Environmental Assessment

for

Banamine Transdermal (flunixin transdermal solution) Pour-On for Cattle

Date	**September 29, 2016**
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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

ABS_{food}	absorption efficiency from food
ABS_{hair}	absorption efficiency from hair
AF	assessment factor
ANOVA	analysis of variance
AUC	area under the curve
BRD	bovine respiratory disease
BW	body weight
BW_{coyote}	coyote body weight
BW_{magpie}	magpie body weight
$BW_{RT\ Hawl}$	red-tail hawk body weight
$^{\circ}C$	degrees centigrade
C_{cow}	flunixin concentration in cattle tissue
C_{food}	flunixin concentration in food
C_{hair}	flunixin concentration in hair
C_{magpie}	flunixin concentration in magpie tissue
$C_{magpie}(t)$	flunixin concentration in magpie tissue at time t
cc	cubic centimeter
cm	centimeter
cm^2	square centimeter
C_0	initial concentration
C_t	concentration at time t
DFI	daily food intake
DFI_{coyote}	daily food intake for coyote
DHI	daily hair intake
DT50	dissipation half-life
e	base e
EA	Environmental Assessment
ECGs	electrocardiograms
ED_{50}	dose of a test substance which results in an effect in 50% of the test species
EIA	Environmental Impact Assessement
FFA	flunixin free acid
FIR	food ingestion rate
$FIR_{normalized}$	normalized food ingestion rate
FPO	flunixin pour-on
FTS	flunixin transdermal solution
g	gram
GL	guideline
GLP	Good Laboratory Practices
h	hour
ha	hectare
IV	intravenous
k	rate constant of decline 1/days
kg	kilogram
km^2	square kilometer
K_{ow}	octanol/water partition coefficient
lb	pound
INAD	Investigational New Animal Drug
lb	pound
LD_5	dose of a test substance which results in a 5% mortality of the test species

LD_{50}	dose of a test substance which results in a 50% mortality of the test species
m	meter
mL	Milliliter
µg	microgram
mg	milligram
NADA	New Animal Drug Application
ng	nanogram
nm	nanometer
NOEL	no-observed effect level
NSAID	non-steroidal anti-inflammatory drug
OECD	Organization for Economic Co-operation and Development
PD	predicted dose
PD_{coyote}	predicted dose for coyote
PD_{magpie}	predicted dose for magpie
$PD_{RT\ Hawk}$	predicted dose for red-tail hawk
PEC	predicted environmental concentration
PK	pharmacokinetics
PNEC	predicted no-effect concentration
PNED	predicted no-effect dose
ppm	parts per million
ROS	reactive oxygen species
RQ	risk quotient
SFO	Simple First Order
t	time
$t_{1/2\ el}$	elimination half-life
US	United States
USDA-APHIS	United States Department of Agriculture Animal and Plant Health Inspection Service
USEPA	United States Environmental Protection Agency
VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
VMP	veterinary medicinal products
w/v	weight/volume
ww	wet weight

1. Description of Proposed Action

Banamine Transdermal (flunixin transdermal solution) Pour-On for Cattle is in development under an Investigational New Animal Drug (INAD) file. Banamine Transdermal contains flunixin meglumine at 83 mg/mL, equivalent to 50 mg/mL of flunixin free acid (FFA) as the single active ingredient. For the purposes of consistency within this Environmental Assessment (EA), flunixin concentrations will be reported as FFA and converted from flunixin meglumine as necessary using a conversion factor of 0.6.

1.1 Target Species and Indications

Banamine Transdermal is proposed for the following indications: (1) for control of pyrexia associated with bovine respiratory disease (BRD) in beef and dairy cattle, (2) for the control of pyrexia associated with acute bovine mastitis in lactating dairy cattle, (3) for the control of pain associated with bovine interdigital phlegmon (foot rot, or acute interdigital necrobacillosis, or infectious pododermatitis) in beef and dairy cattle, and (4) for the control of post-operative pain and inflammation associated with cautery dehorning in calves. This product will be dispensed by prescription.

1.2 Dosage and Route of Administration

Banamine Transdermal is administered topically as a pour-on. The recommended dose is 3.3 mg FFA/kg BW administered as a single dose. This is equivalent to 1.5 mg/lb or 3 mL per 100 lb. The total dose should not exceed 3.3 mg FFA/kg (1.5 mg/lb or 3 mL/100 lb) BW.

1.3 Pharmacological Properties

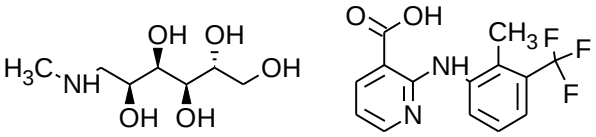
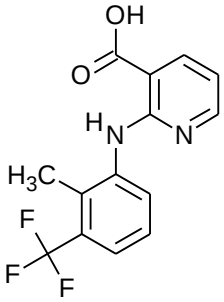
Flunixin is a non-steroidal anti-inflammatory drug (NSAID), and a non-narcotic analgesic with antipyretic activities. In veterinary medicine, it is typically formulated as the meglumine salt to increase water solubility. However, *in vivo*, flunixin meglumine is rapidly converted to the free acid. FFA demonstrates potent inhibition of the cyclo-oxygenase system involved in the inflammatory pathway. The resultant decrease in production of certain inflammatory mediators accounts for its analgesic, anti-pyretic, and anti-inflammatory properties.

2. Identification of Substances that are the Subject of the Proposed Action

2.1 Active Pharmaceutical Ingredient

Flunixin meglumine is a salt formulation that increases the water solubility of the active ingredient, FFA, which is also known as flunixin. The identification information for each substance is presented in Table 2-1.

Table 2-1. Identification of substances

	Flunixin Meglumine	Flunixin
IUPAC Name[a]	2-{[2-Methyl-3-(trifluoromethyl)phenyl]amino}nicotinic acid-1-deoxy-1-(methylamino)-D-glucitol (1:1)	2-{[2-Methyl-3-(trifluoromethyl)phenyl]amino}nicotinic acid
Synonym	2-[[2-methyl-3-(trifluoromethyl) phenyl]amino]-3-pyridinecarboxylic acid meglumine salt	2-{[2-methyl-3-(trifluoromethyl)phenyl]amino}pyridine-3-carboxylic acid
CAS Registry Number	42461-84-7	38677-85-9
Molecular Formula	$C_{14}H_{11}F_3N_2O_2 \cdot C_7H_{17}NO_5$	$C_{14}H_{11}F_3N_2O_2$
Molecular Weight	492 g/mol	296 g/mol
Structure		

[a] Source: http://www.chemspider.com

2.2 Excipients

Flunixin Meglumine Transdermal Solution for Cattle also contains pyrrolidone, propylene glycol dicaprylocaprate, glyceryl monocapyrlocaprate, levomenthol, and FD&C Red No. 40. Table 2-2 lists the ingredients of Banamine Transdermal. Excipients in the formulation are not expected to cause adverse effects in non-target organisms or result in significant environmental impacts. Therefore, the excipients are not considered in the environmental assessment.

Table 2-2. Ingredients of Banamine Transdermal

Flunixin meglumine
Pyrrolidone EP
FD&C Red No. 40 (Allura Red AC E 129)
Levomenthol EP
Propylene glycol dicaprylocaprate EP (Propylene glycol dicaprylate/dicaprate NF)
Glycerol monocaprylate EP (Mono- and diglycerides NF)

2.3 Use of Lidocaine Prior to Cautery Dehorning of Calves

Lidocaine, a potent, short-acting local anesthetic, is a prescription drug that can only be used by, or under the order of, a licensed veterinarian. Veterinarians typically administer lidocaine prior to dehorning calves as a standard practice of care and will recommend that lidocaine be used in conjunction with Banamine Transdermal when it is prescribed for the control of pain and inflammation associated with cautery dehorning. Depending on the size of the calf, 2 to 7 mL of 2% lidocaine may be injected close to the base of each horn bud to block the nerves and pain experienced during cautery dehorning. Based on the intended use of lidocaine as an anesthetic in calves in conjunction with flunixin transdermal solution for dehorning calves, the environmental exposure of lidocaine in the environment is expected to be limited and is not expected to result in significant environmental impacts. Therefore, lidocaine will not be evaluated further in this EA.

3. Ecosystem at the Site of Introduction

3.1 Background

Oaks et al. (2004) identified the NSAID diclofenac as the cause of renal failure and visceral gout leading to substantial population declines in Oriental white-backed vultures (*Gyps bengalensis*) in Pakistan. These authors were able to experimentally reproduce diclofenac residues and renal disease in the vultures by direct oral exposure and through feeding of tissue from diclofenac-treated livestock. This raised a potential safety concern for the use of flunixin in cattle. Cuthbert et al. (2007) conducted surveys of veterinarians and zoos where NSAIDs were used, establishing that mortality from renal disease and gout occurred after treatment of a number of species of birds with different NSAIDs. Flunixin meglumine was associated with mortalities of *Gyps* vultures and other scavenging birds and raptors, with a reported mortality of 30% (7 of 23 cases). Cuthbert et al. (2007) thus raised the issue that the veterinary use of flunixin in southern Asia could result in problems similar to those caused by diclofenac. The doses for the treatment-related deaths were not well characterized, however. The finding, on a game hunting reserve in Spain, of a carcass of a Eurasian griffon vulture (*Gyps fulvus*) with visceral gout and high flunixin levels in the liver and kidney raises the possibility that flunixin can cause similar organ effects to diclofenac (Zorrilla et al. 2014).

Scavenging as a route of exposure has also been described for North American raptors that were killed by the organophosphate drug famphur when topically applied to livestock (Henny et al. 1985, 1987) for the control of cattle grubs and to reduce cattle lice infestations. These investigators also identified additional exposure routes, reporting that magpies died after consuming hair from cattle that had been treated with famphur and that secondary poisoning of raptors occurred after they consumed these disabled or dead magpies.

These findings concerning the toxicity of NSAIDs and the identification of several exposure pathways for potentially sensitive receptors that are relevant for flunixin transdermal solution therefore warrant an evaluation of the environmental impacts for this product, as described below.

3.2 Approach

The assessment of environmental impacts for Banamine Transdermal Pour-On for Cattle is performed in accordance with the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines (GLs) “Environmental impact assessments (EIA’s) for veterinary medicinal products (VMPs) — Phase I” (VICH GL 6; Guidance for Industry 89; VICH 2000) and “Environmental impact assessments for veterinary medicinal products (VMPs) — Phase II guidance” (VICH GL 38; Guidance for Industry 166; VICH 2004).

Typically, the assessment of environmental impacts for VMPs proceeds through a tiered process, with Phase I being a screening-level assessment and Phase II a more complex evaluation. The approach incorporates the assessment of exposure and of effects if needed, which are then integrated to characterize risk. In the latter case, data from environmental effects studies are used to calculate the predicted no-effect concentrations (PNECs) for each relevant taxonomic level. Information on the use pattern of the VMP is used to calculate the predicted environmental concentrations (PECs) for each relevant environmental compartment (e.g., soil, water). The PECs are compared to the PNECs to determine risk quotients (PEC/PNEC; RQs). If the RQ ≥ 1, further refinement of the PECs is conducted.

Due to reported toxicity of NSAIDs (especially diclofenac) to birds, and given the potential for avian exposure following the use of topically administered drug products in cattle (as seen with the drug famphur), the EA for Banamine Transdermal proceeds directly to a Phase II risk assessment to specifically address the potential for risks to avian receptors in the environment. In addition, toxicity to non-target mammalian receptors and exposure via ingestion of exposed birds is addressed. As such, modifications to the typical Phase II process have been made. This EA focuses on exposures of birds that may ingest hair or tissue from flunixin-treated cattle, exposures of predators and scavengers that may feed on those birds, and exposures of scavengers that may ingest the carcasses of flunixin-treated cattle.

Information about the effects of flunixin on birds and mammals is presented in Section 4. The exposure pathways are described in Section 5. Section 6 provides the risk characterization.

4. Effects Assessment

4.1 Avian Species

As discussed above, population declines in Oriental white-backed vultures in Pakistan linked to diclofenac have prompted concerns about the effects of NSAIDs on birds and resulted in several publications on the subject (e.g., Oaks et al. 2004; Swan et al. 2006). Some information is available from the therapeutic use of NSAIDs on birds, although detailed data on flunixin use are minimal. The mode of action of toxicity and the difference in sensitivity among avian species for flunixin are hypothesized to be similar to that of diclofenac, as discussed below.

4.1.1 Pathology

According to Cuthbert et al. (2007), flunixin appears to carry a high risk of renal damage in birds. Flunixin and other NSAIDs are listed as drugs with known potential for nephrotoxicity by Pollock (2006). According to Paul-Murphy and Ludders (2001), renal ischemia and tissue damage are the most serious complications of the therapeutic use of flunixin in birds. The Eurasian griffon vulture carcass found with high levels of flunixin in the liver and kidney (Zorrilla et al. 2014) had symptoms of severe visceral gout, including abnormal white precipitates, consistent with urate deposits, on the surface of the liver and pericardium and on the capsule and cut surface of the kidney. The pathology of flunixin in avian species thus is identical to that described for diclofenac (Meteyer et al. 2005). Acute necrosis of proximal convoluted tubules was reported by these investigators for Oriental white-backed vultures that died of renal failure when they ingested diclofenac in tissues of domestic livestock. Mostly, lesions were extensive with large urate aggregates obscuring renal architecture. Extensive urate precipitation on the surface and within organ parenchyma (visceral gout) was found consistently. It was hypothesized that impaired production of prostaglandins may alter smooth muscle control of the renal portal valve and shunt blood from the renal cortex.

Naidoo and Swan (2009) used studies in chickens and a single African white-backed vulture (*Gyps africanus*) to present a hypothesis about the mode of toxic action of diclofenac. They reported that diclofenac is toxic to renal tubular epithelial cells following 12 hours of exposure, due to an increase in production of reactive oxygen species (ROS). However, while ROS formation is a plausible mechanism, the chicken cell cultures were exposed continuously for 12 hours, which does not account for the relatively short half-life (0.6 hours) of diclofenac in the chicken (i.e., chicken toxicity was not related to long-term exposure). Diclofenac was observed to decrease the transport of uric acid even after drug withdrawal, depriving cells of an important anti-oxidant and potentially explaining the signs of toxicity seen 48 hours after the drug was completely excreted by chickens. High concentrations of uric acid were also observed in all domestic fowl that died after intramuscular dosing with diclofenac (Naidoo et al. 2007).

Effects on the kidneys, as well as other organs, have been noted upon necropsy in studies in which birds were orally dosed with flunixin meglumine. In a sponsor-supported study to determine the acute oral LD_{50} of flunixin meglumine to northern bobwhite, *Colinus virginianus* (Hubbard and Beavers 2013, Appendix 8), birds that died from exposure were found to have abnormal findings in the following organs: gastrointestinal tract (hyperemia in the wall of the crop, primarily empty gastrointestinal tract, brownish fluid or white chalk-like plaques in the abdominal cavity), kidneys (enlarged, pale, mottled), heart (pale heart, white chalk-like plaques in the pericardium), spleen (enlarged, white chalk-like plaques present), and liver (pale, friable). These observations are consistent with the findings of Zorrilla et al. (2014). Not all of the northern bobwhite that died on study exhibited all symptoms.

Similar symptoms, amongst others, were observed by Slepetys and Campbell (1986), Appendix 13, in broiler chickens orally exposed to flunixin meglumine. White, filmy adhesions in the abdominal cavity and on the liver were the most notable gross changes. White foci were also found on the abdominal fat, lungs, kidney, heart, and pericardium. Lungs contained fluid, displayed congestion, edema, and pleuritis, and were friable in texture. Kidneys were enlarged, discolored, friable, had accentuated tubules, and displayed nephritis. Livers were enlarged and congested. In an earlier study, Slepetys et al. (1985), Appendix 12, found that chickens orally exposed to flunixin meglumine exhibited pulmonary congestion and petechiation and yellow mottling of the liver, with sporadic incidences of pale thyroids and isolated incidences of hepatic hyperemia and enlargement.

4.1.2 Species-Dependent Susceptibility

Sensitivity to NSAIDs appears to vary significantly among bird species, and this seems to be the hallmark of the cyclooxygenase-2 inhibitors (Rattner et al. 2008). *Gyps bengalensis* is very sensitive to diclofenac, with a calculated oral LD_{50} of 0.098–0.225 mg/kg BW (Oaks et al. 2004; Swan et al. 2006). Although an LD_{50} was not determined, diclofenac was also toxic to *Gyps africanus* and *Gyps fulvus* at 0.8 mg/kg BW, and it has been suggested that diclofenac is probably toxic to all eight vulture species within the *Gyps* genus (Swan et al. 2006). Based on population counts, Galligan et al. (2014) speculated that diclofenac is also toxic to vulture species outside the genus *Gyps*, namely the Egyptian vulture (*Neophron percnopterus*) and the red-headed vulture (*Sarcogyps calvus*). A recent finding by Sharma et al. (2014) indicates that diclofenac is also toxic to the Steppe eagle, *Aquila nipalensis*, another member of the *Accipitridae* family which includes hawks, eagles, buzzards, and Old World vultures. Sharma et al. (2014) found extensive visceral gout in two specimens of *A. nipalensis*, and the co-occurrence of diclofenac in the one specimen that was tested.

In contrast, Rattner et al. (2008) found that, for turkey vultures (*Cathartes aura*), diclofenac had no toxic effects up to 25 mg/kg BW, over 100 times the LD_{50} for *Gyps* vultures. Turkey vultures are members of the Cathartidae family, which includes New World vultures and condors. The LD_{50} for diclofenac for domestic fowl was 9.8 mg/kg BW for an intramuscular dose and thought to be as high as 19.6 mg/kg BW for an oral dose (Naidoo et al. 2007). Finally, the pied crow (*Corvus albus*) demonstrated no signs of toxicity following oral treatment with diclofenac at a dose of 10 mg/kg BW (the highest dose tested) (Naidoo et al. 2011). This species is in the family, *Corvidae*, as is the magpie.

Data to compare the species-specific susceptibility of birds to flunixin are less definitive, but limited information is available from a survey conducted by Cuthbert et al. (2007). Cuthbert et al. (2007) compiled information requested from 31 zoos, wildlife rehabilitation centers, and veterinarians worldwide on their experiences of treating birds with NSAIDs, including flunixin. Mortality with post-mortem clinical signs of visceral gout and/or renal failure was observed in 7 out of 24 flunixin-treated birds (Table 4-1), but no mortalities were noted in species that are resident to the US (i.e., bald eagle, red-tailed hawk, and Harris hawk). Unfortunately, this article did not provide information on the dose, route of administration, and duration of treatment for each species.

Table 4-1. Toxicity of flunixin in scavenging birds and raptors following therapeutic treatment

Mortality with clinical signs of visceral gout and/or renal failure (dose range: 1.0–4.5 mg/kg BW)	No Mortality (dose range: 0.5–12.0 mg/kg BW)
Rüppell's Vulture (*Gyps rueppellii*)	Rüppell's Vulture (*Gyps rueppellii*)
European Black Vulture (*Aegypius monachus*) (3 birds)	European Black Vulture (*Aegypius monachus*)
	Griffon Vulture (*Gyps fulvus*)
	Bald Eagle (*Haliaeetus leucocephalus*)[a]
	Red-tailed Hawk (*Buteo jamaicensis*) (5 birds)[a]
	Harris Hawk (*Parabuteo unicinctus*) [a]
	Bateleur (*Terathopius ecaudatus*)
	Andean Condor (*Vultur gryphus*) (2 birds)
Marabou Stork (*Leptoptilos crumeniferus)*	Marabou Stork (*Leptoptilos crumeniferus)*
	White Stork (*Ciconia ciconia*) (2 birds)
African Spoonbill (*Platalea alba*)	
Red-legged Seriema (*Cariama cristata*)	

From data provided in Cuthbert et al. (2007)
[a] Species resident in the US.

In the report of Zorrilla et al. (2014), a carcass of *Gyps fulvus* was found on a game hunting reserve in Spain and examined forensically. The carcass was in good body condition with well-developed undamaged plumage, and based on rigor mortis stage, was estimated to have died approximately 5-6 hours before examination. Symptoms of severe visceral gout were noted and the median flunixin level was 2.7 and 6.5 mg/kg in the liver and kidney, respectively. The level of exposure, or route of exposure, resulting in this mortality was not able to be determined but suspected by the authors to be through scavenging of a dead agricultural animal that was treated with flunixin shortly before death or through ingestion of a meal containing a particularly high flunixin concentration. Another possible explanation could be the intentional poisoning of the vulture, as indeed, deliberate wildlife poisoning is well-documented in Spain (Sanchez-Barbudo et al. 2012, cited in Zorrilla et al. 2014).

4.1.3 Pharmacokinetics and Metabolism of Flunixin in Birds

The pharmacokinetics (PK) of flunixin in avian species is described by Baert and De Backer (2003). These investigators established PK parameters in pigeon, duck, turkey, ostrich, and chicken following a single intravenous administration of 1.1 mg/kg BW of commercially available flunixin (Finadyne®) [converted to 0.66 mg FFA/kg BW]. Baert and De Backer (2003) found that the clearance of flunixin is more variable and generally more rapid in birds than mammals. The elimination half-life ($t_{1/2\ el}$) in plasma for the five bird species tested ranged from less than an hour for pigeon, duck, turkey, and ostrich to 5.52 hours for chicken. The mean residence time (the average amount of time that flunixin spends in plasma) was highest for chicken, at 6.66 hours. Baert and De Backer (2003) concluded that flunixin, as well as the other NSAIDs they studied, is eliminated rapidly in most bird species.

The PK of flunixin in chicken was also investigated by Musser (2010) following intravenous and oral administration of flunixin meglumine at a dose of 5 mg/kg BW [converted to 3 mg FFA/kg BW].[1] No adverse effects were observed in the birds. The $t_{1/2\ el}$ in plasma following oral administration was 6.1 hours, similar to that reported by Baert and De Backer (2003). The time to maximal concentration in plasma was 1.5 hours, indicating that flunixin is well absorbed. The oral bioavailability of flunixin was calculated to be 69.9%; however, the analytical methods used were not validated. Musser et al. (2013) investigated the PK of flunixin meglumine after intravenous administration of 5.0 mg/kg BW [converted to 3 mg FFA/kg BW][1] to two species of pet birds. In budgerigars, the elimination half-life was 0.72 hours, and the mean residence time was 0.73 hours. In Patagonian conures (variety of parakeet), the $t_{1/2\ el}$ was 0.91 hours, and the mean residence time was 1.20 hours. Table 4-2 summarizes PK data for various avian species and illustrates the rapid elimination of flunixin from the plasma.

