevo 18% is administered subcutaneously to cattle at a single dose of 1 mL/100 lb of body weight (4 mg/kg body weight), there is a rapid absorption with a $T_{max}$ of 45 minutes after injection and a high absolute bioavailability (78.9%). Tildipirosin has a limited plasma protein binding (approximately 30%), allowing for a high volume of distribution (approximately 50 L/kg). Tildipirosin has a long mean terminal half-life of approximately 9 days, implicating a long persistence in plasma and target tissues at levels that far exceed those in plasma. The prolonged periods of antibiotic exposure afforded by Zuprevo exceeds $MIC_{90}$ values of *M. haemolytica, P. multocida* and *H. somni* for about 14 to 21 days in the lungs. Zuprevo 18% as a treatment drug stands alone for its speed of action, high tissue concentration and longevity in lung tissues.

## References

[1]Menge, M. et al., Pharmacokinetics of tildipirosin in bovine plasma, lung tissue, and bronchial fluid (from live, non-anesthetized cattle). J Vet Pharm Therap. DOI: 10.1111/J. 1365-2885, 2011.1349.x. The correlation between pharmacokinetic data and clinical effectiveness is unknown.

[2]Menge, M; Rose, M; Bohland, C; Zschiesche, E; Kilp, S; Metz, W; Allan, M.; Röpke, R; Nürnberger, M. Pharmacokinetics of tildipirosin in bovine plasma, lung tissue, and bronchial fluid (from live, nonanesthetized cattle). *J Vet Pharm Ther*. DOI: 10.1111/j.1365-2885.2011.01349.x.

[3]Nowakowski *et al* (2004) *Vet Ther* 5:60-74.

[4]Huang *et al* (2009) *J Vet Pharm Ther* (published online Sep 09).

Zuprevo Technical Monograph ZUPCA-17, 2012.



MERCK Animal Health

# ZUPREVO™ 18%

## *(Tildipirosin)*

Injectable Solution for Cattle

**ANTIMICROBIAL DRUG:**
180 mg of tildipirosin/mL

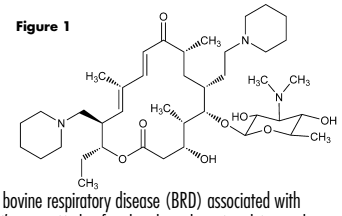
For subcutaneous injection in beef and non-lactating dairy cattle only. Not for use in female dairy cattle 20 months of age or older or in calves to be processed for veal.

**CAUTION:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

**DESCRIPTION:** Zuprevo™ 18% is a ready-to-use sterile injectable solution containing tildipirosin, a semi-synthetic macrolide antibiotic. Each mL of Zuprevo18% contains 180 mg of tildipirosin as the free base, 82.5 mg citric acid monohydrate and 400 mg propylene glycol, and water qs with citric acid monohydrate added to adjust pH.

**CHEMICAL NOMENCLATURE AND STRUCTURE:**
Tildipirosin is the nonproprietary name for (11E,13E)-(4R,5S,6S,7R,9R,15R,16R)-6-(4-Dimethylamino-3,5-dihydroxy-6-methyl-tetrahydro-pyran-2-yloxy)-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-piperidin-1-yl-ethyl)-15-piperidin-1-ylmethyl-oxacyclohexadeca-11,13-diene-2,10-dione. The empirical formula is $C_{41}H_{71}N_3O_8$. The chemical structure of tildipirosin is shown below.

**Figure 1**



**INDICATIONS:** Zuprevo 18% is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* in beef and and non-lactating dairy cattle, and for the control of respiratory disease in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica, P. multocida,* and *H. somni.*

**DOSAGE AND ADMINISTRATION:** Inject subcutaneously as a single dose in the neck at a dosage of 4 mg/kg (1 mL/100 lb) body weight (BW). Do not inject more than 10 mL per injection site. Do not puncture the stopper of the respective vial size more than the tested number of punctures, shown in Table 1.

Clinical field studies indicate that administration of Zuprevo 18% (tildipirosin) Injectable Solution is effective for the control of respiratory disease in beef and non-lactating dairy cattle at "high risk" of developing BRD. Calves at high risk of developing BRD typically experience one or more of the following risk factors:

- Commingling from multiple sale barns/sources
- Extended transport times and shrink
- Exposure to wet or cold weather conditions or wide temperature swings
- Stressful arrival processing procedures (such as castration, dehorning, or branding)
- Recent weaning and poor vaccination history

**Table 1 Number of punctures tested in the in-use study for the respective vial sizes**

| Vial size [mL] | Number of punctures tested in the in-use study |
|---|---|
| 50 | 8 |
| 100 | 8 |
| 250 | 16 |

**WARNINGS: FOR USE IN ANIMALS ONLY. NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. TO AVOID ACCIDENTAL INJECTION, DO NOT USE IN AUTOMATICALLY POWERED SYRINGES WHICH HAVE NO ADDITIONAL PROTECTION SYSTEM. IN CASE OF HUMAN INJECTION, SEEK MEDICAL ADVICE IMMEDIATELY AND SHOW THE PACKAGE INSERT OR LABEL TO THE PHYSICIAN.**

Avoid direct contact with skin and eyes. If accidental eye exposure occurs, rinse eyes with clean water. If accidental skin exposure occurs, wash the skin immediately with soap and water. Tildipirosin may cause sensitization by skin contact.

For technical assistance or to report a suspected adverse reaction, call: 1-800-219-9286.
For customer service or to request a Material Safety Data Sheet (MSDS), call: 1-800-211-3573.
For additional Zuprevo 18% information go to www.zuprevo.com.
For a complete listing of adverse reactions for Zuprevo 18% reported to CVM see:
http://www.fda.gov/AnimalVeterinary/SafetyHealth.

**DO NOT USE ZUPREVO 18% IN SWINE.** Fatal adverse events have been reported following the use of tildipirosin in swine. NOT FOR USE IN CHICKENS OR TURKEYS.

**RESIDUE WARNING:** Cattle intended for human consumption must not be slaughtered within 21 days of the last treatment. Do not use in female dairy cattle 20 months of age or older. Use of this drug product in these cattle may cause milk residues. A withdrawal period has not been established in pre-ruminating calves. Do not use in calves to be processed for veal.

**PRECAUTIONS:** The effects of Zuprevo 18% on bovine reproductive performance, pregnancy and lactation have not been determined. Swelling and inflammation, which may be severe, may be seen at the injection site after administration. Subcutaneous injection may result in local tissue reactions which persist beyond the slaughter withdrawal period. This may result in trim loss of edible tissue at slaughter.

**CLINICAL PHARMACOLOGY:** Similar to other macrolides, tildipirosin inhibits essential bacterial protein biosynthesis with selective binding to ribosomal subunits in a bacteriostatic and time-dependent manner. Tildipirosin may be bactericidal against certain isolates of *M. haemolytica* and *P. multocida.*

The following plasma pharmacokinetic (PK) properties of tildipirosin have been observed following a subcutaneous injection at a dose of 4 mg/kg BW in the neck:

**Table 2 Summary of pharmacokinetic characterization of tildipirosin administered subcutaneously to calves at a dose of 4 mg/kg BW.**

| Parameter | Average | SD |
|---|---|---|
| $C_{max}$ (ng/mL) | 767* | 284 |
| $T_{max}$ (hr) | 0.75* | 0.43 |
| $AUC_{0\text{-}last}$ (hr·ng/mL) | 21017** | 3499 |
| $AUC_{0\text{-}inf}$ (hr·ng/mL) | 24934** | 3508 |
| $t_{1/2}$ (hr) | 210** | 53 |

* Value based on all 14 animals
** Value based on 8 animals that were slaughtered at 504 hr post-treatment.
$C_{max}$: Maximum observed plasma concentration
$T_{max}$: Time at which Cmax was observed

$AUC_{0\text{-}last}$: Area under the plasma concentration versus time curve measured from time zero to the last sample with tildipirosin concentrations exceeding the limit of quantification of the analytical method

$AUC_{0\text{-}inf}$: AUC estimated from time zero to time infinity $t_{½}$: Terminal elimination half life

Due to the extensive partitioning of macrolides into tissues and because of their multi-fold greater concentrations in bronchial fluid relative to that observed in the blood, plasma drug concentrations underestimate concentrations at the site of action[1]. This is shown for tildipirosin in the following table, where bronchial fluid samples were collected in live, healthy calves, and compared to the concentrations in plasma observed in these same animals:

**Table 3 Bronchial fluid-to-plasma ratio of tildipirosin in non-anesthetized cattle following a subcutaneous injection at a dose of 4 mg/kg BW in the neck**

| Time (hours) | Bronchial fluid (BF) concentration (ng/g) | | Plasma (P) concentration (ng/mL) | | BF/P Ratio |
|---|---|---|---|---|---|
| | Average | SD | Average | SD | |
| 4 | 1543 | 895 | 297 | 81.8 | 5.20 |
| 10 | 2975 | 1279 | 242 | 96.7 | 12.3 |
| 24 | 3448 | 1433 | 136 | 53.9 | 25.4 |
| 72 | 3489 | 1712 | 70.7 | 29.0 | 49.3 |
| 96 | 1644 | 2024 | 60.2 | 29.0 | 27.3 |
| 120 | 1619 | 1629 | 52.3 | 19.9 | 30.9 |
| 240 | 1937 | 1416 | 27.1 | 10.8 | 71.5 |
| 336 | 1225 | 1682 | 26.1 | 9.2 | 47.0 |
| 504 | 935 | 1032 | 16.8 | 1.7 | 55.6 |

Tildipirosin concentrations in bronchial fluid collected *in vivo* from non-anesthetized cattle reflect the bacterial exposure to drug concentrations at the site of action.