Table 4-2. Summary of elimination half-lives of flunixin in different bird species

Species	Elimination Half-life (hours)	Route	Source
Birds			
Ostrich	0.17	Intravenous	Baert and De Backer (2003)
Mallard	0.43	Intravenous	Baert and De Backer (2003)
Turkey	0.54	Intravenous	Baert and De Backer (2003)
Pigeon	0.62	Intravenous	Baert and De Backer (2003)
Budgerigar	0.73	Oral	Musser et al. (2013)
Patagonian conures	0.91	Oral	Musser et al. (2013)
Chicken	5.52	Intravenous	Baert and De Backer (2003)
Chicken	6.1	Oral	Musser (2010)

4.1.4 Acute Toxicity of Flunixin to Birds

An acute oral toxicity study was conducted to determine the LD_{50} of flunixin meglumine to northern bobwhite quail (Hubbard and Beavers 2013, Appendix 8). This study was conducted under regulations for Good Laboratory Practices (GLP) and according to Organization for Economic Co-operation and Development (OECD) Guideline 223, which uses a sequential testing procedure to optimize dose placement while minimizing the number of birds tested. The northern bobwhites were females, 37–43 weeks of age, and weighed from 184 to 235 g at the time of dosing. Birds were dosed once with flunixin meglumine, which was dissolved in deionized water, via oral intubation and observed at least twice daily for signs of toxicity for 21 days. Stage 1 consisted of one bird exposed to each of four doses of flunixin meglumine, expressed as the salt form (4.24 mg/kg BW to 212 mg/kg BW); Stage 2 consisted of 10 doses (139 to 1,188 mg/kg BW), with one bird per dose; and Stage 3 consisted of two doses (191 and 579 mg/kg BW), with five birds per dose. The control group, consisting of five birds, received the same volume of water (4 mL/kg BW) as the dosed birds.

[1] It is unclear from the Musser 2010 and 2013 studies whether the doses reported were as flunixin meglumine or as FFA; conservatively, it was assumed that the dose was administered as flunixin meglumine.

All mortality occurred within the first 4 days of dosing and delayed onset toxicity was not observed. There was 20% (1 of 5) mortality at the 191 mg/kg BW dose, 60% (3 of 5) at the 579 mg/kg BW dose, and 100% (1 of 1) mortality at the 224, 361, 582, 738, 936 and 1188 mg/kg BW doses. No mortality or signs of toxicity occurred at or below doses of 177 mg/kg BW (equal to 106 mg FFA/kg BW), while signs of toxicity or mortality were observed in all treatment groups >177 mg/kg BW. Birds at higher doses that survived yet showed signs of toxicity displayed the symptoms on the day of dosing (within 6 hours of dosing), including shallow and rapid respiration, ruffled appearance, lethargy, and a reduced reaction to external stimuli. These birds did not display these symptoms after the day of dosing and appeared normal for the remaining duration of the test. There was a slight loss of mean body weight in surviving birds exposed to 458 and 579 mg/kg BW and some reduction in feed consumption, but these effects resolved by Day 3. Gross necropsy findings indicative of acute flunixin toxicity were identified in organs such as the gastrointestinal tract, kidney, liver, heart, and spleen of the birds that died (see Section 4.1.1).

The LD_{50} for northern bobwhite was 375 mg/kg BW for flunixin meglumine. Adjusting to the dose of FFA using a conversion factor of 0.6, the acute oral LD_{50} for FFA was calculated to be 225 mg/kg BW, with a 95% confidence interval of 122 to 474 mg/kg BW. The LD_5 was 66 mg FFA/kg BW, with a 95% confidence interval of 0.9 to 122 mg/kg. Data from this study provide the primary basis for determination of the PNEC of flunixin to birds in this EA. Additional information is presented below.

The toxicity of flunixin to chickens following oral administration has been investigated in two sponsor-conducted GLP studies (Slepetys et al. 1985, Appendix 12; Slepetys and Campbell 1986, Appendix 13). In a preliminary oral safety study, Slepetys et al. (1985), Appendix 12, determined toxicological effects of flunixin meglumine when orally administered to broiler chickens. Flunixin meglumine was dissolved in distilled water and administered by esophageal intubation to chickens, weighing 1.064 to 1.559 kg. The doses were 0 (vehicle control), 0.5, 1.5, and 2.5 mg flunixin meglumine/lb BW (corresponding to 0, 0.66, 2.0 and 3.3 mg FFA/kg BW). There were 10 male and 10 female birds per dose group. Birds were dosed once daily for 2, 4, 7, or 9 days. No mortalities were reported in this study; thus, a 9-day LD_{50} of >3.3 mg FFA/kg BW is indicated from the study data.

The second study of Slepetys and Campbell (1986), Appendix 13, was also designed to investigate the toxic syndrome of flunixin meglumine in chickens when administered orally. Flunixin meglumine was dissolved in distilled water and orally administered by esophageal intubation to broiler-type chickens, weighing 0.538 to 0.892 kg. Twelve male and female chickens were dosed at 22, 44, or 66 mg flunixin meglumine/kg BW (equivalent to 13.2, 26.4, and 39.6 mg FFA/kg), while five male and three female birds of the same age and similar weight served as controls. After dose administration the birds were observed for 9 days. Within 48 hours following treatment, three birds had died in the 13.2 mg FFA/kg BW group, four birds had died in the 26.4 mg FFA/kg BW group (no survivors in this group), and three birds died in the 39.6 mg FFA/kg BW group. This study was insufficiently documented, and the data are considered inadequate to calculate the $LD_{50.}$ Toxicity was characterized by decreased food consumption, increased serum creatine phosphokinase, and liver and kidney enlargement. Gross pathology was characterized by white foci, adhesions, and discoloration of organs. Histopathology was characterized by inflammation, necrosis, and changes in the bone and marrow.

Published data on the toxicity of flunixin in birds are sparse. Musser (2010) reported that no adverse effects were observed following a single oral treatment of chickens at a dose of 3 mg

FFA/kg.[2] Available information on the tolerability of flunixin following therapeutic treatment is from unknown exposure routes (Cuthbert et al. 2007) or other than oral administration (Baert and De Backer 2003; Cuthbert et al. 2007; Machin et al. 2001; Machin 2005; Paul-Murphy and Ludders 2001; Werther 2001) and cannot be used to calculate an oral LD_{50}.

4.2 Mammalian Species

4.2.1 Pharmacokinetics and Metabolism of Flunixin in Cattle and Dogs

Bourry (2010, 2011), Appendix 2, examined the plasma concentration profile of flunixin in cattle. The half-life following a single intravenous administration was 4.2 hours, while the half-life following a single transdermal application was 7.8 hours.

In a study conducted in six adult dogs, the elimination half-life of intravenously administered flunixin meglumine was 3.7 hours (Hardie et al. 1985). Blood samples were taken at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours following administration of 1.1 mg flunixin/kg, and the 12-hour data fit to a 2-compartment model.

4.2.2 Tissue Residues of Flunixin in Cattle

Byrd (1990), Appendix 3, intravenously administered ^{14}C-flunixin meglumine at a dose of 3.3 mg FFA/kg BW to two steers and two heifers, once a day for three consecutive days. The animals were sacrificed 12 hours after the final dose, and residual flunixin was measured in tissues. Average flunixin concentrations were highest in liver and kidney tissue, and substantially lower in fat and muscle. The average concentrations of FFA in liver, kidney, muscle, and fat were 3.789, 2.495, 0.008, and 0.056 ppm, respectively.

Heird (1996), Appendix 7 intravenously administered ^{14}C- flunixin meglumine at a dose of 3.6 mg FFA/kg BW to twelve Hereford crossbred cattle, once a day for three consecutive days. Groups of three animals were sacrificed at 24, 48, 72, and 96 hours after the final dose, and residual flunixin concentrations were measured in tissues. Mean liver tissue concentrations declined from 1.95 mg FFA/kg at 24 hours after final dose to 0.39 mg FFA/kg at 96-hours post-final dose. Similarly, kidney tissue concentrations declined from 1.42 mg FFA/kg at 24 hours to 0.25 mg/kg at 96 hours. Flunixin concentrations in muscle and fat were very low, even shortly after dosing (0.023 mg FFA/kg and <0.039 mg FFA/kg, respectively, at 24 hours), and concentrations in muscle fell to <0.019 mg FFA/kg at 48−96 hours after the final dose. Heird's finding that residues decline rapidly after flunixin administration is consistent with Bourry (2010, 2011), Appendix 2, who found a half-life in cattle of 4.2 hours for intravenous administration at 2.2 mg FFA/kg BW and 7.8 hours following topical administration at 5 mg FFA/kg BW.

In the study by Crouch (2013), Appendix 6, twelve male and twelve female Angus, commercial cross-bred, Charolais cattle received a single topical application of 3.9 mg FFA/kg. Tissues were collected from two animals of each sex at 24, 48, 72, 96, 120, and 168 hours post dose. Liver, kidney, omental/renal fat, leg muscle, and muscle at the application site were sampled. Muscle samples at the site of application were further divided into "core" (on either side of the backbone) or "ring" (adjacent to the core samples). As seen in the other studies discussed previously, the highest concentrations were observed in the liver and kidney, with substantially lower concentrations in muscle and fat. Concentrations at the site of application ("core" and

[2] It is unclear from the Musser 2010 study whether the doses reported were as flunixin meglumine or as FFA; conservatively, it was assumed that the dose was administered as flunixin meglumine and was, thus appropriately converted to FFA.

"ring" samples) were higher than concentrations far from the application site (e.g., leg muscle). Concentrations were highest at 24 hours post dose and then declined. Mean tissue concentrations of flunixin residues measured over time are shown in Table 4-3.

Table 4-3 Summary of mean flunixin residues in cattle tissue after administration of flunixin transdermal solution (Crouch 2013, Appendix 6)

Post-Dose (hours)	Mean (± standard deviation) flunixin concentrations in cattle tissue (µg FFA/kg)				
	Liver	Kidney[1]	Leg Muscle	Application Site Muscle	
				Core	Ring
24	643 (± 275)	1154 (± 667)	14.2 (± 4.6)	104 (± 121)	75.8 (± 65.6)
48	142 (± 70.5)	130 (± 49.8)	5.13 (± 0.5)	12.2 (± 8.3)	23.8 (± 36.9)
72	97.5 (± 49.5)	66.4 (± 35.8)	3.40 (± 0.6)	11.4 (± 5.2)	3.48 (± 0.5)
96	81.6 (± 21.5)	41.6 (± 11.4)	2.94 (± 0.1)	6.03 (± 5.7)	4.55 (± 2.6)
120	58.7 (± 8.4)	36.0 (± 19.8)	1.91 (± 0.3)	1.99 (± 0.4)	1.82 (± 0.7)
168	36.6 (± 22.6)	26.2 (± 20.3)	1.50 (± 0.5)	1.85 (± 0.8)	1.34 (± 0.4)

[1] Values for kidney are corrected for recovery; all other values are uncorrected.

4.2.3 Acute Toxicity of Flunixin in Mammals

The acute toxicity of flunixin transdermal solution when given orally as a single dose to female rats was investigated in a GLP study performed according to OECD Guideline 425 (Smedley 2013, Appendix 14). Mortality, moribundity, clinical signs, body weight gains, and gross necropsy were evaluated. The limit test phase resulted in mortality in 2 of 3 rats at 2000 mg/kg BW and was discontinued. The up/down study phase was initiated at 550 mg/kg BW, and using an approximate multiplier dose progression of 3.2, at 2000 mg/kg BW. A single female rat was dosed at each evaluation point, and the study terminated when five reversals were observed among six tests. No mortality was observed in the three rats dosed at 550 mg/kg BW. Clinical signs included piloerection, hypothermia, hunched posture, ocular discharge, and small feces; no significant gross necropsy findings were observed. One animal at this dose level had a slight loss in body weight during the first week. Three of the four rats dosed at 2000 mg/kg BW in the up/down phase either had to be euthanized moribund or died by day 6 of the study. Clinical signs included thin appearance, hunched posture, piloerection, hypothermia, partial palpebral closure, decreased activity, rough coat, urine stain, and few/small feces. The rat that survived showed a transient loss of body weight during the first week of the study. Gross necropsy findings included abnormal content of the gastrointestinal tract and adhesions within the abdominal cavity involving the uterine horn, colon, and small intestine. The estimated LD_{50} for rats based on the data from this study was 2000 mg/kg BW flunixin transdermal solution, with 95% confidence limits of 614.6 to 5110 mg/kg BW. The flunixin transdermal solution was tested at 5% FFA w/v, so this equals an estimated LD_{50} of 100 mg FFA/kg BW, with 95% confidence limits of 30.7–255.5 mg FFA/kg BW.

The acute oral toxicity of flunixin meglumine was investigated in mouse, rat, and guinea pig (Castellano et al. 1971, Appendix 4). A single bolus of the test substance was orally administered to animals at one of three dose levels and animals were observed for a period of 14 days. Mortalities in mice occurred between 45 minutes and 5 days, in rats between 48 hours and 10 days, and in guinea pigs between 2 hours and 7 days. The acute oral LD_{50} values for the

mouse, rat, and guinea pig were based on the 14-day data and were 249.4, 53.3, and 468.3 mg flunixin meglumine/kg BW, respectively. Using a factor of 0.6, these values correspond to 149.6, 32.0, and 281.0 mg FFA/kg BW, respectively.

The acute oral toxicity of flunixin in rats and mice was investigated in another study (Castellano et al. 1976, Appendix 5). The acute oral LD_{50}s after 14 days of observation following a single administration were 68 and 78 mg FFA/kg BW for male and female rats, respectively. For mice, LD_{50}s were 197 mg FFA/kg BW for males and an estimate of 113 mg FFA/kg BW for females.

The acute oral toxicity of flunixin has been investigated in dogs, which were treated at doses of 150, 250, 300, and 350 mg FFA/kg (Baker et al. 1971, Appendix 1). None of the treated animals died, and emesis occurred at the three highest doses, so a definitive LD_{50} could not be derived. The dose of 150 mg/kg did not result in emesis or cause mortality, so it can be assumed that the acute oral LD_{50} of flunixin in dogs is >150 mg FFA/kg BW.

4.2.4 Chronic Toxicity of Flunixin in Dog

In dogs, a 3-month repeated-dose study was performed with flunixin meglumine by oral gavage (Mertens 1999, Appendix 9). The dose levels employed were equivalent to 0, 0.01, 0.05, 0.15, 0.4, and 0.6 mg FFA/kg BW/day. A total of 10 animals per dose group were included (5 males and 5 females). Animals were dosed daily for at least 91 consecutive days. Plasma concentrations of FFA peaked within 0.5 hours after dosing and were below the limit of quantitation within approximately 24 hours. No compound-related changes were observed on survival, clinical condition, body weights, food consumption values, or organ weights in any of the test groups. Hematology, biochemistry, and urinalysis parameters were unaffected by treatment. Flunixin meglumine administration had no effect on ophthalmic, electrocardiographic, or physiologic examinations. No treatment-related abnormalities were evident at post mortem on either gross pathology or histopathology. A no-observed-effect level (NOEL) for dogs of 0.6 mg FFA/kg BW/day was derived from this GLP-compliant study.

4.3 Avian and Mammalian Predicted No Effects Dose

Predicted no effects doses[3] (PNEDs) for avian and mammalian receptors are presented in Table 4-4. According to VICH GL 38, toxicity data on representative non-target ecological receptors are used to establish PNECs, for comparison to PECs, or in this case PNEDs, which are compared to predicted doses (PDs). The PNEDs are determined from acute toxicity endpoints such as the LD_{50} or ED_{50}, divided by an appropriate assessment factor (AF) to account for uncertainties such as intra- and inter-laboratory and species variation, the need to extrapolate from laboratory study results to the field, and to extrapolate from short-term to long-term toxicity. AFs between 10 and 1,000 are recommended in VICH GL 38, with an AF of 1,000 designed to be conservative and protective, and applied when limited data are available (e.g., acute toxicity data are used to predict chronic effects).

The PNED used in this EA for avian species is derived from the acute LD_5 value of 66 mg FFA/kg measured in a study with northern bobwhite quail (Hubbard and Beavers 2013, Appendix 8) and an AF of 10. The acute LD_5, which is considered equivalent to an acute no observed effects level (NOEL), was used instead of the acute LD_{50} because reliable data for this endpoint were available and allow greater predictability for protecting the test population. The

[3] PNEDs are calculated in this EA instead of predicted no-effect concentrations (PNECs), which are recommended in VICH GL 38, because the toxicity endpoints are based on the administered dose rather than exposure concentrations.

AF recommended in VICH GL 38 for short-term exposures was reduced from 1,000 to 10 for several reasons. First, only acute exposures are evaluated because flunixin has a short half-life in birds (see Table 4-2) and flunixin is not persistent on the hair of cattle and will decline quickly over time (see Table 5-2; Schieber et al., 2014; Appendix 10). Second, the LD_5 is used to calculate the PNED instead of the endpoint that is traditionally used (LD_{50}), allowing us to further reduce the AF because it is more conservative. Therefore, only an AF of 10 was needed to account for species-to-species variability. An AF of 10, when relying on quail toxicity data, is supported by Mineau et al. (2001). In this article, Mineau et al. (2001) concluded that a species-to-species extrapolation factor of 8.61 should be sufficiently protective of birds more sensitive than northern bobwhite quail when assessing the relative acute risk of different pesticides (or in this case, drugs) to birds. Therefore, the PNED for avian species is 6.6 mg FFA/kg BW (Table 4-4).

The PNED for mammals (coyotes) is derived from the LD_{50} value of 100 mg FFA/kg BW calculated for rats (Smedley 2013, Appendix 14). Of the mammalian species tested, rats were the most sensitive to flunixin. The AF recommended in VICH GL 38 for short-term exposures was reduced from 1,000 to 100 because it was not necessary to evaluate chronic exposures, as flunixin is rapidly metabolized in mammals (i.e., Bourry 2010, 2011, Appendix 2 and Byrd 1990, Appendix 3) and will decline substantially over 24 hours. Therefore only acute exposures are expected. An AF of 100, however, still accounts for the species-to-species variation, limited laboratory data, and other uncertainties. Therefore, using an AF of 100, the resulting PNED is 1 mg FFA/kg BW (Table 4-4). This is considered a conservative PNED for evaluating coyote exposures because flunixin was much less toxic to dogs than to rats.

Table 4-4. Variables used to calculate the PNEDs for avian and mammalian species

Receptor / species	Reference	Toxicity Endpoint	Effect level (mg FFA/kg BW)	Assessment factor	PNED (mg FFA/kg BW)
Avian / northern bobwhite quail	Hubbard and Beavers (2013), Appendix 8	LD_5	66	10	6.6
Mammal / rat	Smedley (2013), Appendix 14	LD_{50}	100	100	1

5. Exposure Assessment

Several exposure pathways and receptors are considered relevant for evaluation of the environmental impact following the use of Banamine Transdermal. Specifically, this assessment considers exposure to flunixin for black-billed magpies (*Pica pica hudsonia*), red-tailed hawks (*Buteo jamaicensis*), bald eagles (*Haliaeetus leucocephalus*), and coyotes (*Canis latrans*). Rationale for selection of these receptors of concern as well as the identification of the main potential exposure pathways is detailed in the following sections. As described below, magpies may experience primary exposure by ingesting cattle hair containing flunixin residues. Magpies, bald eagles, and coyotes may experience primary exposure by ingesting cattle carcasses containing flunixin residue. Although bald eagles and coyotes may also ingest treated hair while scavenging carcasses, these animals would preferentially consume soft tissue and organs from the underbelly, and would scavenge in packs; therefore ingestion of the treated hair (which is found along the midline of the backs of cattle) would be relatively small and not a significant route of exposure for any individual scavenger. Red-tailed hawks and coyotes may experience secondary exposure by preying or scavenging on magpies containing flunixin residues. The species selected as ecological receptors for this EA are representative of scavenging passerines, carnivorous and scavenging raptors, and scavenging and carnivorous mammals. The main potential exposure pathways and receptors are illustrated in Figure 5-1.

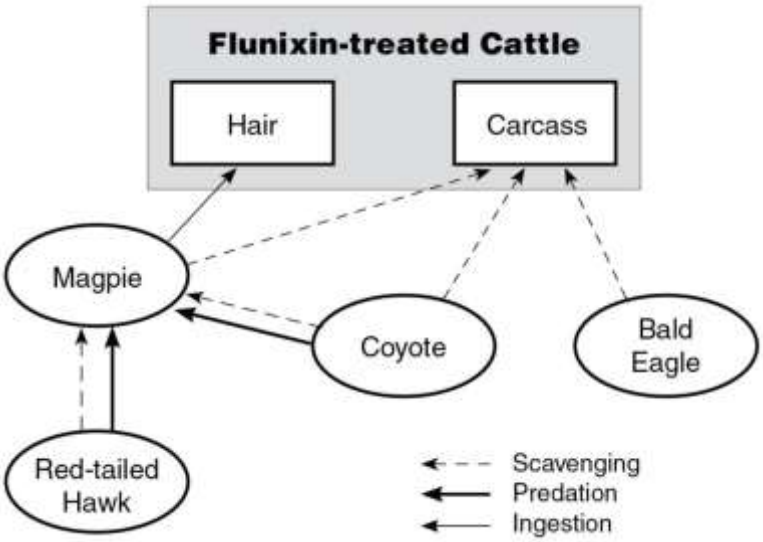


Figure 5-1. Potential environmental exposure pathways for Banamine Transdermal Pour-On for Cattle

5.1 Receptors

Information relevant to the exposure pathways (e.g., range, habitat, food preferences, behavior, etc.) is presented for each receptor in the following sections.

5.1.1 Magpies

Magpies were identified as a receptor of concern because they commonly feed on insects found on cattle and have been known to ingest cattle hair. This hair ingestion pathway is unique for

magpies, in contrast to other receptors. They have also been observed scavenging on dead livestock. Through these mechanisms, magpies could be exposed via ingestion of flunixin-treated cattle hair or cattle tissue containing flunixin residues from cattle that have been treated with flunixin. Risk calculated for magpies is expected to be representative of omnivorous birds.

Linsdale (1946) reports that magpies can be found around livestock herds, mainly due to the presence of dung- and carrion-inhabiting insects. Sometimes magpies examine large animals for parasites, and when opportunity offers, they open sores or cuts and eat the flesh of the animal itself. Furthermore, magpies have been reported to be affected by the drug famphur (an organophosphate insecticide) topically applied to cattle (Henny et al. 1985; Hanson and Howell 1981), establishing the plausibility of this pathway.

5.1.1.1 Range and Habitat

Magpies are passerine birds in the family Corvidae. Two magpie species are present in the US: the black-billed magpie and the yellow-billed magpie (*Pica nutalli*). The black-billed magpie ranges throughout the cold shrub-steppe region of the western US and Canada, reaching west from North and South Dakota to the non-coastal portions of the Pacific Northwest and including southern areas of Alaska (Kalmbach 1927). The range of the yellow-billed magpie does not overlap with that of the black-billed magpie, and is limited to portions of California (Reynolds 1995). Both magpie species are essentially non-migratory, and have similar feeding and behavioral characteristics. The yellow-billed magpie may be even more insectivorous than the black-billed magpie (Reynolds 1995). According to Linsdale (1946), magpies avoid deep forests and dry, open plains, preferring areas with clearings for foraging on the ground and nearby thickets and trees for cover and nesting. They generally occupy riparian areas associated with meadows, grasslands, and sagebrush (Linsdale 1946). In Europe, breeding magpies hold a territory of about 5 ha year round, with non-breeding birds forming flocks with a home range of up to 20 ha (University of Hertfordshire 2011); range information is not known for North America (Trost 1999).

5.1.1.2 Diet

Magpies are highly omnivorous. In a detailed, though dated, study, Kalmbach (1927) found 402 different dietary items in his examination of the stomach contents of 547 magpies. These birds predominantly ingest animal matter when it is available, but will incorporate substantial plant matter components if necessary. The animal component of the diet ranges from 92% in May when animal matter is abundant, to slightly less than 40% in winter when animal matter is less available (Kalmbach 1927). The animal matter portion of the magpie's diet is primarily composed of insects (36% of the food source for adult birds, on an annual basis) such as grasshoppers, weevils, ground beetles, carrion beetles, caterpillars, bees, ants, flies, dragonflies, etc. There are also significant contributions to the diet from carrion and small mammals (e.g., shrews, cottontail rabbits, mice, pocket gophers, ground squirrels) (Kalmbach 1927).

Magpies generally forage on the ground, and most of the insects found in their gizzards were ground-dwelling insects (Henny et al. 1985; Kalmbach 1927). Grasshoppers form a large component of the magpie diet, especially in September when they are abundant and consumed to the exclusion of other dietary components. According to Kalmbach (1927), carrion makes up approximately 14% of the food for adults on an annual basis, but this figure is higher in the winter (approximately 30%) when insects are less abundant.

5.1.1.3 Relevant Behavior

Magpies have long-documented associations with large animals that may be mutualistic, commensalistic, and parasitic. In Colorado, magpies have been observed picking parasites off the heads and backs of Rocky Mountain mule deer, American wapiti, and Rocky Mountain bighorn (Linsdale 1946). Magpies are also known to feed on ticks on moose in Alberta, sometimes caching (storing) them alive (Trost 1999). Magpies may also eat parasites, such as maggots or warble grubs, off the backs of livestock (Linsdale 1946).

Magpies commensally associate with cattle and other livestock while searching for food, such as insects under dung piles or errant grain that is more abundant around livestock (Linsdale 1946). Criddle (1923) reported observations of magpies resting overnight on the backs of cattle. However, Henny et al. (1985) observed only one incidence of a magpie perching on the back of a cow during more than 2 months of field activities in the study discussed below. Sporadically, magpies have been reported as attacking mildly injured or even healthy livestock (Kalmbach 1927). Reported magpie attacks on cattle, horses, sheep, and swine are often triggered by fresh wounds, such as new brands, shearing nicks, friction sores, or parasite wounds. Stock-molesting by magpies is usually reported in winter and early spring, but may be so severe as to cause the incapacitation or death of the livestock (Kalmbach 1927). However, this behavior is reported to be an uncommon occurrence, except possibly during severe winters when food for magpies is scarce (Link 2005). Magpies also readily feed on carrion and carrion fly larvae, including on the carcasses of livestock. From anecdotal observations reported in Linsdale (1946), magpies were observed feeding on the carcasses of horses, elk, and buffalo, which they attacked first by picking out the eyes, next the eyelids and lips, and then the neck.

Cattle hair may be unintentionally ingested in the course of feeding around, on, or off cattle. Kalmbach (1927) reported that magpie stomachs contained the hair of horses and cattle, the wool of sheep, and bristles of hogs. However, the nutritional value of hair is low at best, and the crude protein it is composed of is not readily available (Henny et al. 1985). Horsehair has also been reported to be used by magpies to line nests (Kalmbach 1927). It is possible that cattle hair may also be used for this purpose.

Henny et al. (1985) investigated the gizzard contents from 13 magpies that had died due to exposure to famphur (an organophosphate insecticide) used as a pour-on cattle treatment during October of two consecutive years. The average gizzard content consisted of 51% vegetable matter, 37% animal matter, and 12% cattle hair. Cattle hair was found in all gizzards (comprising <1% to 50% of the gizzard contents) except one that was empty. Henny et al. (1985) noted that other authors have observed hair of horses and cattle, the wool of sheep, and the bristles of hogs in magpie gizzards. Cattle hair is common throughout the pastures on objects such as fences, feed bunkers, and rubbing posts, but the proportion of hair ingested directly from the backs of treated cattle versus shed hair from other sources, and the reason for ingesting cattle hair, is unknown.

5.1.2 Red-Tailed Hawks

The red-tailed hawk was selected as a representative ecological receptor because it is a well-studied species that may be exposed to flunixin through predation or scavenging of magpies (secondary exposure). For example, Linsdale (1946) compiled evidence that magpies may be preyed upon by prairie falcons, goshawks, sharp-shinned hawks, and Cooper's hawks. Red-tailed hawks found dead in central Washington, eastern Oregon, and northwestern Colorado (one per location) were believed to be secondarily poisoned by eating magpies contaminated

with famphur (Henny et al. 1985, 1987). Risk calculated for red-tailed hawks is expected to be representative of a non-specific avian magpie predator or scavenger.

5.1.2.1 Range and Habitat

Red-tailed hawks belong to the avian order *Accipitriformes*, and are one of the most widespread and commonly observed birds of prey (raptors) in North America. They are present in central and southern Alaska, throughout central and southern Canada, through the entire contiguous US, and in Mexico and other parts of Central America (Preston and Beane 1993). In the northern reaches of their range, red-tailed hawks are present only during the breeding season, while in the southern extent of their range (roughly south of the US/Canadian border), they are present throughout the year.

As would be expected with such broad distribution, red-tailed hawks occupy a wide variety of habitats including scrub desert, plains, montane grassland, agricultural fields, pastures, urban parkland, broken coniferous and deciduous woodland, and tropical rainforest (Preston and Beane 1993).

5.1.2.2 Diet

Red-tailed hawks generally feed on small to medium-sized prey, largely composed of mammals such as voles, mice, rabbits, snowshoe hares, jackrabbits, and ground squirrels (Preston and Beane 1993). They also eat birds, including pheasants, bobwhites, and passerines, as well as reptiles, amphibians, insects, and fresh carrion. Individual prey items may weigh anywhere from 15 to 2,000 g (Preston and Beane 1993). Dietary composition studies in Alberta, Canada, north-central Oregon, and central California found that avian prey makes up 1.3%–27% of the red-tailed hawk diet (USEPA 1993) and includes waterfowl, grouse, rock partridge, western meadowlark, and other birds.

5.1.2.3 Relevant Behavior

Opportunistic hunters, red-tailed hawks will generally target whatever prey is abundant and readily available (USEPA 1993; Preston and Bean 1993). Red-tailed hawks prefer to forage in open areas with scattered perch sites, such as patches of trees or other structurally similar features. Most hunting (60%–80%) is conducted from a perch, but red-tailed hawks occasionally also hunt by cruising over open spaces at low altitudes or by soaring at higher altitudes (Preston and Beane 1993). If sufficiently small, prey will be carried to a feeding perch for consumption. At the perch, small mammals are swallowed whole, and birds are decapitated and plucked before being eaten (Preston and Beane 1993). Large prey items may be dragged to a protected area where they can be fed on prior to abandonment. Red-tailed hawks typically do not return to abandoned prey, but in cold weather, a red-tail hawk may feed on a carcass for several days (Preston and Beane 1993).

5.1.3 Bald Eagles

The bald eagle was selected as a representative ecological receptor, because it is a well-studied species that could be exposed to flunixin by scavenging carcasses of cattle that have been treated with flunixin. Bald eagles were selected over other avian scavengers as they are a species of cultural and ecological importance, and formerly were an endangered species in the United States. Furthermore, according to Henny et al. (1987), deaths of bald eagles (two in California and one in Idaho) were associated with scavenging on carcasses of cattle treated with famphur, thus establishing the plausibility of this pathway. Bald eagles could also be

potentially exposed to flunixin via ingestion of treated cattle hair during scavenging; however, this is not identified as a major exposure pathway as scavenging animals would preferentially consume soft tissue or organs from the underbelly of the cattle rather than tissue along the backline of the cattle, where treatment would occur.

Risk calculated for bald eagles is representative of an avian carnivorous scavenger.

5.1.3.1 Range and Habitat

North America's second largest bird of prey, bald eagles, are sea eagles in the genus *Haliaeetus*. The wintering range of bald eagles is predominantly along the coasts of Alaska and British Columbia, and in the contiguous US. Bald eagles typically winter at elevations below 500 m along major river systems, including those of the Chesapeake Bay, the Pacific Northwest, Klamath Basin, Oregon-California, and the intermountain west (Buehler 2000). Suitable wintering habitat is determined by the presence of a food supply, protection from inclement weather, and lack of human disturbance (Buehler 2000). Wintering bald eagles do not maintain territories and may roost communally in large aggregations (USEPA 1993).

Bald eagles generally breed in somewhat more northern climes than their wintering habitat, and breeding occurs throughout subarctic and temperate Canada and Alaska. In the contiguous US, breeding is concentrated in the Pacific Northwest, along the southeast coast, and in Florida (Buehler 2000). Breeding habitat is commonly located near coasts, estuaries, rivers, lakes, and reservoirs. Territories are established during the breeding season, and territory size varies with habitat and food availability. Typical territory sizes are 1–2 km^2, with the smallest reported territory size (i.e., greatest nesting density) of 0.05 km^2 found on Kruzof Island, Alaska (Buehler 2000).

5.1.3.2 Diet

An opportunistic forager, bald eagles prefer fish, but will prey on a variety of mammal, bird, and reptile species. The average dietary composition of nestlings from 20 studies conducted across the bald eagle range revealed that 56% of the diet was fish, 28% birds, 14% mammals, and 2% other (Buehler 2000). Given the bald eagle's relatively poor fishing abilities, live fish prey must be available near the water surface. Fish species typically captured live include gizzard shad, threadfin shad, and white bass (Buehler 2000). Bald eagles may also eat squirrels, raccoons, muskrats, hares, reptiles, amphibians, crustaceans, and variety of birds (e.g., waterfowl, Canada geese, gulls, great blue herons) (Buehler 2000; USEPA 1993). Available vulnerable or dead prey may provide significant dietary contributions for bald eagle, even if they are only sporadically available. For example, bald eagles may take advantage of stunned fish near hydroelectric facilities, fish kills (e.g., resulting from temperature variation or disease), spawning salmon, or waterfowl killed or injured by hunters (Buehler 2000). Bald eagles will eat carrion of all sorts (including road kill and domestic livestock) and can be found foraging at dumps in Alaska (Buehler 2000; USEPA 1993).

The proportion of carrion relative to live prey consumed by the bald eagle is unclear, but potentially significant. The Wildlife Exposure Factors Handbook (USEPA 1993) describes bald eagles as primarily carrion eaters that will also take live prey. High dietary fractions of carrion, however, are not present in the handbook's tables that detail the dietary composition of bald eagles. The only specific mention in the handbook of a potential carrion dietary fraction was based on relative foraging effort rather than proportion of ingested food. In this case, bald eagles in the Pacific Northwest spent 57% of their foraging time pursuing live prey, 24% of their time scavenging, and 19% pirating food from others (USEPA 1993).

Buehler (2000) describes two studies that provide additional information. In one study conducted in Chesapeake Bay, dead fish comprised 25%–67% of all fish captured by bald eagles. Another study found that 53% of bald eagle prey brought to nests on San Juan Island, Washington were European rabbits, mostly via foraging of road kill. The lack of precision regarding the proportion of carrion consumed by bald eagle may result from the difficulty of distinguishing live versus dead prey using standard field methods (e.g., stomach contents, nest leavings, pellet castings). However, it appears evident that the opportunistic bald eagle is likely to feed on carrion if it is available, and such carrion may form a substantial part of the diet when available.

5.1.3.3 Relevant Behavior

Bald eagles generally nest in trees, but may also nest on cliff faces or on the ground when trees are unavailable. Characteristic roost sites are clusters of mature deciduous trees in riparian areas, with little human disturbance and near foraging areas (Buehler 2000). While wintering, bald eagles may congregate in large groups with shared foraging areas and communal night roosts.

Bald eagles use three strategies to procure food: hunting live prey, scavenging carrion, and stealing from other birds (e.g. hawks, gulls, ospreys, herons, and mergansers) and from mammals (e.g., sea otters, coyotes, foxes) (Buehler 2000; USEPA 1993). Live hunting is conducted in flight, from perches, while wading in the water, from the ground (infrequently), and cooperatively with other bald eagles. Bald eagles may also exhibit intraspecies piracy, which seems to be determined mostly by size, hunger, and relative position, and not by age (Buehler 2000).

High-quality foraging areas are determined more by the presence of diverse, abundant, and/or vulnerable prey than by the proximity to water (Buehler 2000). Good foraging conditions exist where there is minimal human disturbance and easily caught live fish or available carrion. Bald eagles will readily consume carrion, irrespective of whether it is fish, bird, or mammal, where access to the ground is disturbance-free (Buehler 2000). Bald eagles usually bring prey back to a perch for consumption, but small prey may be consumed in flight, and large prey or carrion (e.g., salmon, duck, goose, deer, and occasionally domestic cows, sheep, and pig carcasses) may be consumed onsite (Buehler 2000). Although the bald eagle is not known to cache food, large carcasses may be fed on repeatedly (Buehler 2000). Bald eagles may also gorge themselves, filling up their crop and digesting the food over several days (Buehler 2000).

5.1.4 Coyotes

Found throughout North America, the coyote is an opportunistic predator and scavenger in the *Canidae* family. The coyote was selected as a representative ecological receptor, because it is a common species that could be exposed to flunixin by scavenging or preying on exposed magpies (secondary exposure) and treated cattle carcasses. Coyotes could also be potentially exposed to flunixin via ingestion of treated cattle hair during scavenging; however, this is not identified as a major exposure pathway as coyotes typically feed on a carcass at the flanks or behind the ribs; the internal organs such as the liver, heart, and lungs are eaten first. In contrast, treated hair would occur along the backline of the cattle. Risk calculated for coyotes is representative of a mammalian carnivorous predator or scavenger.

5.1.4.1 Range and Habitat

Coyotes are highly adaptable animals that are distributed throughout the contiguous US, Canada, and Mexico. They are found in habitats ranging from the arctic to the tropics, including in deserts, swamps, tundra, grasslands, brush, and forests (Green et al. 1994). Inhabiting areas with both low and high human disturbance, coyote densities may be highest in suburban areas where human activities subsidize their diets with trash, cultivated fruit trees, pets, and livestock (Fedriani et al. 2001).

Being territorial animals, coyotes maintain ranges of different sizes depending on food abundance. Coyote densities can range from 0.2 to 3.0 coyotes/km^2 (Knowlton et al. 1999; Fedriani et al. 2001). Although they are not migratory, coyotes may disperse from their social group when they become adults or when food is scarce (Knowlton et al. 1999).

5.1.4.2 Diet

Coyotes are opportunistic and adaptable predators and scavengers that will consume whatever food source is most available (Van Vuren and Thompson 1982). Their diet generally targets small to medium sized prey, and may include rodents, rabbits, carrion, ungulates (fawns), insects (grasshoppers), livestock, and poultry (Green et al. 1994). Coyotes often kill young and inexperienced animals, or old, sick, or weakened animals, but will also take healthy prey (Green et al. 1994). They will readily scavenge carcasses, including those of livestock (Green et al. 1994).

The composition of the coyote diet varies with habitat, seasonally, and from year to year (Meinzer et al. 1975; Van Vuren and Thompson 1982). Cattle may form part of the coyote diet when coyotes prey on calves or scavenge cattle carcasses. In an analysis of coyote scat in the Wichita Mountains Wildlife Refuge in Kansas, Holle (1978) found that cattle comprised approximately 14% of the diet when measured by volume in scat. While performing a similar study in Texas, Meinzer et al. (1975) found that carrion comprised an average of 6% of the coyote diet as determined through scat, and 21% of the diet as determined through stomach contents. Carrion found in scat and stomach contents was likely predominantly bovine in origin, because livestock carcasses were discovered near almost all the scat or stomachs in which carrion was observed. Since coyote kills were not reported by local ranchers, the authors concluded that these cattle carcasses were likely the result of disease rather than predation.

5.1.4.3 Relevant Behavior

Coyotes are most active at night and during the early morning (Green et al. 1994). Livestock predation by coyotes peaks in the spring and summer when livestock are born and increases again when young coyotes disperse to form new territories in the fall.

Coyotes typically feed on a carcass at the flanks or behind the ribs; the internal organs such as the liver, heart, and lungs are eaten first. Calves are usually killed by eating into the anus or abdominal area. If more food is available than can be consumed, coyotes will cache food for later use. Such caches can be substantial and include complete jackrabbits, and heads, shoulders, and hindquarters of 15- to 20-lb lambs or kid goats (Knowlton et al. 1999).

5.2 Exposure Calculations

For this EA, all exposures of non-target receptors to flunixin occur through ingestion of hair from treated cattle or ingestion of a food source (i.e., carrion or prey). Therefore, the PD for the receptor is analogous to the PEC, which is normally used to develop RQs, per VICH GL 38. The

exposure scenarios are calculated for individual receptors with the conservative assumptions that, on a given day, (1) the ingested hair or food source was obtained from a single source (e.g., a bird ingests flunixin from a single treated cow), (2) the receptor consumed its entire meal for the day in one sitting (e.g., the bird's gizzard contains only treated hair or only carrion from treated cattle), and (3) flunixin is completely bioavailable in the cattle hair and food sources (i.e., cattle or magpie tissue) for uptake by the receptor (e.g., magpie, red tailed hawk, bald eagle, or coyote).

The equation used to calculate the PD for exposure pathways involving ingestion of cattle hair is given by Equation 5-1. The equation used to calculate the PD for exposure pathways involving ingestion of food sources containing flunixin is given by Equation 5-2 based on the dietary exposure calculation presented in USEPA (1993).

Equation 5-1:

$$PD = \frac{DHI \times C_{hair} \times ABS_{hair}}{BW}$$

Where

PD	=	predicted dose, flunixin ingested via hair (mg/kg BW)
DHI	=	daily hair intake (kg hair ww)
C_{hair}	=	flunixin concentration in hair (mg FFA/kg hair ww)
ABS_{hair}	=	absorption efficiency from ingested hair (unitless)

Equation 5-2:

$$PD = \frac{DFI \times C_{food} \times ABS_{food}}{BW}$$

Where

PD	=	predicted dose, flunixin ingested via food (mg/kg BW)
DFI	=	daily food intake (kg food ww)
C_{food}	=	flunixin concentration in food (mg FFA/kg food ww)
ABS_{food}	=	absorption efficiency from food (unitless)
BW	=	body weight (kg)

5.2.1 Exposure of Magpies

Magpies can be exposed to flunixin through ingestion of hair from treated cattle and cattle tissue, both with flunixin residues. These two exposure routes for magpies are described in the following sections.

5.2.1.1 Exposure via Ingestion of Cattle Hair

As shown in Equation 5-1, flunixin exposure from cattle hair is a function of daily hair intake (DHI), flunixin concentration in hair (C_{hair}), and the absorption efficiency from hair (ABS_{hair}). These factors are discussed in the following sections.

5.2.1.1.1 Daily Hair Intake

To derive an upper estimate of the amount of hair that could be ingested by magpies, it was necessary to first estimate the volume of gizzard contents in a magpie's gizzard and then to determine the mass of cattle hair that could be realistically ingested and fill this volume. The maximum DHI would be equivalent to the mass of hair that could fill the predicted volume of the

magpie gizzard contents (i.e., gizzard contents containing 100% cattle hair). These determinations are discussed below.

Determination of Gizzard Content Volume

The results of Yom-Tov (1975), a study conducted in carrion crows, were used to estimate the volume of a magpie's gizzard content in the absence of data specifically for magpies. The data for crows is appropriate because magpies and crows are both members of the *Corvidae* family and demonstrate similar feeding habits. In this study, the volume of gizzard contents was determined for a subset of 40 nestling crows and plotted against body weight. The maximum volume of gizzard contents observed in each 50 g weight class (e.g., 1-50 g, 51-100 g, etc.) up to 600 g was considered to be the maximum volume for that weight class and a theoretical maximal inner gizzard volume was calculated, as shown as Line A in Figure 5-2. From these data, Yom-Tov (1975) estimated that the gizzards of most birds were 65-87% full at the time of analysis. The linear regression for the relationship between body weight (y) and gizzard volume (x) was described as: $y = 44.8x - 97.14$ ($r=0.82$, $P<0.001$). Line A represents the theoretical maximal gizzard volume, and does not represent the line equation of the data.

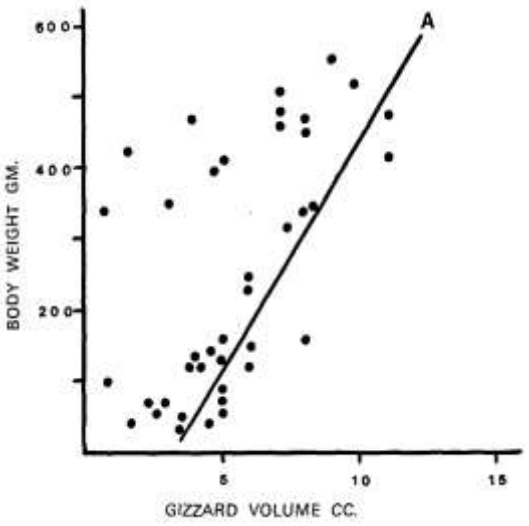


Figure 5-2. Relationship between body weight and gizzard volume of 40 nestling crows (from Yom-Tov, 1975).

The linear equation determined by Yom-Tov (1975) was used to estimate the gizzard volume of a magpie that weighs 173.2 g. As shown below in Equation 5-3, the estimated volume of gizzard contents for a magpie is 6.0 cc (6 mL). Although the equation was based on gizzards that were approximately 65-87% full and may under predict the maximum volume of a magpie's gizzard, 6 mL can be considered to be a typical volume of food that may be observed in a magpie's gizzard.