[1]Nightingale, C.H. (1997) Pharmacokinetics and pharmacodynamics of newer macrolides. The Pediatric Infectious Disease Journal, 16, 438-443.

**MICROBIOLOGY:** Tildipirosin has shown *in vitro* and *in vivo* antibacterial activity against the bacteria *M. haemolytica, P. multocida,* and *H.somni,* three pathogens associated with BRD.

The minimum inhibitory concentrations (MICs) of tildipirosin against the indicated BRD pathogens were determined using the methods described in the M31-A2 standard of the Clinical and Laboratory Standards Institute (CLSI) and are shown in Table 4.

The MICs of tildipirosin were determined for isolates of *M. haemolytica, P. multocida,* and *H. somni* obtained from two BRD field studies. In both studies, tested isolates of *M. haemolytica* and *P. multocida* were obtained from nasopharyngeal swabs taken prior to treatment from all study animals. Tested isolates of *H. somni* were obtained from nasopharyngeal swabs taken prior to treatment from all study animals and from nasopharyngeal swabs taken from saline-treated animals classified as treatment failures.

**Table 4 Tildipirosin minimum inhibitory concentration (MIC) values* of indicated pathogens isolated from BRD field studies in the U.S.**

| Indicated Pathogens | Year of isolation | Study | Number of isolates | MIC50** (µg/mL) | MIC90** (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|---|---|---|
| *Mannheimia haemolytica* | 2007 | Treatment | 484 | 1 | 2 | 0.25 to >32 |
| | 2007 to 2008 | Control | 178 | 1 | 1 | 0.25 to >32 |
| *Pasteurella multocida* | 2007 | Treatment | 235 | 0.5 | 1 | 0.12 to >32 |
| | 2007 to 2008 | Control | 273 | 0.5 | 1 | ≤0.03 to 4 |
| *Histophilus somni* | 2007 | Treatment | 33 | 2 | 4 | 1 to 4 |
| | 2007 to 2008 | Control | 32 | 2 | 4 | 1 to >32 |

* The correlation between *in vitro* susceptibility data and clinical effectiveness is unknown.
** The lowest MIC to encompass 50% and 90% of the most susceptible isolates, respectively.

**EFFECTIVENESS:** In a multi-location field study, calves with naturally occurring BRD were treated with tildipirosin. The treatment success rate of the tildipirosin-treated group was compared to the treatment success rate in the saline-treated control group. A treatment success was defined as a calf not designated as a treatment failure from Day 1 to 13 and with normal attitude, normal respiration, and a rectal temperature of <104°F on Day 14. The treatment success rate was significantly higher (p=0.003) for the tildipirosin-treated group (229/300, 76%) compared to the saline-treated control group (96/200, 32%). There were no BRD-related deaths in the tildipirosin-treated group compared to a 7% (21/300) BRD-related mortality rate in the saline-treated group.

In another multi-location field study, calves at high risk for developing BRD were administered tildipirosin. The treatment success rate of the tildipirosin-treated group was compared to the treatment success rate in the saline-treated control group. A treatment success was defined as a calf not designated as a treatment failure based on clinical respiratory and attitude scoring and, if necessary, rectal temperature measurement of <104°F through the end of the study (Day 14). The treatment success rate was significantly higher (p=0.0001) for the tildipirosin-treated group (305/386, 79%) compared to the saline-treated group (197/387, 51%). There were three BRD-related deaths during the study (one tildipirosin-treated calf and two saline treated calves).

**ANIMAL SAFETY:** A target animal safety study was conducted using Zuprevo 18% administered in 5-month-old cattle as three subcutaneous doses of 4, 12, or 20 mg/kg BW given 7 days apart (1X, 3X, and 5X the labeled dose). Animals remained clinically healthy during the study at the labeled dose. Injection site swelling and inflammation, initially severe in some animals, was observed that persisted to the last day of observation (21 days after injection). No other drug-related lesions were observed macroscopically or microscopically at the labeled dose.

A separate injection site tolerance study was conducted using Zuprevo 18% in 5- to 9-month-old cattle administered as a single subcutaneous injection of 10 mL. Injection site swelling and inflammation, initially severe in some animals, was observed that persisted to the last day of observation (35 days after injection). No other drug-related clinical signs were observed.

**STORAGE CONDITIONS:** Do not store above 30°C (86°F). Do not freeze. The maximum storage time after first puncture is 28 days at or below 25°C (77°F).

**HOW SUPPLIED:** Zuprevo 18% is supplied in 50, 100 and 250 mL, amber glass, sterile, multi-dose vials.
U. S. Patent: 6,514,946
NADA 141-334, Approved by FDA
Use Only as Directed

Rev. 03/12
Made in Germany
Distributed by: Intervet Inc d/b/a Merck Animal Health, Summit, NJ 07901

MERCK Animal Health