Equation 5-3:

$$y = 44.8x - 97.14$$
$$173.2\ g = 44.8(gizzard\ volume) - 97.14$$
$$gizzard\ volume = 6.0\ cc = 6.0\ mL$$

Where

y = bodyweight (g)
x = gizzard content volume (cc)

Determination of Mass of Cattle Hair Fitting into Gizzard Volume of 6 mL

After estimating the volume of food that may be observed in a magpie's gizzard (6 mL), it was necessary to determine the amount of cattle hair that could fill that volume in order to calculate a maximum DHI. Intervet conducted a pivotal study (Sczesny 2015, Appendix 11) to determine the weight of wet, compressed cattle hair that could fill a volume of 6 mL. Holstein Friesian black pied male cattle were used in the study. Ten hair samples were prepared according to the following steps. Cattle were brushed along the backline and upper flanks (areas typically treated with flunixin transdermal solution) with a curry comb, avoiding dirty spots. The hair removed from the cattle was then immersed in tap water. Sub-samples of wet hair were then added to ten, 20-mL syringes. The syringe plungers were pushed down to release excess liquid and then released slightly to allow the manually compressed hair to expand based on release of residual stress. Volumes of hair equal to 6 mL were determined and then removed from the syringes and weighed (wet weights). The hair samples were dried overnight in an oven at 27°C and then weighed again to determine the weight of the oven-dried hair. The weights of the wet and oven-dried hair samples including means, standard deviations, and 90% confidence interval on the means were calculated and are presented in Table 5-1.

Table 5-1. Weights determined for wet and dry cattle hair fitting into a 6 mL volume (Sczesny 2015, Appendix 11)

Replicate	Weight of wet hair (g)	Weight of dried hair (g)
1	1.053	0.580
2	1.777	0.804
3	1.250	0.672
4	1.111	0.571
5	1.259	0.651
6	0.976	0.505
7	1.116	0.561
8	0.957	0.477
9	1.431	0.737
10	1.309	0.626
Mean	**1.224**	**0.618**
Standard deviation	**0.246**	**0.101**
Upper 90th percentile confidence bound on the mean	**1.331**	**0.663**

Determination of Daily Hair Intake (DHI) for Magpies

For the purposes of this EA, it is assumed that cattle hair would be dry when ingested. The DHI for magpies can be estimated using the results shown in Table 5-1. However, these results alone could underestimate the equivalent weight of natural cattle hair because it is assumed that (1) all moisture was removed from the oven-dried hair and (2) natural cattle hair reportedly has inherent moisture content of approximately 13.4% (Allen et al. 1964). Therefore, the mass of the dried hair reported by Sczesny (2015), Appendix 11, was corrected to account for inherent moisture that is naturally present in cattle hair (0.134). Substituting the upper 90th percentile confidence bound on the mean of the oven-dried hair (0.663 g), the upper 90th percentile confidence bound on the mean of natural cattle hair potentially ingested by magpies is calculated to be 0.766 g (7.66×10^{-4} kg)(Equation 5-4).

Equation 5-4:

$$moisture\ content\ of\ natural\ cow\ hair = \frac{natural\ weight - dried\ weight}{natural\ weight}$$

$$0.134 = \frac{natural\ weight\ g - 0.663g}{natural\ weight\ g}$$

$$0.134 = 1 - \frac{0.663\ g}{natural\ weight\ g}$$

$$-0.866 = -\frac{0.663\ g}{natural\ weight\ g}$$

$$natural\ weight\ g = \frac{0.663\ g}{0.866}$$

$$natural\ weight\ g = 0.766\ g$$

The upper limit of the 90% confidence bound on the mean natural hair weight (0.766 g) is used to represent the DHI if 100% of the gizzard content was cattle hair. However, this fraction is reduced for the exposure scenarios in the EA because it is highly unlikely that a magpie would ingest such a large quantity of cattle hair, partially, due to the fact that hair has no nutritional value. There is only one study available in the literature that reports the fraction of cattle hair found in magpie gizzards. Henny et al. (1985) observed cattle hair in the gizzards of 13 magpies, ranging from <1 to 50% of the gizzard contents, with a mean of 12% of the gizzard content. Yet, because there are no other data available, the results of Henny et al. (1985) are used in this EA with the conservative assumption that the all gizzards were full at the time of analyses. Therefore, the initial DHI is assumed to be 50% of gizzard content, based on the maximum fraction reported by Henny et al. (1985). Using this assumption, the initial DHI is 0.383 g of cattle hair, as shown in Equation 5-5.

Equation 5-5:

$$initial\ DHI = maximum\ DHI \times 0.50$$
$$initial\ DHI = 0.766\ g \times 0.50$$
$$initial\ DHI = 0.383\ g$$

5.2.1.1.2 Flunixin Concentration in Cattle Hair

A study was conducted to determine the concentration of flunixin found in cattle hair subsequent to treatment with Banamine Transdermal at the therapeutic dose. In the pivotal study (Schieber et al. 2014, Appendix 10), six male and six female Hereford breed beef cattle were subjected to a single dermal application of flunixin transdermal solution (3.3 mg FFA/kg BW); another group contained two male and two female cattle that were not dosed. The product was administered topically in a narrow strip along the dorsal midline from the withers to the tail-head, as it should be administered in practice. Hair was collected from the three zones (A, B, and C) shown below in Figure 5-3, at 1, 3, 6, 12, 24, 48, 72, and 168 hours post dose. The flunixin concentration in the hair from the treated site was measured in the samples collected at each time point. Hair samples were weighed and then analyzed for FFA using a validated LC/MS-MS method.

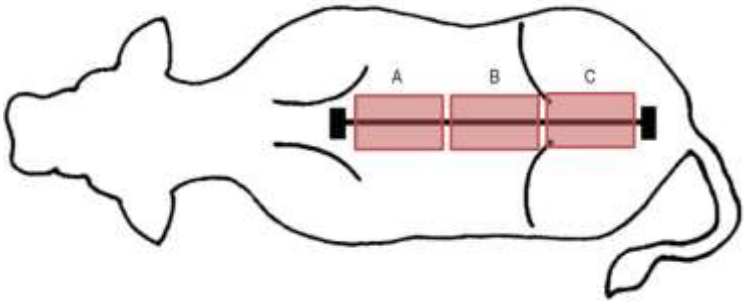


Figure 5-3. Treatment line and zones for hair sample collection (Schieber et al. 2014, Appendix 10)

The results of the study are summarized in Table 5-2. Relatively high levels of FFA were detected in the cattle hair up to 12 hours after dosing, with the highest mean flunixin concentrations measured at 3 hours post dose, after which the concentrations declined. By 168 hours post dose, concentrations were only about 6%–8% of the highest concentrations measured. Based on these data, the initial PD for magpies was calculated using the mean flunixin concentrations in hair observed at 3 hours post treatment (C_{hair} = 13,812 mg FFA/kg hair). The estimated dissipation half-life (DT50) of the flunixin concentration on cattle hair is 22.1 hours. The DT50 was estimated based on the concentrations of flunixin measured in cattle hair for individual cattle (Schieber et al. 2014, Appendix 10). As the 3-hour values are the peak concentrations measured during the study, the DT50 calculations started with this time point. The rate constant and DT50 were calculated as shown in Equation 5-6 and Equation 5-7, respectively, using CAKE software[4], version 3.1. Acceptable fit of this simple first order (SFO) kinetic to the dataset is indicated by a R^2 value of 0.7927.

Equation 5-6:

$$C_t = C_0 e^{-kt}$$

Where:

C_t = concentration at time t
C_0 = initial concentration
e = base e
k = rate constant of decline 1/days
t = time

Equation 5-7:

$$DT50 = \frac{\ln(2)}{k}$$

[4] https://showcase.tessella.com/products/cake/#.V1fmSv5Prcs

Table 5-2. Average flunixin concentrations observed in cattle hair over time across all cattle, and including all zones (Schieber et al. 2014, Appendix 10)

Time post dose (hours)	Mean (± standard deviation) flunixin concentrations in hair (mg FFA/kg ww)
1	10,005 (± 3,617)
3	13,812 (± 3,474)
6	12,629 (± 2,800)
12	11,012 (± 3,033)
24	5,784 (± 3,012)
48	3,630 (± 1,908)
72	2,446 (± 982)
168	800 (± 708)

5.2.1.1.3 Absorption Efficiency from Cattle Hair

In the absence of data for the receptor species on the absorption efficiency of flunixin from hair, it is assumed that complete absorption can occur, resulting, conservatively, in a value of 1 for ABS_{hair}.

5.2.1.1.4 Magpie Body Weight

For the purpose of this EA, the magpie BW was based on the average weight of second-year and older male (186.5 g) and female (159.9 g) black-billed magpies in the western US, from data presented by Trost (1999) and Birkhead (1991), with an overall average weight of 0.1732 kg.

5.2.1.1.5 Predicted Dose for Magpies from Ingestion of Treated Cattle Hair

The predicted dose of flunixin to magpies through the ingestion of treated cattle hair is calculated in Equation 5-8. The values for each parameter in Equation 5-8 are presented in Table 5-3. The PD for magpies ingesting flunixin from the ingestion of cattle hair is 30.5 mg FFA/kg BW.

Equation 5-8:

$$PDmagpie = \frac{DHI \times C_{hair} \times ABS_{hair}}{BW}$$

$$PDmagpie = \frac{3.83x10^{-4}kg \times 13{,}812\frac{mg\ FFA}{kg} \times 1}{0.1732\ kg\ bw}$$

$$PDmagpie = 30.5\ \frac{mg\ FFA}{kg\ bw}$$

Table 5-3. Assumptions for calculation of the initial predicted dose of flunixin for magpies via ingestion of cattle hair

Parameter	Value	Source
DHI, daily hair intake (kg hair ww)	3.83×10^{-4}	Equation 5-5
C_{hair}, concentration in hair (mg FFA/kg ww)	13,812	Schieber et al. (2014), Appendix 10; highest average flunixin concentration in hair measured at 3 hours post dose
ABS_{hair}, absorption efficiency from ingested hair (unitless)	1	Default in absence of other information
BW_{magpie}, magpie body weight (kg)	0.1732	Trost (1999)

5.2.1.2 Exposure via Ingestion of Cattle Tissue

If a cow dies following treatment with Banamine Transdermal and is not quickly disposed of, cattle tissue containing flunixin could be consumed by scavengers, including magpies. As shown in Equation 5-2, flunixin exposure from a food source (i.e., cattle tissue) is a function of DFI, flunixin concentration in tissue, and absorption efficiency from tissue. Values used for each of these parameters are calculated below.

5.2.1.2.1 Daily Food Intake (DFI) for Magpies

There is no specific value given for the DFI of magpies by USEPA (1993). Therefore, the DFI for magpies was estimated using allometric metabolic equations developed by Nagy (2001). For omnivorous birds such as magpies, DFI can be described using Equation 5-9. Based on the assumption used in this EA that the average magpie weights 0.1732 kg, the DFI is calculated to be 0.053 kg wet weight (ww), which is approximately 31% of the BW.

Equation 5-9:

$$DFI = (2.094 \times [BW \times 1000\,g/kg]^{0.627}) \times \frac{1\,kg}{1000\,g}$$

$$DFI_{magpie} = (2.094 \times [0.1732 \times 1000\,g/kg]^{0.627}) \times \frac{1\,kg}{1000\,g}$$

$$DFI_{magpie} = 0.053\,kg\,ww$$

5.2.1.2.2 Flunixin Concentration in Cattle Tissue

In exposure pathways whereby receptors ingest cattle tissue containing flunixin residues, the concentration of flunixin in food (C_{food}) in Equation 5-2 is equivalent to the concentration of flunixin in the cow tissue (C_{cow}), as presented in Equation 5-10:

Equation 5-10:

$$C_{food} = C_{cow}$$

To predict C_{cow}, the results of Crouch (2013), Appendix 6, were used (summarized in Section 4.2.2). In this study, liver, kidney, fat, leg muscle, and muscle tissue at the application site were collected and analyzed from two animals of each sex at 24, 48, 72, 96, 120, and 168 hours post dose. The highest mean tissue concentrations of flunixin residues were observed in the liver and kidney at 24 hours post dose and decreased over time (results presented in Table 4-3). The residue data collected from liver at 48 hours post dose (142 µg FFA/kg, 0.142 mg FFA/kg) was chosen for C_{cow} because the liver has the highest reported flunixin concentrations in this study, at the 48-hour time period. Further, the 48 hour time point was chosen because it is assumed that cattle will not die and/or be scavenged prior to this time. It would be expected that very sick cattle (i.e., near death) would be under close observation by a farmer or veterinarian and would likely be in a hospital pen rather than a pasture or feedlot. Thus, the potential for a cow to die and then be scavenged less than 48 hours after treatment is considered to be very low.

Therefore, C_{food} = C_{cow} = 0.142 mg FFA/kg tissue.

5.2.1.2.3 Absorption Efficiency from Cattle Tissue

In the absence of data on the absorption efficiency of flunixin from tissue, it is assumed that complete absorption can occur, resulting in a value of 1 for ABS_{food}. This means that all of the flunixin that reaches the cattle carcass can be absorbed by the magpie upon ingestion of the carcass.

5.2.1.2.4 Predicted Dose for Magpies via Ingestion of Cattle Tissue

The predicted dose for magpies exposed to flunixin residues by ingesting the carcasses of treated cattle is calculated in Equation 5-11. The values for each parameter in Equation 5-11 are presented in Table 5-4. The PD for magpies ingesting flunixin from the ingestion of cattle tissue is 0.0435 mg FFA/kg BW.

Equation 5-11:

$$PDmagpie = \frac{DFI \times C_{food} \times ABS_{food}}{BW}$$

$$PDmagpie = \frac{0.053\ kg \times 0.142\ \frac{mg\ FFA}{kg} \times 1}{0.1732\ kg\ bw}$$

$$PDmagpie = 0.0435\ \frac{mg\ FFA}{kg\ bw}$$

Table 5-4. Assumptions for calculation of the initial predicted dose of flunixin for magpies via ingestion of cattle tissue

Parameter	Value	Source
DFI, daily food intake (kg ww)	0.053	Equation 5-9
C_{food}, concentration in cattle tissue (mg FFA/kg ww)	0.142	Crouch (2013), Appendix 6; flunixin concentrations measured at 48 hours post dose in liver
ABS_{food}, absorption efficiency from food (unitless)	1	Default in absence of other information
BW_{magpie}, magpie body weight (kg)	0.1732	Trost (1999)

5.2.1.3 Summary of PDs for Magpies

The results of the calculations for both magpie exposure route scenarios are presented inTable 5-5. These calculations provide the initial PDs, as refinement factors are not yet considered.

Table 5-5. Initial predicted doses for magpie exposures

Exposure Scenario	Initial Predicted Dose (mg FFA/kg BW)
Ingestion of hair from treated cattle	30.5
Ingestion of cattle tissue	0.0435

The exposure scenarios for magpies ingesting hair and tissue are considered to be separate events in this EA. Although magpies could potentially ingest cattle hair and cattle tissue simultaneously when searching for insects on the back of a live cow, this scenario did not need to be evaluated because the exposure scenario of magpies ingesting only cattle hair is considered to be the worst-case (i.e. the PD from hair is orders of magnitude higher than the PD from tissue).

5.2.2 Exposure of Red-Tailed Hawks

Red-tailed hawks may be exposed to flunixin through the ingestion of magpies. This exposure route is described in the following sections.

5.2.2.1 Ingestion of Magpie Tissue

Magpies exposed to flunixin, especially if they manifest sublethal or lethal effects, may be preyed on or scavenged, respectively, by red-tailed hawks. As shown in Equation 5-2, flunixin exposure from ingestion of a food source (e.g., magpie tissue) is a function of the DFI, flunixin concentration in tissue, and absorption efficiency from tissue. Values for each of these parameters are calculated below.

5.2.2.1.1 Daily Food Intake for Red-Tailed Hawks

For red-tailed hawks, the Wildlife Exposure Factors Handbook (USEPA 1993) presents species-specific DFI values, or food ingestion rates (FIRs), from a 1956 study of captive, outdoor, red-tailed hawks in Michigan. This study reported FIRs for adult red-tailed hawks of 0.086 g/g-day for males in summer, 0.10 g/g-day for males in winter, and 0.11 g/g-day for females in winter (Craighead and Craighead 1956, cited in USEPA 1993). The FIR selected for this EA of 0.105 g/g-day (kg food/kg BW-day) was calculated by averaging the winter FIRs for adult males and females. The selection of the higher, winter FIRs over the single summer FIR (available only for females) results in a more conservative FIR for the EA.

To calculate daily food intake, the body weight-normalized FIR presented in USEPA (1993) must be adjusted using body weight. USEPA (1993) presents three sources of body weights for adult red-tailed hawks. These values reflect BWs for red-tailed hawks in Michigan/Pennsylvania, southwest Idaho, and Ohio. All three sources give similar body weights for adults: 957–1,204 g for males and 1,154–1,235 g for females. The largest body weights were selected for this EA, resulting in a more conservative assessment. The body weights of 1,204 g for males and 1,235 g for females were reported for adult red-tailed hawks in Ohio (Springer and Osborne 1983, cited in USEPA 1993) and resulted in an average body weight for red-tailed hawks of 1.22 kg. Using this average body weight, food intake for a single day was calculated as 0.128 kg ww using Equation 5-12.

Equation 5-12:

$$DFI = FIR_{normalized} \times BW$$

$$DFI = 0.105\ kg/kg \times 1.22\ kg = 0.128\ kg$$

5.2.2.1.2 Flunixin Concentration in Magpie Tissue

In exposure pathways whereby receptors ingest magpies containing flunixin residues, the concentration of flunixin in food sources (C_{food}) in Equation 5-2 is equivalent to the concentration of flunixin in the magpie (C_{magpie}). Because tissue residue concentrations of flunixin in magpies have not been measured, C_{magpie} can be estimated using Equation 5-13.

Equation 5-13:

$$C_{magpie}(t) = PD_{magpie} \times 2^{-\left(\frac{t}{t_{\frac{1}{2}el}}\right)}$$

Where:

C_{magpie} (t)	=	concentration of flunixin in magpie tissue at time t (Note: this equals C_{food} for the red-tailed hawk exposure)
PD_{magpie}	=	predicted dose to magpies, cattle hair exposure (mg FFA/kg BW)
$t_{1/2\ el}$	=	elimination half-life (unitless)
t	=	time since magpie ingested predicted dose (hours)

Flunixin concentrations as a result of potential exposure from use on cattle have not been measured in magpies and must be estimated. Based on the low K_{ow} value of 1.34 (Vincent 1990, Appendix 15) and short half-life in birds (Table 4-2), flunixin is not expected to accumulate in magpie tissue. A repeated-dose study in dogs (Mertens 1999, Appendix 9) also showed that flunixin did not accumulate. Because it is not bioaccumulative, the greatest flunixin concentration present in a magpie was assumed to be no greater than the highest PD_{magpie} calculated (30.5 mg FFA/kg bw, Equation 5-8) at 3 hours post dose. This tissue concentration assumes that exposure occurs through ingestion of cattle hair, as this route of exposure yields the highest PD. It also assumes that the magpie is exposed to an entire day's worth of flunixin exposure instantaneously with no metabolism and/or excretion of the compound.

Because it is unlikely that a magpie would be consumed (i.e., preyed upon or scavenged) immediately after instantaneously consuming an entire day's worth of flunixin exposure, the initial magpie tissue concentration represents a highly conservative estimate. It is more likely that some time would elapse between these events during which metabolism and excretion in the magpie would occur, prior to predation or scavenging by a red-tailed hawk. Therefore, to be more realistic but remain conservative, it was assumed that a magpie experiencing symptoms of toxicity would be more susceptible to predation and that these symptoms would be evident within 1 hour after the magpie's exposure. Conservatively, red-tailed hawks were assumed to prey or scavenge on magpies one hour after magpie exposure.

It is also assumed that flunixin metabolism in the magpie would begin shortly after ingestion of flunixin and continue until the magpie is preyed upon or until its time of death for this exposure route. The elimination half-life of flunixin in magpies is not known, so a conservative, representative avian metabolic half-life was estimated by calculating the upper 90th percentile confidence bound on the mean half-life from the data for several avian species (Table 4-2). There were two elimination values presented for chicken; however, only the more conservative of the two (6.1 h) was included in the calculation to ensure that each species was equally represented when calculating the mean, as shown in Table 5-6. Based on these data, the elimination half-life of flunixin in magpies is estimated to be 2.5 hours.

Table 5-6. Elimination half-life data used to estimate the elimination half-life in magpies

Bird Species	Elimination Half-life (hours)	Route	Source
Ostrich	0.17	Intravenous	Baert and De Backer (2003)
Mallard	0.43	Intravenous	Baert and De Backer (2003)
Turkey	0.54	Intravenous	Baert and De Backer (2003)
Pigeon	0.62	Intravenous	Baert and De Backer (2003)
Budgerigar	0.73	Oral	Musser et al. (2013)
Patagonian conures	0.91	Oral	Musser et al. (2013)
Chicken	6.1	Oral	Musser (2010)
Mean	**1.4**		
Standard deviation	**2.1**		
Number of values	**7**		
One-sided Student's t value at α=0.1	**1.44**		
Upper 90th percentile confidence bound on the mean	**2.5**		

Table 5-7 presents values used in Equation 5-14 for calculating the flunixin concentration in magpie tissue (C_{magpie}). The C_{mapgie} under the initial scenario is 23.1 mg FFA/kg ww**.**

Equation 5-14:

$$C_{food} = C_{magpie}(t) = PD_{magpie} \times 2^{-\left(\frac{t}{t_{\frac{1}{2}el}}\right)}$$

$$C_{magpie}(t) = 30.5\ mg\,FFA/kg\,BW \times 2^{-\left(\frac{1}{2.5}\right)} = 23.1\ mg/kg\,ww$$

$$C_{magpie}(t) = 23.1\ mg\,FFA/kg\,ww$$

Table 5-7. Assumptions for calculation of the initial predicted flunixin concentration in magpie tissue

Parameter	Value	Source
PD, initial predicted dose for magpies via ingestion of cattle hair (mg FFA/kg BW)	30.5	Equation 5-8
$t_{1/2\,el}$, elimination half-life (hours)	2.5	Table 5-6
t, time since magpie ingested predicted dose (hours)	1	Conservative assumption

5.2.2.1.3 Absorption Efficiency from Magpie Tissue

In the absence of data on the absorption efficiency of flunixin from tissue, it is assumed that complete absorption can occur, resulting in a value of 1 for ABS_{food}. This means that all the flunixin within the magpie can be absorbed by the red-tailed hawk upon ingestion of the magpie.

5.2.2.2 Calculation of Predicted Doses for Red-Tailed Hawks from Ingestion of Magpies

Red-tailed hawks can be exposed to flunixin by ingesting magpie tissues that contain flunixin residues. The PD for red-tailed hawks ingesting magpies was calculated using Equation 5-2 (presented previously):

$$PD = \frac{DFI \times C_{food} \times ABS_{food}}{BW}$$

PDs for red-tailed hawks were calculated in Equation 5-15 assuming that red-tailed hawks ate a diet composed entirely of magpies (e.g., C_{food} in the equation above equals C_{magpie}) that all had consumed the maximal dose of flunixin via hair from treated cattle. Table 5-8 presents the values for each parameter used to calculate the initial PD. The initial PD for red-tailed hawks is 2.43 mg FFA/kg BW.

Equation 5-15:

$$PD_{RT\ hawk} = \frac{DFI \times C_{food} \times ABS_{food}}{BW}$$

$$PD_{RT\ hawk} = \frac{0.128\ \text{kg} \times 23.1\ \frac{\text{mg FFA}}{\text{kg}} \times 1}{1.22\ \text{kg}}$$

$$PD_{RT\ hawk} = 2.43\ \text{mg FFA/kg BW}$$

Table 5-8. Assumptions for calculation of initial predicted dose of flunixin for red-tailed hawks ingesting magpies

Parameter	Value	Source
DFI, daily food intake (kg ww)	0.128	USEPA (1993), Equation 5-12
C_{food} = C_{magpie}, concentration in magpie tissue (mg FFA/kg ww)	23.1	Equation 5-14
ABS_{food}, absorption efficiency from food (unitless)	1	Default in absence of other information
$BW_{RT\ hawk}$, body weight (kg)	1.22	Average of males and females in Ohio, Springer and Osborne (1983), as cited in USEPA (1993)

5.2.3 Exposure of Bald Eagles

Bald eagles may be exposed to flunixin through the ingestion of cattle tissue from carcasses. This exposure route is described in the following sections.

5.2.3.1 Ingestion of Cattle Tissue

If a cow dies following treatment with Banamine Transdermal and is not quickly disposed of, cattle tissue containing flunixin may be consumed by scavengers such as the bald eagle. As shown in Equation 5-2, flunixin exposure from ingestion of a food source (e.g, cattle tissue) is a function of the DFI, flunixin concentration in tissue, and absorption efficiency from tissue.

5.2.3.1.1 Food Ingestion Rate for Bald Eagles

The Wildlife Exposure Factors Handbook (USEPA 1993) presents three species-specific FIRs for bald eagles. These values reflect FIRs of captive bald eagles in Utah in winter, free-flying bald eagles in Washington, and free-flying bald eagles in Connecticut. The FIRs were identical for free-flying adult bald eagles in Washington and Connecticut at 0.12 g/g-day. Because this rate was developed for free-flying birds and two research groups independently arrived at the same number, this FIR (0.12 kg/kg BW food ingested in a single day) was selected for this EA.

To calculate DFI, the body-weight-normalized FIR presented in USEPA (1993) was adjusted using body weight. USEPA (1993) presents a single source for body weights of adult bald eagles. This source, Wiemeyer (1991), reported body weights for adult male and female bald eagles of 3.0 kg and 4.5 kg, respectively. Using an average body weight for males and females of 3.75 kg, DFI was calculated as 0.45 kg ww using Equation 5-16.

Equation 5-16:

$$DFI = FIR_{normalized} \times BW$$

$$DFI = 0.12\ kg/kg\ BW \times 3.75\ kg = 0.45\ kg\ ww$$

5.2.3.1.2 Flunixin Concentration in Cattle Tissue

Bald eagles were assumed to be exposed to flunixin in cattle tissues at the same concentrations at which magpies are exposed. As described in Section 5.2.1.2.2, the concentration of flunixin in cattle tissue was estimated using the highest flunixin concentration observed in cattle tissue (liver) 48 hours after dosing (average liver concentration of 0.142 mg FFA/kg ww). Therefore, C_{food} = C_{cow} = 0.142 mg FFA/kg tissue.

5.2.3.1.3 Absorption Efficiency from Cattle Tissue

In the absence of data on the absorption efficiency of flunixin from tissue, it is assumed that complete absorption can occur, resulting in a value of 1 for ABS_{food}. This means that all of the flunixin that reaches the cattle tissue can be absorbed by the bald eagle upon ingestion of the carcass.

5.2.3.2 Calculation of Predicted Doses for Bald Eagles Ingesting Cattle Tissue

The initial PD for bald eagles was calculated assuming that bald eagles eat a diet composed entirely of cattle carcasses from cattle that were treated with flunixin 48 hours before they were scavenged. The PD for bald eagles exposed to flunixin residues from ingesting the carcasses of

treated cattle was calculated using Equation 5-17. Table 5-9 presents the values for each parameter. The initial PD for bald eagles ingesting cattle tissue is 0.0170 mg FFA/kg BW.

Equation 5-17:

$$PD_{bald\ eagle} = \frac{DFI \times C_{food} \times ABS_{food}}{BW}$$

$$PD_{bald\ eagle} = \frac{0.45\ kg \times 0.142\ \frac{mg\ FFA}{kg} \times 1}{3.75\ kg}$$

$$PD_{bald\ eagle} = 0.0170\ mg\ FFA/kg\ BW$$

Table 5-9. Assumptions for calculation of the initial predicted dose of flunixin for bald eagles ingesting cattle tissue

Parameter	Value	Source
DFI, daily food intake (kg ww)	0.45	USEPA (1993), Equation 5-16
C_{food}, concentration in cattle tissue (mg FFA/kg ww)	0.142	Crouch (2013), Appendix 6, flunixin concentrations measured at 48 hours post dose in liver
ABS_{food}, absorption efficiency from food (unitless)	1	Default in absence of other information
BW_{eagle}, body weight, kg	3.75	Average for males and females from Wiemeyer (1991), as cited in USEPA (1993)

5.2.4 Exposure of Coyotes

Coyotes can be exposed to flunixin through the ingestion of magpies (exposed to flunixin via hair or tissue from treated cattle) or cattle tissue with flunixin residues from cattle treated with flunixin. These two exposure routes are described in the following sections.

5.2.4.1 Ingestion of Magpie and Cattle Tissue

Coyotes can scavenge or prey on magpies containing flunixin if the magpies manifest lethal or sublethal effects, respectively, from exposure. They may also consume cattle tissue containing residual concentrations of flunixin if treated cows die and are not quickly disposed of. As shown in Equation 5-2, flunixin exposure from magpie or cattle tissue is a function of the DFI, flunixin concentration in tissue, and absorption efficiency from tissue.

5.2.4.1.1 Food Ingestion Rate for Coyotes

The DFI for coyotes was estimated using allometric metabolic equations developed by Nagy (2001). For carnivorous mammals such as coyotes, the DFI can be described using Equation 5-18. For the purpose of this EA, the coyote body weight was based on the average weight of adult males (14 kg) and females (13 kg) in eastern states as reported by Green et al. (1994). This average, 13.5 kg, is slightly larger and thus more conservative, than the average weight of

10.5–13 kg for adult coyotes in western states (Green et al. 1994). The DFI is calculated to be 1.49 kg ww.

Equation 5-18:

$$DFI = (0.469 \times [BW \times 1000\,g/kg]^{0.848}) \times \frac{1\,kg}{1000\,g}$$

$$DFI_{coyote} = (0.469 \times [13.5\,kg \times 1000\,g/kg]^{0.848}) \times \frac{1\,kg}{1000\,g}$$

$$DFI_{coyote} = 1.49\,kg\,ww$$

5.2.4.1.2 Flunixin Concentration in Magpie Tissue

Coyotes were assumed to be exposed to flunixin in magpie tissue at the same concentrations as red-tailed hawks. As described in Section 5.2.2.1.2, the concentration of flunixin in magpie tissue was estimated by assuming that magpies were preyed on 1 hour after their exposure to the maximal PD for magpies, accounting for a metabolic half-life of 2.5 hours. Thus, the flunixin concentration in magpie tissue is 23.1 mg FFA/kg ww.

5.2.4.1.3 Flunixin Concentration in Cattle Tissue

Coyotes were assumed to be exposed to flunixin in cattle carcass tissue at the same concentration as are magpies and bald eagles. As described in Section 5.2.1.2.2, the concentration of flunixin in cattle tissue was estimated using the highest flunixin concentration observed in cattle tissue (liver) 48 hours after dosing (average liver concentration of 0.142 mg FFA/kg ww).

5.2.4.1.4 Absorption Efficiency from Magpie and Cattle Tissue

In the absence of data on the absorption efficiency of flunixin from tissue, it is assumed that complete absorption can occur, resulting in a value of 1 for ABS_{food}. This means that all the flunixin residues in magpie and cattle tissue can be absorbed by the coyote when ingested.

5.2.4.2 Calculation of Predicted Doses for Coyotes Ingesting Magpies and Cattle Tissue

The PD for coyotes is calculated following Equation 5-2 presented previously:

$$PD = \frac{DFI \times C_{food} \times ABS_{food}}{BW}$$

PDs for coyotes were calculated assuming that coyotes ate a diet composed entirely of either magpies (all of them maximally exposed through hair from flunixin-treated cattle) or cattle carcasses (all from cattle treated with flunixin) in Equation 5-19 and Equation 5-20, respectively. A coyote with a diet composed of both magpies and cattle carcass tissue would have a PD between those calculated for cattle-carcass-only and magpie-only diets. Table 5-10 presents the

values for each parameter used to calculate the coyote PD. The PDs for coyotes ingesting magpies and cattle tissue are 2.55 and 0.0157 mg FFA/kg BW, respectively.

Equation 5-19 (magpie tissue ingestion):

$$PD_{coyote} = \frac{1.49\ kg \times 23.1\ \frac{mg\ FFA}{kg} \times 1}{13.5\ kg}$$

$$PD_{coyote} = 2.55\ mg\ FFA/kg\ BW$$

Equation 5-20 (cattle tissue ingestion):

$$PD_{coyote} = \frac{1.49\ kg \times 0.142\ \frac{mg\ FFA}{kg} \times 1}{13.5\ kg}$$

$$PD_{coyote} = 0.0157\ mg\ FFA/kg\ BW$$

Table 5-10. Assumptions for calculation of the initial predicted dose of flunixin for coyotes ingesting exposed magpies and cattle tissue from treated cattle

Parameter	Value	Source
DFI, daily food intake (kg ww)	1.49	Nagy (2001), Equation 5-18
C_{food}, concentration in magpie tissue (mg FFA/kg ww)	23.1 (1 hour after ingestion of maximal PD by magpie, 2.5 h elimination half-life)	Equation 5-14
$C_{food\ 2}$, concentration in cattle tissue (mg FFA/kg ww)	0.142	Crouch (2013), Appendix 6, flunixin concentrations measured at 48 hours post dose in liver
BW_{coyote}, body weight (kg)	13.5	Green et al. (1994)

6. Risk Characterization

Effects on ecological receptors as a result of exposure to predicted doses as calculated in Section 5 were evaluated using the effects data described in Section 4. The exposure and the effects assessments were combined to estimate risks to non-target receptors by dividing the PD by the PNED to find the RQ (Equation 6-1). RQs less than one indicate that adverse effects on receptors are unlikely to occur. RQs greater than 1 usually indicate an unacceptable level of risk that requires further evaluation. Initial RQs are presented in the following sections, followed by refined RQs where appropriate.

Equation 6-1:

$$RQ = \frac{PD}{PNED}$$

Where

RQ = risk quotient (unitless)
PD = predicted dose of flunixin (mg FFA/kg BW)
PNED = predicted no effect dose (mg FFA/kg BW)

6.1 Avian Risk Characterization

This EA evaluated risk to avian receptors from dietary exposure to flunixin residues in cattle tissue and cattle hair from cattle topically treated with Banamine Transdermal and through the food chain. Magpies, red-tailed hawks, and bald eagles were used as surrogate species to estimate the risk from flunixin to scavenging passerines and scavenging and carnivorous raptors. RQs for avian receptors are presented in Table 6-1.

6.1.1 Initial Risk Quotients for Magpies

Magpies could potentially be exposed to flunixin through two different pathways: ingestion of cattle hair or ingestion of cattle tissue. Several conservative assumptions were implemented in the calculations for the initial PDs. The initial PD for magpies via the ingestion of cattle hair assumed that magpies ingested a mass of hair equal to 50% of maximal gizzard contents three hours after the cattle were treated, which contained flunixin residues at the highest concentration of flunixin observed in treated cattle hair (Schieber et al. 2014, Appendix 10). Additionally, it was assumed that this amount of hair was ingested all at once and that the full concentration of flunixin in the hair was readily available for absorption by the magpie.

The initial PD for magpies exposed via the ingestion of cattle tissue assumed that the full diet consisted of cattle tissue (i.e., liver tissue) from treated cattle because the liver tissue contained flunixin residues at the highest average tissue concentration observed at the 48-hour time point in the tissue residue study (Crouch 2013, Appendix 6). It was also assumed that cattle would not die or be scavenged less than 48 hours after treatment. Furthermore, it was assumed that the full concentration of flunixin in the ingested tissues was readily available for absorption by the magpie. Initial PDs calculated for magpies for each pathway are shown in Table 5-5. The PNED for avian receptors was calculated based on the LD_5 obtained in an acute oral study conducted with northern bobwhite quail (Table 4-4). The initial PDs, PNED, and RQs for magpies are presented in Table 6-1. Risks are acceptable for magpies ingesting cattle tissue

(RQ = 0.0066), but are unacceptable for magpies ingesting cattle hair (RQ = 4.6). Therefore, refinements for this exposure pathway were needed and are discussed in Section 6.3.

6.1.2 Initial Risk Quotients for Red-Tailed Hawks

Red-tailed hawks could be exposed to flunixin by preying on or scavenging exposed magpies. The initial PD for red-tailed hawks was calculated assuming that the diet consisted entirely of magpies and that all the ingested magpies had ingested a mass of hair equal to 50% of maximal gizzard contents three hours after the cattle were treated, which contained flunixin residues at the highest concentration of flunixin observed in treated cattle hair. Additionally, it was assumed that this amount of hair was ingested all at once and that the full concentration of flunixin in the hair was readily available for absorption by the magpie. Further, it was assumed that ingestion of magpies by red-tailed hawks occurred only one hour after the magpies were exposed to flunixin and that the full amount of flunixin in the magpie tissues was immediately bioavailable to the hawk (see Table 5 8). Using this PD and the PNED based on northern bobwhite, the initial RQ was determined (Table 6 1). An acceptable level of risk was indicated for red-tailed hawks ingesting exposed magpies (RQ = 0.37), even using very conservative assumptions. Therefore, refinements are not needed for this exposure pathway.

6.1.3 Initial Risk Quotients for Bald Eagles

Bald eagles could be exposed to flunixin through scavenging tissue from cattle carcasses. The initial PD for bald eagles (see Table 5-9) was calculated using the assumption that bald eagles would eat a diet composed entirely of cattle carcasses from cattle that were treated with flunixin 48 hours before they were scavenged and that tissue ingested contained flunixin residues at the highest average tissue concentration observed in the tissue residue study (Crouch 2013, Appendix 6). Using this PD and the PNED based on northern bobwhite, the initial RQ was determined (Table 6-1). The risk was at an acceptable level for bald eagles ingesting carcass tissues from exposed cattle (RQ = 0.0026), even using very conservative assumptions. Therefore, further refinements are not needed for this exposure pathway.

Table 6-1. Initial RQs for avian receptors exposed to flunixin

Exposure Scenario	Initial PD (mg FFA/kg BW)	PNED (mg FFA/kg BW)	Initial RQ (PD/PNED)
Magpie exposed from ingestion of cattle hair	30.5	6.6	4.6
Magpie exposed from ingestion of cattle tissue	0.0435	6.6	0.0066
Red-tailed hawks exposed from ingestion of magpies	2.43	6.6	0.37
Bald eagles exposed from ingestion of cattle tissue	0.0170	6.6	0.0026

6.2 Mammalian Risk Characterization

This EA evaluated risk to mammalian receptors from dietary exposure through direct consumption of tissues from cattle topically treated with Banamine Transdermal or through secondary exposure through ingestion of magpies with flunixin residues. Coyotes were used as

the surrogate species to estimate the risk from flunixin to carnivorous and scavenging mammals.

6.2.1 Initial Risk Quotients for Coyotes

Coyotes could be exposed to flunixin through predation or scavenging of exposed magpies or through scavenging of tissue from cattle carcasses. Several conservative assumptions were implemented in the calculations for the initial PDs. The initial PD for the pathway involving ingestion of magpies was calculated assuming that the coyote diet consisted entirely of magpies and that all the ingested magpies had ingested a mass of hair equal to 50% of maximal gizzard contents three hours after the cattle were treated (i.e., initial exposure pathway for magpies ingesting hair), which contained flunixin residues at the highest concentration of flunixin observed in treated cattle hair. Additionally, it was assumed that this amount of hair was ingested all at once and that the full concentration of flunixin in the hair was readily available for absorption by the magpie. Further, it was assumed that ingestion of magpies occurred only one hour after they were exposed to flunixin, and that the full amount of flunixin in the magpie tissues was immediately bioavailable to the coyote. The initial PD for coyotes exposed via the ingestion of cattle tissue assumed that the full diet consisted of cattle tissue (i.e. liver tissue) from treated cattle that had died 48 hours after being treated, and that the tissue ingested contained flunixin residues at the highest average tissue concentration observed in the tissue residue study at the 48-hour time point (Crouch 2013, Appendix 6). Furthermore, it was assumed that the full concentration of flunixin in the ingested cattle tissues was readily available for absorption by the coyote (see Table 5-10). Using these PDs and the PNED based on rats (see Table 4-4), the RQs were determined (Table 6-2). While very little risk is indicated for ingestion of cattle tissue (RQ =0.0157), there is an indication of unacceptable risk associated with coyotes ingesting exposed magpies (RQ =2.55), thus requiring further evaluation. Refinements of the RQ for this pathway are discussed in Section 6.3.

Table 6-2. Initial RQs for coyote exposed to flunixin

Exposure Scenario	Initial PD (mg FFA/kg BW)	PNED (mg FFA/kg BW)	Initial RQ (PD/PNED)
Coyotes exposed from ingestion of magpies	2.55	1	2.55
Coyotes exposed from ingestion of cattle tissue	0.0157	1	0.0157

Although the RQ is equal to 2.55 for coyotes ingesting magpies (Table 6-2), a coyote would need to consume approximately 9 magpies within 1 hour of the magpies ingesting the flunixin. Additionally, those 9 magpies would need to all be in close proximity to each other and have all ingested enough cattle hair to fill 50% of their gizzards with treated hair, approximately 3 hours after a cow was dosed (Equation 6-2). The likelihood of this scenario occurring would be very low.

Equation 6-2: Number of magpies that must be consumed for the RQ = 2.55

$$\#\ of\ magpies = \frac{initial\ PDcoyote\ \times BWcoyote}{concentration\ in\ magpie\ tissue\ \times BWmagpie}$$

$$\#\ of\ magpies = \frac{2.55 \frac{mg\ FFA}{kg\ BW} \times 13.5\ kg\ BW}{23.1\ \frac{mg\ FFA}{kg\ BW} \times 0.1732\ kg}$$

$$\#\ of\ magpies = \frac{34.4\ mg\ FFA\ ingested\ by\ coyote}{4\ mg\ FFA\ in\ each\ magpie}$$

$$\#\ of\ magpies = 8.6$$

Alternatively, as calculated in Equation 6-3, a coyote would need to consume 3.4 magpies to be at a risk level that would potentially be considered unacceptable (RQ = 1). Based on the assumptions used to estimate PD_{magpie}, the likelihood of this scenario occurring would still be low, but more probable. Therefore, refinements for this exposure pathway are discussed in Section 6.3.

Equation 6-3: Number of magpies that must be consumed for the RQ = 1

$$\#\ of\ magpies = \frac{initial\ PDcoyote\ \times BWcoyote}{concentration\ in\ magpie\ tissue\ \times BWmagpie}$$

$$\#\ of\ magpies = \frac{1.0 \frac{mg\ FFA}{kg\ BW} \times 13.5\ kg\ BW}{23.1\ \frac{mg\ FFA}{kg\ BW} \times 0.1732\ kg}$$

$$\#\ of\ magpies = \frac{13.5\ mg\ FFA\ ingested\ by\ coyote}{4\ mg\ FFA\ in\ each\ magpie}$$

$$\#\ of\ magpies = 3.4$$

6.3 Refined Risk Quotients for Pathways of Concern

RQs less than 1 were calculated for exposure pathways based on ingestion of tissue from flunixin-treated cattle for magpies, bald eagles, and coyotes. This indicates that no unacceptable risks are expected from the use of Banamine Transdermal to these representative ecological receptors through this exposure pathway. However, initial RQs greater than 1 were calculated for magpies ingesting cattle hair (RQ = 4.6) and for coyotes ingesting exposed magpies (RQ = 2.55). Therefore, further assessment of these exposure pathways and appropriate refinements to the predicted dose are presented in the following sections to refine the associated risk.

6.3.1 Calculation of Refined Predicted Dose and Risk Quotient for Magpies Ingesting Cattle Hair

Refinements are needed only for the magpie exposure pathway involving the ingestion of cattle hair (RQ = 4.6), because the RQ for the cattle tissue ingestion pathway is already below 1. As previously discussed, several conservative assumptions were implemented in the calculations for the initial PD for magpies exposed via ingestion of treated cattle hair. For instance, the initial PD assumed that magpies ingested a mass of hair equal to 50% of the maximal gizzard contents three hours after the cattle were treated, which contained flunixin residues at the highest concentration of flunixin observed in treated cattle hair. Although ingestion of cattle hair is likely not deliberate (i.e., cattle hair has little to no nutritional value), there is a possibility, although unlikely, for an individual magpie to be at risk if it ingested an abnormally large amount of hair from the treated area of cattle within a short time after treatment was applied. This scenario would be rare however, especially due to the fact that birds would likely be deterred from contacting cattle immediately after treatment due to the presence of human activity near the treated cattle. Moreover, sick cattle are commonly housed in structures such as hospital pens. Almost all small feedlots (95.6%) and all large feedlots have a hospital pen or area for treatment or housing of sick animals, and 74.8% of feedlots always or usually treat animals in a hospital pen and keep them there for 24 hours or more (USDA APHIS 2000). Therefore, sick cattle will likely be housed in structures such as hospital pens that may hinder contact between the cow and a magpie. Additionally, a magpie can land on a cow at any time after it has been treated, not just 3 hours post treatment.

To estimate a refined PD, the fraction of gizzard content presumed to be cattle hair (i.e., DHI), is reduced from 50% to 12% to represent a more realistic quantity of hair that could be ingested. As discussed previously, in a study by Henny et al. (1985), the average gizzard content of 13 magpies consisted of 88% food content and 12% cattle hair, while the maximum percent of cattle hair observed was 50% of the gizzard contents (observed in a single individual). Therefore, a more realistic estimation of the percentage of gizzard contents that may be typically expected to consist of cattle hair would be 12%. This assumption can be used to refine the maximum DHI (i.e., 100% of gizzard content is cattle hair) from 7.66×10^{-4} kg to 9.19×10^{-5} kg hair, as calculated in Equation 6-4.

Equation 6-4:

$$\begin{aligned} &refined\ DHI \\ &= \ total\ weight\ of\ cattle\ hair\ filling\ 6\ mL\ volume \\ &\times \ percentage\ of\ gizzard\ contents\ expected\ to\ consist\ of\ cattle\ hair \end{aligned}$$

$$refined\ DHI\ = \ 7.66\ \times\ 10-4\ kg\ hair\ \times\ 0.12$$

$$refined\ DHI\ = \ 9.19\ \times\ 10-5\ kg$$

Additionally, concentrations of flunixin measured in cattle hair after treatment were not static over time and were observed to decline rapidly between 12 and 168 hours post-treatment (Table 5-2). Therefore, it is more realistic to capture the range of time-dependent PDs and RQs for potential doses magpies may be exposed to via ingestion of cattle hair depending on the length of time between the treatment and the time of hair ingestion. Average flunixin concentrations observed in cattle hair over time are shown in Table 5-2**.**

An example calculation using the 3-hour observed concentration in hair (13,812 mg FFA/kg hair) is presented in Equation 6-5. Input parameters for Equation 6-5 are shown in Table 6-3. Using this same equation for each time point, the PDs and RQs for magpies ingesting hair from treated cattle at different time periods after treatment are presented in Table 6-4.

Equation 6-5:

$$refined\ PD_{magpie} = \frac{refined\ DHI \times C_{hair} \times ABS_{hair}}{BWmagpie}$$

$$refined\ PD_{magpie} = \frac{\left[9.19\ x\ 10^{-5}\ kg\ hair \times \left(13{,}812\ \frac{mg\ FFA}{kg\ hair}\right) \times 1\right]}{0.1732\ kg}$$

$$refined\ PD_{magpie} = 7.33\ mg\ FFA/kg\ BW$$

Table 6-3. Assumptions for calculation of the refined predicted dose for magpies via ingestion of cattle hair

Parameter	Value	Source
Refined DHI, daily hair intake (kg ww)	9.19×10^{-5}	Equation 6-4
C_{hair}, concentration in hair (mg FFA/kg ww)	Range (800-13,812)	Table 5-2; Average flunixin concentrations observed in cattle hair over time in Schieber et al. (2014), Appendix 10
ABS_{hair}, absorption efficiency from ingested hair (unitless)	1	Default in absence of other information
BW_{magpie}, magpie body weight (kg)	0.1732	Trost (1999)

Table 6-4. Refined risk quotients for magpies ingesting cattle hair at different time periods post treatment

Time post dose (hours)	Refined PD (mg FFA/kg BW)	PNED (mg FFA/kg BW)	Refined RQ
1	5.31	6.6	0.80
3	7.33	6.6	1.1
6	6.70	6.6	1.0
12	5.84	6.6	0.89
24	3.07	6.6	0.46
48	1.93	6.6	0.29
72	1.3	6.6	0.20
168	0.42	6.6	0.06

Thus, using a more realistic estimation of the percentage of gizzard contents that may be typically expected to consist of cattle hair (i.e., 12% versus 50% of maximum gizzard content) and accounting for the change in flunixin concentrations in cattle hair that will be present over time, refined risk quotients are all less than or equal to 1.1 (Table 6-4). This indicates that the risk to magpies from this exposure route may occur primarily within the first 6 hours of the cattle being dosed under the refined scenario. But overall, the risk to magpies is low, especially in the days following treatment.

Although two refined RQs were at or slightly above 1.0, it should be noted that several conservative assumptions were used to calculate the refined RQs for individually exposed magpies. Risks to individual magpies are likely less than quantitatively defined due to the following highly conservative assumptions that were used in the dose estimation of flunixin to magpies via ingestion of cattle hair:

- It was assumed that the full amount of hair is ingested in a very short duration and would not be metabolized; however, it is likely that it would take several minutes to hours for a magpie to ingest large quantities of hair, and the flunixin would already be partially metabolized due to the rapid rate of flunixin metabolism in birds (see Table 4-2).
- It was assumed that the magpie searched for food items (i.e., insects) only from within the treated area on the back of a standing cow and did not forage outside of this zone or leave the back of the individually treated cow, and that all the hair ingested by a magpie was exclusively from the area that was treated with flunixin. This is considered to be very conservative because (1) it is more likely that the magpie would continually move about on the cow, move to other cows that may not be treated, and search for food on the ground rather than remaining in one place for a long period of time,[5] (2) only

[5] A YouTube video captured the interaction between a single magpie and approximately six Highland cattle in Canterbury, England. The magpie moves around the ground and on the dorsal aspect of the cattle. It does not spend a substantial amount of time on the back of cattle, where Banamine Transdermal would be present, nor does it spend much time on a single cow. https://www.youtube.com/watch?v=qWQG80-H5cA

a relatively small portion of the back of a cow would be covered with flunixin as compared to the overall surface area on the dorsal aspect (e.g., head, neck, back, rump) of a standing cattle that a magpie could land on,[6] and (3) it has been suggested that large quantities of ingested cattle hair are more likely obtained from fence posts and barbed wire or dead cattle, where the hair falls off more easily,[7] and not from the back of a live cow where flunixin transdermal solution would be applied.

- It was assumed that the full concentration of flunixin in the hair was readily available immediately for complete absorption by the magpie. No data on the oral absorption efficiency of flunixin from ingestion of treated cattle hair are available.

6.3.2 Calculation of Refined Predicted Dose and Risk Quotient for Coyotes

Refinements are needed only for the exposure pathway to coyotes involving the ingestion of magpies (RQ = 2.55), because the RQ for the cattle tissue ingestion pathway is already below 1. Several conservative assumptions were implemented in the calculations for the initial PD for coyotes ingesting magpies presented in Section 6.2. For example, it was assumed that the coyote's diet consisted entirely of exposed magpies and that ingestion of magpies occurred only 1 hour after they were exposed to flunixin. However, a range of possible exposure scenarios can be used to estimate several PDs and RQs for coyotes ingesting magpies.

In Table 6-5 below, the RQs for coyotes were calculated based on two varying assumptions: (1) the fraction of ingested cattle hair by magpies can range from 10 to 50% of the gizzard content, and (2) the time at which the coyote ingests the magpies (i.e., to prey upon or scavenge) can range from 1 to 24 hours after the magpie has consumed the cattle hair (i.e., flunixin would have time to be metabolized by the magpie before it is consumed by a coyote). For all calculations for the table, it was assumed that all magpies had ingested cattle hair from treated cattle 3 hours after being dosed (i.e., highest flunixin concentrations observed in hair by Schieber et al. 2014, Appendix 10).

Using these exposure assumptions, 25 out of the 30 scenarios resulted in RQs at or below 1 (Table 6-5), which is considered an acceptable risk. If a coyote ingests a magpie at a time interval of 6 hours or longer after the magpie has ingested flunixin, the RQs are all below 1. If a coyote ingests a magpie less than 6 hours after the magpie ingests flunixin residues, RQs are only above 1 if the magpie ingests large quantities of hair that are well above the average fraction of hair found in magpie gizzards (12%) by Henny et al. (1985). Based on this analysis of a range of exposure scenarios, the scenarios where the RQ is greater than 1 are unlikely to occur because we do not think that those combinations of assumptions are likely to co-occur. Therefore, the risk to mammals is acceptable.

[6] Schieber et al. (2014) reported that the area of spread of the flunixin transdermal solution was approximately 23% of the dorsal aspect.

[7] Personal communication with a Wildlife Biologist at the USDA-APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, CO; November 5, 2013.

Table 6-5. Refined RQs for coyotes exposed to flunixin residues in magpies that had ingested varying amounts of flunixin 3 hours after cattle were treated

Fraction of gizzard content that is cattle hair (%)	Refined Risk Quotients				
	Time interval between magpies ingesting flunixin and coyotes ingesting magpies*				
	1 hour	3 hours	6 hours	12 hours	24 hours
50 (assumed initial DHI)	**2.55** (Initial RQ)	**1.5**	0.64	0.12	0.004
40	**2.0**	**1.2**	0.51	0.10	0.003
30	**1.5**	0.90	0.38	0.07	0.003
20	**1.0**	0.60	0.26	0.05	0.002
12 (assumed refined DHI)	0.61	0.35	0.15	0.03	0.001
10	0.51	0.29	0.13	0.02	<0.001

* The metabolism of flunixin in the magpie is accounted for in this time interval (Equation 5-13)
Bold numbers signify RQ≥1.0

6.4 Population-level Assessment

Throughout this EA, this risk assessment focused on the exposures and risk of flunixin to individual receptors (i.e., individual birds or coyotes). But it is important to also consider the potential risk that Banamine Transdermal may have at the population level. In general, if there are no unacceptable risks to individual receptors, as was the case herein for all receptors that were evaluated, after refinement of the exposure assessment and RQs, then it is also expected there would be no adverse effects to populations of these same receptors. There are possible exceptions for chemicals that may bioaccumulate or persist for long periods *in vivo* or in the environment, resulting in long-term exposures, but that is not the case for flunixin, which is very quickly metabolized by both birds and mammals (i.e., elimination half-life less than 6 hours, as discussed in Section 4) and dissipates quickly from the cattle hair (DT50 of 22.1 hours; Schieber et al. 2014, Appendix 10). Therefore, it is highly unlikely that flunixin would produce any adverse population level (e.g., reproductive) effects if it does not cause effects on individuals.

Although risks are not expected at the population level, is worth noting that the home range of a magpie (or any receptor) would typically extend well beyond the perimeter of any particular farm. Therefore, it is unlikely that the receptor population (or even the individual receptor) would be in search of food only on the farm. Additionally, any potential risk to the population will be further reduced because only a fraction of the herd will be treated, thus reducing the likelihood of a magpie landing on a treated cow. Banamine Transdermal is proposed for four indications: (1) for control of pyrexia associated with BRD in beef and dairy cattle, (2) for the control of pyrexia associated with acute bovine mastitis in lactating dairy cattle, (3) for the control of pain associated with bovine interdigital phlegmon (foot rot, or acute interdigital necrobacillosis, or infectious pododermatitis) in beef and dairy cattle, and (4) for the control of post-operative pain and inflammation associated with cautery dehorning in calves. For each individual indication, only a minor fraction of the herd is expected to be treated at any one time. If flunixin transdermal solution is used to treat multiple indications on a single farm, the fraction of the herd treated overall (i.e., over the entire period the herd is on the farm) could potentially increase

substantially; however, because the four indications are independent and not interrelated (i.e., they are not causally related), the percentage of the entire herd that is treated at any particular time with Banamine Transdermal is expected to be small. Therefore, while it is likely that a magpie landing on a cow will land on a cow that has been treated at some time in the past with flunixin, it will still be a rare event for that magpie to land on a cow that has been treated recently and still has flunixin remaining in the hair at concentrations high enough to cause adverse effects.

6.5 Cumulative Impact Assessment

The potential for the environmental introduction of flunixin from multiple approved animal drug products containing flunixin was considered. Flunixin is approved for use in several food producing animals (i.e., cattle, horses, and swine) as a paste, injectable, and top dress. For these approved products, the only risk associated with these products would be from receptors ingesting cattle tissue. When taking the pour-on formulation into consideration with the other approved products, the risk to non-target receptors would be greatest from the injectable formulations based on tissue residue data. For example, the concentration of flunixin residues in the liver at 24 hours post dose were three-times greater when administered intravenously (1950 µg/kg; Heird 1996, Appendix 7) than when administered as a pour-on (643 µg/kg; Crouch 2013, Appendix 6). This would result in RQs calculated in this EA for receptors ingesting cattle tissue to increase three-fold. However, the RQs would still be no greater than 0.048 (i.e., the highest RQ was from coyotes ingesting cattle tissue). Additionally, residue concentrations in the cattle hair are much greater than in the tissues, so the exposure to flunixin from directly ingesting cattle hair containing Banamine Transdermal would result in the highest risk compared to all other flunixin products. The hair ingestion exposure route has already been evaluated in this EA, and it has been concluded that this exposure route would not result in unacceptable effects on non-target receptors. Therefore, it is concluded that exposures from a combination of approved uses of flunixin in different formulations and different species will not pose a substantial risk to non-target receptors.

6.6 Conclusions

Based on the data, assumptions, and calculations presented in this EA, the use of Banamine Transdermal Pour-On for Cattle is not expected to cause any significant impacts on avian or mammalian ecological receptors, including the following exposure pathways and representative receptors:

- Ingestion of hair from flunixin-treated cattle by magpies
- Consumption of tissue from flunixin-treated cattle by magpies, bald eagles, and coyotes
- Consumption of magpies that were exposed to flunixin via ingestion of cattle hair or cattle tissues from treated cattle by red-tailed hawks and coyotes.

7. Alternatives to the Proposed Action

The only alternative to the proposed action is the “no action” alternative, which would be the failure to approve the new animal drug application (NADA) for Banamine Transdermal (flunixin transdermal solution) Pour-On for Cattle. However, based on our analysis in this EA, we do not believe that significant environmental impacts will occur from this action; therefore, the “no action” alternative was eliminated from consideration.

8. Agencies and Persons Consulted

This EA was prepared with input and assistance from members of the Environmental Safety Team in the Office of New Animal Drug Evaluation in FDA's Center for Veterinary Medicine.

9. List of Preparers

This document was prepared by Josie Nusz and Jane Staveley of Exponent as well as Dr. Gregor Scheef of MSD Animal Health Innovation GmbH.

10. Certification

The undersigned official certifies that the information presented in this EA is true, accurate, and complete to the best of their knowledge.

Gregor Scheef — 29-Sep-16

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Appendices: Summaries of Sponsor-Owned Studies

Appendix 1

Reference:

Baker, F.W., F. Vymetal, and F.G. Fielder. 1971. Acute oral toxicity of SCH 14714 in dogs. Intervet Study Number: P-3978

Title:

Acute Oral Toxicity of SCH 14714 in Dogs

Name of Company:	**Study number:**	**Report date:**
Schering Corporation	3978	September 2, 1971

Study type: Acute oral toxicity in the dog

Name of test material:	**Name of formulated product:**
SCH 14714, free acid	Not applicable

Test design:

The toxicity of a single oral dose of SCH 14714 to dogs was investigated. Four adult male beagle dogs were administered SCH 14714 in a gelatin capsule after 24-hour fasting. Dosing was via oral capsule at 4 dose levels of 150 mg/kg (1 dog), 250 mg/kg (1 dog), 300 mg/kg (1 dog), or 350 mg/kg (1 dog). No vehicle control or untreated group was included. The dogs were checked periodically throughout the day of administration and three times a day thereafter for 5 weeks. Urinalysis for protein (albumin) and occult blood was conducted for the 300 and 350 mg/kg dose groups.

Statistical analysis:

Statistical tests were not performed. None of the animals died and an LD_{50} could not be derived.

Summary of findings:

At 150 mg/kg, no adverse effects were reported during the 5 week observation period. The weight of the dog was reported as 7.3 kg.

At 250 mg/kg, vomiting was noted on the first day after treatment with the occurrence of blood on one occasion. No other adverse effects were reported during the 5 week observation period. The weight of the dog was reported as 5.2 kg.

At 300 mg/kg, vomiting was noted on the first day after treatment. The dog was catheterized, and a urine sample was examined for occult blood and albumin. No occult blood was detected; a small amount of albumin was detected. The amount of protein was considered usual for dogs of this colony. No other adverse effects were reported during the 5 week observation period. The weight of the dog was reported as 10.4 kg.

At 350 mg/kg, vomiting was noted on the first day after treatment. The dog was catheterized, and a urine sample was examined for occult blood and albumin. No occult blood was detected; a moderate amount of albumin was detected. The amount of protein was considered usual for dogs of this colony. No other adverse effects were reported during the 5 week observation period. The weight of the dog was reported as 9.0 kg.

Study conducted by: Schering Corporation	**Compliance with GLP:**
Author:	No
Baker, F.W., Vymetal, F., and Fielder, F.G.	**If no, justification:**
Address:	Non-guideline study, informational purposes only.
Schering Corporation	
Bloomfield, New Jersey	

Appendix 2

Reference:

Bourry, A. 2010. Evaluation of plasma concentration profile of flunixin (SCH 14714) following a single intravenous dose of Finadyne® (Banamine®) and a single topical application of flunixin transdermal solution to cattle in a crossover study. Intervet study number: EX-05331-00. Release No. RE-14136. 12-July-2010.

Bourry A. 2011. Final report amendment No. 1: Evaluation of plasma concentration profile of flunixin (SCH 14714) following a single intravenous dose of Finadyne® (Banamine®) and a single topical application of flunixin transdermal solution to cattle in a crossover study. Intervet study number: EX-05331-00. Release No. RE-14916, amended report dated 11-October-2011.

Title:

Evaluation of Plasma Concentration Profile of Flunixin (SCH 14714) following a Single Intravenous Dose of Finadyne® (Banamine®) and a Single Topical Application of Flunixin Transdermal Solution to Cattle in a Crossover Study

Name of Company:	**Study number:**	**Report date:**
Intervet Pharma R&D SA Intervet Schering-Plough Animal Health	EX-05331-00	July 12, 2010 October 11, 2011 Amendment No. 1

Study type: Pharmacokinetics in Cattle

Name of test material:	**Name of formulated product:**
SCH 14714, Flunixin as NMG (N-methyl glucamine) salt. Flunixin NMG: 8.3% w/v = Flunixin free acid:5.0 w/v *i.e.* 50 mg/mL 2.2 mg SCH 14714 (Flunixin) free acid/kg BW for the IV route 5 mg SCH 14714 (Flunixin) free acid/kg BW for the transdermal route of administration.	Finadyne® Injectable / Banamine ® (2.2 mg/kg BW) Flunixin Transdermal Solution (5 mg/kg BW)

Test design:

The study was performed as a randomized, two-period two-sequence cross-over design with a 14-days wash-out period between treatments. Twelve cattle (Charolais) were assigned randomly to Groups I and II (3 males and 3 females per group). In Period 1, Group I cattle received the IV dose and Group II cattle received the topical dose; following the washout period, these treatments were reversed for Period 2.

Animals were housed in separate free stalls with natural ventilation and free access to light from outside. The stall was covered with straw, where animals had the possibility to lay down. The stall was cleaned daily (corridor and trough) and fresh straw was added on bedding every 2-4 days. Environmental monitoring consisted of daily temperature and relative humidity records. Temperature ranged from 2-10°C during the first period of sampling and

0-6°C during the second period of sampling. Relative humidity ranged from 86-88% during the first period of sampling and 91-95% during the second period of sampling. The cattle were fed hay *ad libitum* and food supplement supplied once daily. The animals had continuous access to fresh potable tap water via drinking bowls. Animals were acclimatized for a period of 7 days prior to dosing.

For both study periods the target dose levels were 2.2 mg Flunixin free acid/kg BW for the IV route of administration and 5 mg Flunixin free acid/kg BW for the transdermal route of administration. The target dose levels were based on the individual body weight measured the day before the first and second period.

Animals were observed twice daily for general appearance and behavior. Body weights were determined one day prior to treatment and on Day 14 post treatment. During the first phase, cattle were monitored for transdermal dosing site reaction prior to test substance administration and on days 1, 15 and 16 post-treatment. During the second phase, cattle treated transdermally were monitored for dosing site reactions prior to test substance administration and one day after test substance administration.

Blood samples were collected within 1 hour prior to dosing (T_0) and at 5, 15 and 45 minutes and 1,2,3,4,5,6,9,12,24 and 30 hours following dose administration. At each time point, 8 mL of blood was collected

and kept in an insulated box with cooling elements until processing. Within one hour of collection, blood samples were centrifuged and blood and plasma samples stored below -18°C. Within one week after plasma preparation, samples were transported frozen to the bioanalytical laboratory where they were kept frozen below -18°C until analysis.

Concentrations of Flunixin in plasma were determined with HPLC-UV method. The range of linearity was between 20 and 5000 ng/mL. The lower limit of quantification was 20 ng/mL. The maximum time between sample collection and analysis was 27 days for samples collected during Period 1 and 16 days for samples collected during Period 2. The delay between collection and analysis conformed to the storage stability of Flunixin.

Statistical analysis:

Statistical analysis was originally conducted using SAS® (release 9.1.3). Amendment No. 1 states SAS® (SAS Institute Inc, Cary, NC, USA release 9.2).

Descriptive statistical analyses, where applicable include calculations of mean (arithmetic mean for body weight, rectal temperature, C_{max}, C_0, and AUC; harmonic mean for $t_{1/2}$), standard deviation and median value (t_{max}).

To determine whether or not data from male and female cattle could be pooled for statistical evaluation, the gender effect was first evaluated using ANOVA (AUC, $T_{1/2}$, C_o, C_{max}) or Friedman Test (t_{max}).

Amendment No. 1 addressed the new statistical analysis that had to be conducted in order to correct programming errors that occurred for the plasma concentration profile of Flunixin. The programming error did not affect the descriptive statistics (body weights on Day -1 and Day 14 and rectal temperature in the original report). Only minor changes were induced by this new analysis. Gender-by-treatment interaction was observed for AUC_{inf}, AUC_{last}, and $t_{1/2}$ whereas it was observed for AUC_{inf} only with the wrong statistical model. However, this has no impact on the outcome of the study. Parameters obtained for male and female could be combined within each treatment group.

Summary of findings:

Adverse effects noted in the study included swelling of injection site in one animal which was not attributed to the test substance. Dandruff was observed on Days 15 and 16 of treatment in 5/6 cattle treated transdermally in Phase 1. Alopecia was observed following transdermal application in 2 cattle in Phase 2. It was reported that 2 cattle were nervous at the time of IV dosing. No other adverse effects were reported.

There were no significant gender effects within treatments at a significant level for either the IV or dermal treatment. Therefore data from males and females could be combined for all parameters.

After IV treatment, maximum Flunixin concentrations were observed 5 minutes after injection and decreased quickly until 3 hours post-dosing when a small peak occurred due to a possible enterohepatic recycling of Flunixin. Thereafter, concentration levels steadily decreased to reach an overall mean concentration below 20 ng/mL at 30 hours post-dose. The 2 cattle that were nervous at the time of dosing lead to administration of the test substance not performed fully in the vein. These 2 cattle were excluded from pharmacokinetic evaluation due to low plasma flunixin profiles following IV dosing.

Following transdermal dosing, there was a marked inter-individual variability in the over-all plasma concentration over time. Flunixin concentrations reached the blood stream within 15-45 minutes post-dosing and maximum concentration levels occurred between 3 and 12 hours post-dosing and ranged between 686.9 and 2990.1 ng/mL. Once maximal values were reached, concentration levels decreased steadily to reach an overall mean concentration of 174.4 ± 70.90 ng/mL at 30 hours post-dosing.

Bioavailability was calculated from AUC_{inf} values from 9 cattle. Mean bioavailability was 43.8 ± 9.93% (ranging from 32.1 to 63.8%).

The half-life of Flunixin following IV administration was 4.2 hours. Apparent half-life following transdermal application (5 mg/kg) was 7.8 hours. Transdermal absorption of Flunixin occurred within 15 to 45 minutes post-dose. The transdermal application had a median T_{max} of 6 hours (3-12 hour range). The bioavailability was 43.8 ± 9.93%.

Study conducted by: Intervet Schering-Plough Animal Health **Author:** Bourry, A. **Address:** INTERVET PHARMA R&D SA Rue Olivier de Serres 49071 Beaucouzé France	**Compliance with GLP:** Yes **If no, justification:**

Appendix 3

Reference:

Byrd, J.W. 1990. Sch 14714 - A total residue depletion study using [14C]-flunixin in cattle. Intervet study number: A-24495.

Title:

SCH 14714 – A Total Residue Depletion Study Using [^{14}C]-Flunixin in Cattle

Name of Company:	**Study number:**	**Report date:**
Schering-Plough Animal Health	Research Report A-24495. SBL Project No. 9025b	November 5, 1990

Study type: Cattle Residue Study

Name of test material:	**Name of formulated product:**
SCH 14714 (flunixin) as N-methylglucamine salt	Not applicable

Test design:

The study was conducted to determine the concentration of [^{14}C]-SCH 14714-derived residues in liver, kidney, muscle and fat tissues. Five crossbred cattle (3 males and 2 females) were purchased, quarantined for 3 days (Study Days -17 to -14) and acclimated to the test facility for 13 days (Study Days -13 to 0). The animals were randomly divided into a treatment group (Group 1) containing 2 males and 2 females and a control group (Group 2) containing one male. Group 1 animals were dosed intravenously once per day for three consecutive days with [^{14}C]-flunixin at a rate of 3.3 mg flunixin (free acid) / Kg body weight. Dosing solutions were analyzed via HPLC and radiochemical purity determinations were made. The animals were observed twice daily beginning on Study day -16. Body weights were obtained from each animal at 4 timepoints (Study Day -17, -10, 1 and at termination (Day 3)). 24-hour urine and fecal specimens were collected from each animal in Group 1 at Study Day -4, 1, and 2. A twelve hour urine and fecal sample was collected from each animal in Group 1 following the 3rd dose. Group 1 was terminated twelve hours after the third and final dose (i.e., 60 hours). The following tissues or tissue samples were collected at necropsy: liver (entire), kidney (both), muscle (loin), and fat (omental). Prior to processing the liver, kidney and muscle were trimmed of excess fat, connective tissue, and blood clots.

Statistical analysis:

Not applicable.

Summary of findings:

Animals in Group1 and Group 2 were reasonably healthy throughout the treatment period. Animal 312 (Control Group 2) displayed signs of pink eye but was retained on study in isolation. At necropsy all animals appeared normal. At 24, 48 and 60 hours approximately 23%, 27%, and 18% of the administered dose, respectively, was recovered in the excreta. Over the course of the study, approximately 68.28% of the administered dose was recovered via the urine (24.65%) and feces (43.63%). At termination (60 hours after the first dose, 12 hours after the last dose), liver, kidney, loin muscle, and omental fat tissues were analyzed for FFA. The liver contained the highest total [^{14}C]-flunixin residue levels (3.789 ppm) followed by the kidney (2.495 ppm), fat (0.056 ppm) and muscle (0.008 ppm) (n=4).

Study conducted by: Southwest Bio-Labs, Inc.	**Compliance with GLP:**
Author:	Yes
Byrd, J.W.	**If no, justification:**
Address:	
Southwest Bio-Labs, Inc. 401 N. 17th Street Las Cruces, NM	

Appendix 4

Reference:

Castellano, R.F., A. Fabry, and F.G. Fielder. 1971. Acute toxicity in rats (oral and intraperitoneal), mice (oral and intraperitoneal) and guinea pigs (oral). Intervet study number: P-3962.

Title:

SCH 14714 NMG: Acute Toxicity Studies in Rats (Oral and Intra-Peritoneal), Mice (Oral and Intra-Peritoneal), and Guinea Pigs (Oral).

Name of Company:	**Study number:**	**Report date:**
Schering Corporation	3962 (Tox 127)	June 24, 1971

Study type: Oral and Intraperitoneal Rat LD_{50}; Oral and Intraperitoneal Mouse LD_{50}; Oral Guinea Pig LD_{50}

Name of test material:	**Name of formulated product:**
SCH 14714 [although not stated this is assumed to be as the n-methylglucamine salt, since the description of "white powder" matches the description in the more recent study by Castellano et al.]	Not applicable

Test design:

SCH 14714 was administered to Carworth CFE rats by oral gavage in order to determine the oral LD_{50}. Male rats (10 rats/group) were randomly assigned to 6 dose groups corresponding to 0 (vehicle control), 32, 50, 79, 113 and 200 mg/kg SCH 14714. Female rats (10 rats) were administered 79 mg/kg SCH 14714 for comparative dose assessment. SCH 14714 NMG was prepared in 2.5% aqueous Tween® 80 immediately before administration. Control rats received only vehicle at a rate equivalent to the highest dose. The rats were fasted overnight, weighed and administered the test substance by oral gavage. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

SCH 14714 was administered to Carworth CFE rats intravenously in order to determine the intra-peritoneal LD_{50}. Male rats (10/group) were randomly assigned to 6 dose groups corresponding to 0 (vehicle control), 50, 60, 70, 80, and 90 mg/kg SCH 14714. Female mice (10 mice) were administered 70 mg/kg SCH 14714 for comparative dose assessment. SCH 14714 was prepared in 2.5% aqueous Tween® 80 immediately before administration. Control rats received only vehicle at a rate equivalent to the highest dose. The animals were weighed and administered a single intraperitoneal injection. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

SCH 14714 was administered Carworth CF1 mice by oral gavage in order to determine the oral LD_{50}. Male mice (10/group) were randomly assigned to 12 dose groups (0 (vehicle control), 132, 162, 200, 222, 246, 272 or 302 mg/kg; females (10/group) were dosed at one level (200 mg/kg). SCH 14714 was prepared in 2.5% aqueous Tween® 80 immediately before administration. Control mice received only vehicle at a rate equivalent to the highest dose. The mice were fasted overnight, weighed and administered the test substance by oral gavage. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

SCH 14714 was administered to Carworth CF1 mice intraperitoneally in order to determine the intra-peritoneal LD_{50}. Male mice (10/group) were randomly assigned to 6 dose groups corresponding to 0 (vehicle control), 151, 163, 175, 189, and 203 mg/kg SCH 14714. SCH 14714 was prepared in 2.5% aqueous Tween® 80 immediately before administration. Control mice received only vehicle at a rate equivalent to the highest dose. The animals were weighed and administered a single intraperitoneal injection. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

SCH 14714 was administered to Marland Hartley guinea pigs in order to determine the oral LD_{50}. Male guinea pigs were assigned to one of five groups (6/group) (0 (vehicle control), 346, 380, 416, 456, or 500 mg/kg). SCH 14714 was prepared in 2.5% aqueous Tween® 80 immediately before administration. Control guinea pigs received only vehicle at a rate equivalent to the highest dose. The guinea pigs were fasted overnight, weighed and administered the test substance by oral gavage. The animals were observed for 14 days after the dose for

mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

Statistical analysis:

LD_{50} values were calculated from 14-day data using Finney's "Probit Maximum Likelihood Method."

Summary of findings:

For the oral rat LD_{50} study, all deaths occurred between 2 and 10 days after treatment in males and 4 and 10 days after treatment in females. Clinical observations included anorexia, diarrhea, emaciation, chromorhinorrhea, and pilo-erection in both males and females. The principal necropsy findings included peritonitis from ulceration of the small intestine and enlargement of the spleen with excessive number of cells resembling megakaryocytes. The 14-day oral LD_{50} for male rats was identified as 53.3 mg/kg for SCH 14714.

For the oral mouse LD_{50} study, the majority of deaths occurred 45 minutes to 5 days after treatment in males and 3 to 4 days in females. Hypoactivity was the principal clinical effect in males and females. At necropsy, gross changes were seen in only 2 mice; hemorrhagic gastric mucosa in one and abscessation of the stomach and enlargement of the spleen in another (with excessive number of cells resembling megakaryocytes and normoblasts). The 14-day oral LD_{50} for male mice was calculated as 249.4 mg/kg for SCH 14714.

For the oral guinea pig LD_{50} study, deaths occurred 2 hours to 7 days after treatment. Hypoactivity and slow, deep respiration were the principal clinical effects. Necropsy of the animals which died on study within the first 24 hours revealed distended urinary bladder and blood in the stomach in a large proportion of the animals; two others had gastric ulcers. The 14-day oral LD_{50} for male guinea pigs was calculated as 468.3 mg/kg for SCH 14714.

For the intraperitoneal rat LD_{50} study, the deaths occurred 2 – 11 days after treatment in males and 5 - 6 days after treatment in females. Clinical signs were similar to those seen in the oral study and included melena in both males and females. For animals that died on study, the principal finding at necropsy included ulceration of the small intestine and peritonitis. At necropsy, small thymus gland and enlarged spleen were reported. The enlarged spleens contained numerous cells resembling megakaryocytes. The 14-day intraperitoneal LD_{50} for male rats was identified as 59.4 mg/kg for SCH 14714.

For the intraperitoneal mouse LD_{50} study, all the deaths occurred within one hour after treatment. Clinical signs include hypoactivity, hyperactivity, and ataxia. No meaningful changes were observed at necropsy. The 14-day intraperitoneal LD_{50} for male mice was identified as 164.0 mg/kg for SCH 14714.

Study conducted by:	**Compliance with GLP:**
Author:	No
Castellano, R.F., A. Fabry, and F.G. Fielder	**If no, justification:**
Address:	Study was well conducted and the report was thorough. The only data missing according to current guidelines are the terminal body weights.
Schering Corporation Bloomfield, New Jersey	

Appendix 5

Reference:

Castellano, R.F., J.R. Beall, and W.D. Gray. 1976. SCH 14714 NMG: Acute toxicity studies in rats (oral and intravenous) and mice (oral). Project Report No. P-4390 (Tox 443).

Title:

SCH 14714 NMG: Acute toxicity studies in rats (oral and intravenous) and mice (oral)

Name of Company:	**Study number:**	**Report date:**
Schering Corporation	P-4390 (Tox 443)	February 26, 1976

Study type: Oral Rat LD_{50}; Oral Mouse LD_{50}; Intravenous Rat LD_{50}

Name of test material:	**Name of formulated product:**
SCH 14714 NMG (n-Methylglucamine Salt), described as a white powder	Not applicable

Test design:

SCH 14714 NMG was administered to Charles River CD rats by oral gavage in order to determine the oral LD_{50}. Male and female rats (10 rats/sex/group) were randomly assigned to 6 dose groups (0, (vehicle control), 71, 94, 126, 168 or 224 mg/kg). SCH 14714 NMG was dissolved in distilled water immediately prior to administration. The rats were fasted overnight, weighed and administered the test substance by oral gavage. Control rats received only distilled water. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

SCH 14714 NMG was administered to Carworth CF1 mice by oral gavage in order to determine the oral LD_{50}. Male and female rats (10 rats /group) were randomly assigned to 12 dose groups (0 (vehicle control, 251, 316, 398, 502, or 630 mg/kg in males and 0 (vehicle control), 170, 200, 234, 276, or 324 mg/kg in females). SCH 14714 NMG was dissolved in distilled water immediately prior to administration. The mice were fasted overnight, weighed and administered the test substance by oral gavage. Control mice received only distilled water. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

SCH 14714 NMG was administered to male and female Charles River CD rats in order to determine the intravenous LD_{50}. Male rats were assigned to one of five groups (10 rats/group) (0 (vehicle control), 84, 89, 94 or 100 mg/kg) and female rats were assigned to one of seven groups (10 rats/group) (0 (vehicle control), 84, 89, 94, 100, 106, or 112 mg/kg). SCH 14714 NMG was dissolved in sterile water for injection immediately prior to dosing. The rats were weighed and administered a single intravenous injection at 1 mL/kg. Control rats received sterile water injections at the same volume. The animals were observed for 14 days after the dose for mortality and clinical effects. Necropsy was performed on all animals which died during the study period and on all animals that survived the study period at 14 days after treatment.

Statistical analysis:

Oral LD_{50} values were calculated from 14-day data for rats and mice. The intravenous LD_{50} for rats was calculated from the 3-day data. Calculations were done using Finney's "Probit Maximum Likelihood Method."

Summary of findings:

For the oral rat LD_{50} study, all deaths occurred between 2 and 13 days after treatment. Abdominal distension, pallor, emaciation, and staring coat were the principal clinical observations. Ulcers in the jejunum, intestinal adhesions, and a serous transudate in the peritoneal cavity were seen in most of the rats that died during the study. Ulcers in the jejunum, enlargement of the spleen, and intestinal adhesions were the principal changes noted at necropsy in the rat. The 14-day oral LD_{50} for rats was identified as 113 mg/kg for male rats and 130 mg/kg for female rats for the NMG salt of SCH 14714. The 14-day oral LD_{50} for rats expressed as free acid was calculated to be 68 mg/kg for males and 78 mg/kg for females.

For the oral mouse LD_{50} study, the majority of deaths occurred within 5 days after treatment. Clonic convulsions preceded most of the deaths that occurred within one hour after treatment. Other clinical observations include prostration, slow respiration, and staring coat. At necropsy, the principal changes were ulcers in the jejunum which occurred in mice that died during the study and in one mouse that survived until study termination at two

weeks. The 14-day oral LD_{50} for mice was calculated as 327 mg/kg for males for the NMG salt of SCH 14714. An LD_{50} for female mice could not be calculated because the regression was not statistically significant. However, when the highest dose level was omitted from the calculation, an oral LD_{50} of 189 mg/kg was estimated for female mice for the NMG salt of SCH 14714. The 14-day oral LD_{50} for mice expressed as SCH 14714 free acid was calculated to be 197 mg/kg for males and estimated to be 113 mg/kg for females.

For the intravenous rat LD_{50} study, the majority of deaths occurred within one minute of treatment. At necropsy, no changes occurred in rats that died within one minute. Delayed deaths occurred up to 13 days after treatment. Clinical observations included abdominal distension, pallor, clonic convulsions, and hypoactivity. Changes in other rats that died during the study included ulcers and hemorrhages in the jejunum, intestinal adhesions, and a serous transudate in the peritoneal cavity. Most of the rats which survived the observation period had ulcers in the jejunum and enlargement of the spleen. The intravenous LD_{50} for rats was identified as 90 mg/kg for male rats and 92 mg/kg for female rats for the NMG salt of SCH 14714. The intravenous LD_{50} for rats expressed as SCH 14714 free acid was calculated to be 54 mg/kg for males and 55 mg/kg for females.

In a relative potency analysis, the oral LD_{50} values did not differ significantly between male and female rats, but were significantly different between male and female mice. The intravenous LD_{50} values were not significantly different between the sexes for rats.

Study conducted by:	**Compliance with GLP:**
Schering Corporation	No
Author:	**If no, justification:**
Castellano, R.F., J.R. Beall, and W.D. Gray	Study was well conducted and the report was thorough. The only data missing according to current guidelines are the terminal body weights.
Address:	
Bloomfield, NJ	

Appendix 6

Reference:

Crouch, L.S. 2013. SCH14714: A Final Residue Depletion Study of Flunixin in Beef Cattle Following Administration of Flunixin Transdermal Solution During Winter. Intervet Study Number: S11195-00

Title:

SCH 14714: A Final Residue Depletion Study of Flunixin in Beef Cattle Following Administration of Flunixin Transdermal Solution During Winter

Name of Company:	**Study number:**	**Report date:**
Intervet Inc (d/b/a Merck Animal Health)	S11195-00	February 27, 2013

Study type: Residue Depletion Study

Name of test material:	**Name of formulated product:**
Flunixin N-methyl glucamine (NMG) is the active ingredient	Flunixin Transdermal Solution for cattle

Test design:

The level of the marker residue, flunixin, in liver, kidney, omental/renal fat, leg muscle, and muscle at the application site of cattle after a single administration of the proposed flunixin transdermal solution was investigated during winter. An exaggerated target dose level of 3.9 mg flunixin free acid per kg (118% of proposed) was used. Twelve male and twelve female cattle weighing between 268 to 350 kg were treated with dose levels ranging from 3.90 – 4.05 mg flunixin free acid /kg. The tissues were collected from two animals of each sex at 1, 2, 3, 4, 5 and 7 days post-dose, as well as from an untreated control male. Muscle samples at the site of application were further divided into "core" (on either side of the backbone) or "ring" (adjacent to the core samples). Muscle distant from the application site was sampled from the leg as was fat (combined renal and omental), kidneys, and liver. All tissues were analyzed by a validated LC-MS determinative procedure with the results to be used for calculation of the withdrawal period under EU guidelines. Liver was also analyzed by a validated LC-UV determinative procedure which was a slight modification of the accepted US (FDA-CVM) LC-UV determinative procedure with the results to be used for calculation of the withdrawal period under US guidelines. Flunixin levels in tissue were determined in terms of both corrected and uncorrected for method recovery.

Statistical analysis:

Statistical tests were not performed.

Summary of findings:

Animals were healthy with no adverse effects from the dose administration. Comparison of flunixin levels in males versus female in these tissues does not indicate any apparent sex difference. The highest concentrations were observed in the liver and kidney, with substantially lower concentrations in muscle and fat. Concentrations at the site of application ("core" and "ring" samples) were higher than concentrations far from the application site (e.g. leg muscle). Concentrations were highest at 1 day post dose and then declined. Flunixin levels in tissues indicated that all residue levels were below the method recovery limit by 4 days post-dose except for one application site muscle core sample.

Flunixin levels observed in tissues were reported as follows: Mean values (µg FFA/kg), corrected for method recovery, declined from day 1 to day 7 in the following tissues as follows: 784 to 37.6 (liver), 1154 to 26.2 (kidney), 16.2 to 1.71 (leg muscle), 15.8 to 1.47 (fat), 119 to 2.12 (application site muscle, core), and 86.8 to 1.56 (application site muscle, ring). Mean values (µg FFA/kg), uncorrected for method recovery, declined from day 1 to day 7 in the following tissues as follows: 643 to 36.6 (liver), 14.2 to 1.5 (leg muscle), 104 to 1.85 (application site muscle, core), and 75.8 to 1.34 (application site muscle, ring). At 48 hours, mean uncorrected values (µg FFA/kg) were 142 (liver), 5.13 (leg muscle), 12.2 (application site, core), and 23.8 (application site, ring).

Study conducted by: Intervet Inc (TH) **Author:** Crouch, L.S. **Address:** 2458 No. Chamberlain Street Terre Haute, IN 47805 USA	**Compliance with GLP:** Yes **If no, justification:**

Appendix 7

Reference:

Heird, C.E. 1996. SCH 14714: A total residue depletion study in cattle following intravenous administration of [^{14}C]-SCH 14714 (SN 95708). Intervet study number: SN 95708

Title:

SCH 14714: A Total Residue Depletion Study in Cattle Following Intravenous Administration of [^{14}C]-SCH 14714

Name of Company:	**Study number:**	**Report date:**
Schering-Plough Animal Health	Scherling-Plough Study No. SN 95708 Southwest Bio-Labs, Inc. Study No. 95107B Xenobiotic Laboratories, Inc. Study No. 96051	December 4, 1996

Study type: Cattle Residue Study

Name of test material:	**Name of formulated product:**
Mixture of SCH 14714 (Flunixin, unlabelled) and SCH 14714 N-methylglucamine salt (Flunixin NMG, radiolabeled)	Not applicable

Test design:

The study was conducted to determine the concentration of [^{14}C]-SCH 14714-derived residues in liver, kidney, muscle and fat tissues. Eighteen Hereford crossbred cattle (8 males and 10 females) were acclimated to the test facility and randomly assigned to five treatment groups: Group 1 (2 male and 1 female), Group 2 (1 male and 2 females), Group 3 (2 males and 1 female), Group 4 (1 male and 2 females) and Group 5 (1 female) (control group). One female remained as an extra animal. Animals were quarantined prior to the start of the study from Days -19 to -12. The 14-day acclimation period began during quarantine from Days -13 to 0. Animals in Groups 1-4 were dosed intravenously once daily for 3 consecutive days with 3.6 mg of [^{14}C]-SCH 14714/kg. The specific activity of the dose solutions was determined by HPLC and TLC both pre-dose and post-dose. Animals were observed twice daily for clinical signs and body weights were recorded on Study Days -17, -13, -3, and at the scheduled termination for each animal. A body weight for animal #1008-F was recorded on study Day -1; this animal replaced animal #1006-F due to diarrhea. Urine and feces were collected daily throughout the study. The animals in Groups 1, 2, 3 and 4 were terminated at approximately 24, 48, 72 and 96 hours post dose, respectively. At necropsy, tissues were examined for the presence of gross pathology or abnormalities. Liver, kidneys, muscle and fat were collected from each animal.

Statistical analysis:

Statistical analysis was limited to simple measures of central tendency and/or dispersion such as mean and standard deviation. Dixon's Q-test was used to exclude outliers.

Summary of findings:

Overall the animals appeared healthy throughout the course of the study as evidenced by feed intake, veterinary observations, body weights and daily observations. Clinical effects included observations that most of the animals experienced nasal discharge, many animals had occasional coughs and some animals experienced diarrhea or blood in the feces on occasion. Body weights at Day -3 were 181-280 kg and at necropsy were 165-270 kg. At necropsy, the following observations were made: kidney cyst in male from Group 1, pneumonia lesions on the left lung and light small discoloration on one lobe of the right kidney in male from Group 1, light-mottled-looking small spots throughout both kidneys in female from Group 1, kidney cyst in male from Group 3, old pneumonia lesions on both lungs in female from Group 3, kidney cysts in female of group 4, and liver flukes in female from Group 5.

The highest concentrations of [^{14}C]-SCH 14714 equivalent residues were found in the Group 1 liver tissues (24 hour withdrawal, avg. 1.95 ppm). Group 1 kidney tissue contained the next highest residue levels (1.42 ppm). Mean tissue ^{14}C-residue levels declined with time post final dose: Group 2 (48 hours; liver 0.52 ppm and kidney 0.47 ppm), Group III (72 hours; liver 0.44 ppm and kidney 0.37 ppm) and Group 4 (96 hours; liver 0.39 ppm and kidney 0.25 ppm). Muscle and fat contained very low levels of ^{14}C-residues; averaged concentrations in the muscle 24-hours post-final dose were 0.023 ppm and in the fat were <0.039 ppm. Residues in muscle at 48-96 hours post final dose were found to be <0.019 ppm. Recovery of the cumulative ^{14}C-dose in urine for groups 1, 2,

3, and 4 averaged 39.1%, 37.1%, 40.2%, and 36.6%, respectively; average recovery in feces was 36.5%, 44.3%, 41.4% and 45.9%, respectively.	
Study conducted by: Southwest Bio-Labs, Inc. **Author:** Heird, C.E. **Address:** Southwest Bio-Labs, Inc. 401 N. 17th St., Suite 11 Las Cruces, NM	**Compliance with GLP:** Yes **If no, justification:**

Appendix 8

Reference:

Hubbard, P.M. and J.B. Beavers. 2013. Flunixin meglumine: An acute oral toxicity study with the northern bobwhite (*Colinus virginianus*) using a sequential testing procedure. Wildlife International Study no. 706-101, MAH Study No. S12312-00-FPR-SAF-OT. Wildlife International, Ltd., Easton, MD.

Title: Flunixin meglumine: An acute oral toxicity study with the northern bobwhite (*Colinus virginianus*) using a sequential testing procedure

Name of Company:	**Study number:**	**Report date:**
Merck Animal Health	MAH Study No. S12312-00-FPO-SAF-OT Wildlife Study No. 706-101	April 11, 2013

Study type: Avian acute oral toxicity test, OECD Guideline 223

Name of test material:	**Name of formulated product:**
Flunixin meglumine	Not applicable

Test design:

The test design used a sequential dosing procedure to optimize placement of doses and minimize the number of birds tested for animal welfare reasons. The study had three stages: Stage 1 consisted of one bird exposed to each of four doses of flunixin meglumine, expressed as the salt form (4.24 mg/kg to 212 mg/kg); Stage 2 consisted of 10 doses (139 to 1188 mg/kg), with one bird per dose; and Stage 3 consisted of two doses (191 and 579 mg/kg), with five birds per dose. Birds were dosed once with flunixin meglumine which was dissolved in deionized water, via oral intubation. The control group, consisting of five birds, received the same volume of water (4 mL/kg) as the dosed birds. Dosing solutions were collected and shipped to Merck Animal Health for analysis by HPLC.

The northern bobwhite were females, 37-43 weeks of age, and weighed from 184 to 235 grams at the time of dosing. They were acclimated to the facility for approximately 22 – 28 weeks and to the caging for approximately 3-9 weeks prior to the dosing of each stage. Birds were fasted for approximately 17-18 hours before each dosing.

Birds were housed individually and had access to feed and water *ad libitum*. Birds were observed at least twice daily for signs of toxicity for 21 days. Body weights were recorded at test initiation and on Days 3, 7, 14 and 21. Feed consumption was determined by pen for approximately 24-hour intervals from Day 0 to Day 1, Day 1 to Day 2, and Day 2 to Day 3. Average daily feed consumption was then determined from Day 3 to Day 7, from Day 7 to Day 14, and from Day 14 to Day 21. Gross necropsy was performed on each of the mortalities and all surviving birds at test termination. This included, but was not limited to, a general examination of the exterior of the bird and an examination of the thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastro-intestinal tract, and urogenital system.

Statistical analysis:

The mortality data from all stages was analyzed in order to calculate the LD_{50} along with 95% confidence intervals. Data were analyzed using the computer program SEDEC. The LD_{50} value for this study was calculated by probit analysis. The mortality data from all stages was analyzed in order to calculate a LD_5 value along with 95% confidence intervals. Data were analyzed using the computer program SAS. The LD_5 value for this study was calculated by probit analysis. The calculation of the LD_{50} value between the two programs varies slightly.

Summary of findings:

All dosing solutions met the acceptance limits of 85% to 115% of the nominal doses, except for one sample (dosage level 224 mg/kg), where the recovery of the active substance was determined to be 75% of nominal. There were no mortalities in the control group, and all control birds were normal in appearance and behavior throughout the test. Additionally, there were no mortalities in the 4.24, 15.6, 57.6, 139, 177, 212, 285 and 458 mg/kg treatment groups. There was 20% (1 of 5) mortality at the 191 mg/kg dosage level, 60% (3 of 5) mortality at the 579 mg/kg dosage level and 100% (1 of 1) mortality in the 224, 361, 582, 738, 936 and 1188 mg/kg dosage groups. All mortalities occurred within 4 days of dosing. Delayed onset mortality or toxicity was not identified in any dosed birds.

No signs of toxicity were observed for the birds at the 4.24, 15.6, 57.6, 139 and 177 mg/kg dosage levels. Birds at higher doses that survived yet showed signs of toxicity displayed the symptoms on the day of dosing, including

shallow and rapid respiration, ruffled appearance, lethargy, and a reduced reaction to external stimuli. These birds did not display these symptoms after the day of dosing and appeared normal for the duration of the test. There was a slight loss of mean body weight in surviving birds exposed to 458 and 579 mg/kg and some reduction in feed consumption, but these effects resolved by Day 3. Additional signs of toxicity (depression, prostrate posture, and convulsions) were only observed in birds that ultimately died. Gross necropsy findings indicative of acute toxicity were identified in organs such as the gastrointestinal tract, kidney, liver, heart, and spleen. Birds that died from exposure were found to have effects on the following organs: gastro-intestinal tract (hyperemia in the wall of the crop, primarily empty gastro-intestinal tract, brownish fluid or white chalk-like plaques in the abdominal cavity); kidneys (enlarged, pale, mottled), heart (pale heart, white chalk-like plaques in the pericardium); spleen (enlarged, white chalk-like plaques present); and liver (pale, friable). Not all mortalities exhibited all symptoms.

The LD_{50} for northern bobwhite was 375 mg/kg for flunixin meglumine. Adjusting to the dose of flunixin free acid using a conversion factor of 0.6, the acute oral LD_{50} for flunixin free acid was calculated to be 225 mg/kg, with a 95% confidence interval of 122 to 474 mg/kg. The LD_5 was 66 mg/kg flunixin free acid with a 95% confidence interval of 0.9 to 122 mg/kg.

Study conducted by: Wildlife International, Ltd.	**Compliance with GLP:**
Author:	Yes, except for analytical verification of doses.
Hubbard, P.M. and J.B. Beavers	**If no, justification:**
Address:	
8598 Commerce Drive Easton, MD	

Appendix 9

Reference:

Mertens, J.J.W.M. 1999. SCH 14714-NMG (Flunixin meglumine): A 90-day oral (gavage) toxicity study in dogs. Intervet study number: SN 98217.

Title:

SCH 14714-NMG (Flunixin meglumine): A 90-Day Oral (Gavage) Toxicity Study in Dogs

Name of Company:	**Study number:**	**Report date:**
Schering-Plough Animal Health Corporation	Schering-Plough No. 98217 WIL-97025	February 25, 1999

Study type: Repeated Dose Oral Toxicity Study – Non-rodent

Name of test material:	**Name of formulated product:**
SCH-14714 NMG (Flunixin meglumine)	Not applicable

Test design:

This study was conducted to define a no-observed-adverse-effect (NOAEL) level for orally administered SCH 14714-NMG (Flunixin meglumine) after 90-days of oral administration to beagle dogs. The test article in deionized water was administered orally by gavage once daily for 91-93 days to beagle dogs (5/sex/group) at concentrations of 0 (vehicle control), 0.01, 0.05, 0.15, 0.40 or 0.60 mg/kg/day (flunixin free acid dosage levels). All dogs were observed twice daily for mortality and moribundity. The homogeneity, stability, and concentration of the test material in the dosing formulations were analytically assessed. Clinical examinations were conducted twice daily, at the time of dosing (and immediately following dosing) and one to two hours following dosing. Detailed physical examinations were conducted weekly. Physiological parameters including body temperature, respiratory rate, heart rate and blood pressure were recorded once prior to the initiation of dosing and during weeks 5 and 12. Body weights were recorded at least weekly and food consumption was recorded daily and reported weekly. Clinical pathology evaluations (hematology, serum chemistry, and urinalysis) were performed on all animals twice prior to initiation of dosing and during weeks 4, 8 and 13. In addition, fecal samples for occult blood were collected at multiple time points prior to the initiation of dosing and during weeks 4, 8 and 12. Blood samples for toxicokinetic determinations were drawn from all animals at approximately 0.5, 1, 2, 4 and 24 hours post-dosing on the first day of dosing and on day 89. Ophthalmological examinations were conducted once prior to the initiation of dosing and during week 11. Electrocardiograms (ECGs) were performed once prior to the initiation of dosing and during weeks 5 and 12. Complete necropsies were performed on all animals and selected organs (adrenals, brain, epidydimides, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thymus, and thyroid) were weighed and examined microscopically.

Statistical analysis:

All analyses were conducted using two-tailed tests for minimum significance levels of 5% and 1% comparing the treated groups to the control group by sex. All means were presented with standard deviations and the number of sampling units used to calculate the mean. Statistical analyses were not performed if the number of animals was 2 or less. All statistical tests were performed by a Digital® Microvax® 2400 computer with appropriate programming. Physiological parameters, body weights, body weight changes, food consumption, clinical laboratory values (hematology, serum chemistry and urinalysis) and absolute and relative organ weight data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's Test. Clinical laboratory values for cell types that occur at a low incidence were not subjected to statistical analysis. Histopathology incidence data were evaluated using Fisher's Exact Test.

Summary of findings:

All animals survived the treatment period and no treatment related effects were reported in clinical signs, physiological parameters, body weights, body weight gain, food consumption, hematological parameters, serum chemistry, urinalysis, ophthalmological examinations, ECG evaluations, fecal occult blood analyses, macroscopic examination, absolute or relative organ weights, or histopathological findings in either sex at any dose level. Treatment with SCH 14714-NMG results in dose-related increases in plasma flunixin free-acid values; maximum measured concentrations occurred at 0.5 hours after dosing and level were below the level of quantification by 24 hours after dosing. Concentrations at each time point were similar between the initial dose and day 89, indicating that the repeated administration of the test material did not result in any accumulation. There was no gender differences between the values obtained. Mean plasma flunixin-free acid levels for males and females ranged from 0.0295 to 0.0390 µg/mL in the 0.01 mg/kg dose group, from 0.143-0.158 µg/mL in the 0.05 mg/kg dose group, from 0.424-0.527 µg/mL in the 0.15 mg/kg dose group, from 1.2 to 1.45 µg/mL in the 0.40 mg/kg dose group and from 1.97 to 2.23 µg/mL in the 0.60 mg/kg dose group. The NOAEL in this study was identified as the highest dose tested, 0.60 mg/kg flunixin free acid.

Study conducted by: Wil Research Laboratories, Inc.	**Compliance with GLP:**
Author:	Yes
Mertens, J.J.W.M.	**If no, justification:**
Address:	
WIL Research Laboratories, Inc. 1407 George Road Ashland, OH	

Appendix 10

Reference:

Schieber, T., Lechtenberg, K., and Paalangara, R. 2014. Flunixin Transdermal Solution: A Pivotal Study to Determine the Concentration of Flunixin in Cattle Hair Over a Seven Day Period After a Single Dermal Application at the Therapeutic Dose. Sponsor Reference No. S14002-00

Title:

Flunixin Transdermal Solution: A Pivotal Study to Determine the Concentration of Flunixin in Cattle Hair Over a Seven Day Period After a Single Dermal Application at the Therapeutic Dose

Name of Company:	**Study number:**	**Report date:**
Intervet Inc (d/b/a Merck Animal Health)	S14002-00	2014

Study type: Residue determination study in cattle hair

Name of test material:	**Name of formulated product:**
Flunixin Transdermal Solution (FTS) also known as Flunixin Pour-On (FPO)	5% w/v (50 mg/mL of Flunixin free acid) FTS Solution for pour-on administration (as intended for commercialization).

Test design:

Two treatment groups of Hereford breed beef cattle were used. Group 1, with 6 males and 6 females, was used to determine the concentration of flunixin free acid in (FFA) cattle hair over a seven day period after dosing with a single dermal application of Flunixin Pour On (FPO). Group 2 (2 males and 2 females) were not dosed but were used for the determination of hair density. Cattle in Group 1 were dosed on Day 0, with the therapeutic dose of 3.3 mg FFA/kg body weight, along the dorsal midline. Flunixin hair concentrations were monitored at 1, 3, 6, 12, 24, 48, 72 and 168 hours post dose. The treatment area on the back of the cattle was divided into three approximately equal zones (A, B, and C). Hair samples were placed into pre-weighed, labelled centrifuge tubes and were weighed again after sample collection to calculate the weight of each hair sample. The hair samples were analyzed using LC/MS-MS in order to quantify the amount of flunixin (as free acid) in the cattle hair.

The extent of spread of FPO at the treatment site was also measured at 1, 3, 6 and 12 hours post dose. Before dosing, anatomical measurements were made on the dorsal aspect of 14 cattle (7 males and 7 females). Additionally, the density of hair along the back of the cattle was measured in the 4 cattle in Group 2 by clipping the entire hair in an area of 800 cm^2 (80 cm x 10 cm) along the dorsal mid-line of each animal. The clipped hair was then weighed and the density was subsequently calculated in mg/cm^2.

Statistical analysis:

Statistical analysis was not conducted for this study. Only mean and standard deviations of the flunixin concentrations in the hair samples for all animals at each sampling time point were calculated and reported. Likewise, the mean and standard deviations of the spread of FPO at the treatment site, measurement of the anatomical dimensions on the dorsal aspect of animals and the density of hair on the dorsal aspect were calculated and reported.

Summary of findings:

Relatively high levels of flunixin were detected in hair samples up to 12 hours post dose. The concentration of FFA seemed to peak at 3 hours post dose. By 24 hours post dose the flunixin levels in hair samples showed a significant decline and levels were about 50% less than what was detected in the first 12 hours post dose. At 48 and 72 hours post dose, the flunixin levels in the hair sample showed a steady decline. By 7 days post dose the FFA levels in hair samples had dropped significantly and the levels were approximately 6-8% of that detected during the first 12 hours post dose. Mean (n = 12) FFA concentrations in hair at 1, 3, 6, 12, 24, 48, 72 and 168 hours post dose were 10005, 13812, 12629, 11012, 5784, 3630, 2446, and 800 µg/g, respectively. Concentrations were highest in Zone C.

The area of spread of FPO appears to peak at 3 hours post dose and decreased thereafter. The mean area of spread (cm^2) at 1, 3, 6 and 12 hours post dose was reported as 877.54, 1199.45, 889.15 and 703.24, respectively.

The mean anatomical measurements were reported as follows: distance between the base of the horns = 16.5 cm; distance between the base of the horns and shoulder joint = 61.4 cm; distance between shoulder joint and the ischium = 118.8 cm; distance between the left and right tuber coxae = 37.9 cm; distance between the left and

right ischium = 26.6 cm. The mean (n = 4) density of cattle hair along the back of the cattle was 14.43 mg/cm^2.	
Study conducted by: In-Life: Midwest Veterinary Services, Inc., 1290 County Road M, Suite A, Oakland NE 68045 Bio-Analytical: Intervet Inc. (d/b/a Merck Animal Health), 181 Passaic Avenue, Summit, NJ 07901	**Compliance with GLP:** Yes, with some exceptions regarding test article manufacture, characterization, and stability as well as feed and water analyses **If no, justification:**

Appendix 11

Reference:

Sczesny, S. 2015. Determination of the amount of wet cattle hair fitting into a 6 mL volume (representative of the volume of solid matter content in a magpie gizzard) and determination of its dry weight. Study Number: 115282-00. 02 November 2015.

Title:

Determination of the amount of wet cattle hair fitting into a 6 mL volume (representative of the volume of solid matter content in a magpie gizzard) and determination of its dry weight.

Name of Company:	**Study number:**	**Report date:**
MSD Animal Health Innovation GmbH	S15282-00	02 November 2015

Study type: Study to determine weight of cattle hair potentially ingested

Name of test material:	**Name of formulated product:**
Not applicable	Not applicable

Test design:

Holstein Friesian black pied male cattle were brushed along the backline and upper flanks with a curry comb, avoiding dirty spots, to collect hair. Hair was immersed in tap water. Dripping hair was placed into a 20 mL syringe. The plunger was pushed down at maximum force to release excess water. The plunger was released to allow manually compressed hair to expand slightly, releasing residual stress. The volume of hair following release of residual stress was intended to be 6 mL. Hair was removed or added as needed to attain a 6 mL volume. This hair, equaling a volume of 6 mL, was removed from the syringe and weighed to determine the weight of wet hair. The hair was then dried in a drying chamber overnight at approximately 27 °C and weighed again to determine the weight of dry hair.

The process described above was repeated in order to determine weights for a total of 10 replicates of hair.

Statistical analysis:

Mean, standard deviations, and the upper 90th percentile confidence bound on the mean for the weights of the 10 hair samples were calculated.

Summary of findings:

The weight of wet hair that fits into a 6 mL volume ranged from 0.957 g to 1.777 g. The arithmetic mean for the wetted hair weight was 1.224 g, the standard deviation 0.246 g. The upper 90th percentile confidence bound on the mean of the weight of wetted hair was 1.331g.

The weight of oven-dried hair that fits into a 6 mL volume ranged from 0.477 g to 0.804 g. The arithmetic mean for the oven-dried hair was 0.618 g, the standard deviation 0.101 g. The upper 90th percentile confidence bound on the mean of the oven-dried hair was 0.663 g.

Study conducted by:	**Compliance with GLP:**
MSD Animal Health Innovation GmbH, Zur Propstei, 55270 Schwabenheim, Germany	No, but conducted in GLP-compliant facility **If no, justification:** Not necessary for purposes of study per consultation with CVM

Appendix 12

Reference:

Slepetys, R.M, D.G. Campbell, and R. Lobell. 1985. Preliminary oral safety study of flunixin meglumine in broiler chickens. Intervet study number: A-1-84. Research Report Number A-18723.

Title:

Preliminary Oral Safety Study of Flunixin Meglumine in Broiler Chickens

Name of Company:	**Study number:**	**Report date:**
Schering Corporation	A-1-84	August 15, 1985

Study type: Toxicity to Broiler Chickens

Name of test material:	**Name of formulated product:**
SCH-14714 NMG (Flunixin meglumine)	Not applicable

Test design:

This study was conducted to determine the toxicological effects of flunixin meglumine when administered orally to broiler chickens at elevated levels for extended time periods. The test article in distilled water was administered orally by gavage once daily for 2, 4, 7, or 9 days to six week old White Mountain Cross broiler-type chickens (weighing 1.064 – 1.559 kg) at concentrations of 0 (vehicle control), 0.5, 1.5, or 2.5 mg/lb BW/day. Ten birds of each sex were placed into Group 1 (vehicle control), Group 2 (2.5 mg/lb), Group 3 (1.5 mg/lb) and Group 4 (0.5 mg/lb). All treatments were administered once daily, for up to 9 days of treatment. All chickens were observed for overt toxicity including mortality, moribundity, feather loss, fecal consistency, and appearance/posture. Food and water consumption were measured. The homogeneity, stability, and concentration of the test material in the dosing formulations were not analytically assessed. Hematology and serum chemistry evaluations were performed. Body weights were measured twice pre-treatment and at necropsy. Gross necropsies were performed on all animals and selected organs were weighed.

Statistical analysis:

Parameters were evaluated for biological and toxicological rather than statistical significance.

Summary of findings:

All animals survived the treatment period. Post-treatment feather loss incidences were noted in the 0.5 and 2.5 mg/lb dose groups. Loose feces were noted only in treated animals, with the greatest and most persistent incidence in the 2.5 mg/lb dose group. No treatment related effects were reported in body weights, food consumption, water consumption, hematological evaluations, serum chemistry evaluations, or organ weights either sex at any dose level. Treatment with SCH 14714-NMG resulted in petechiation and yellow mottling of the liver and pulmonary congestion in the treated animals, with sporadic incidences of pale thyroids and isolated incidences of hepatic hyperemia and enlargement. All changes were limited to treated animals. No dose-response was noted and all gross pathology findings occurred late in therapy.

Study conducted by: Schering Corporation	**Compliance with GLP:**
Author:	No
Slepetys, R.M., D.G. Campbell, and R. Lobell	**If no, justification:**
Address:	Study for informational purposes
Schering Corporation, Animal Health Division Animal Health Research Center Allentown, N.J.	

Appendix 13

Reference:

Slepetys, R.M. and D.G. Campbell. 1986. Flunixin meglumine oral toxic syndrome study in broiler chickens. Intervet study number: A-3-84. Research Report Number: A-19051.

Title:

Flunixin Meglumine Oral Toxic Syndrome Study in Broiler Chickens

Name of Company:	**Study number:**	**Report date:**
Schering Corporation	A-3-84 Research Report No. A-19051	March 14, 1986

Study type: Toxicity to Broiler Chickens

Name of test material:	**Name of formulated product:**
SCH-14714 NMG (Flunixin meglumine)	Not applicable

Test design:

This study was conducted to determine the toxicological effects of flunixin meglumine when administered orally to broiler chickens. The test article in distilled water was administered once orally by gavage White Mountain Cross broiler-type chickens (0.538 – 0.892 kg) at concentrations of 0 (vehicle control), 22, 44, or 66 mg/kg flunixin meglumine. Twelve birds (male and female) were dosed; the vehicle control group consisted of 5 male and 3 female birds. All chickens were observed for overt toxicity for 9 days post-treatment. Food and water consumption were measured. The homogeneity, stability, and concentration of the test material in the dosing formulations were not analytically assessed. Hematology and serum chemistry evaluations were performed. Body weights were measured initially, at dosing and termination. Gross necropsies and histopathology were performed on all animals and selected organs (adrenals, brain, pituitary, gonads, heart, kidneys, liver, spleen and thyroid/parathyroid) were weighed.

Statistical analysis:

Parameters were evaluated for biological and toxicological rather than statistical significance.

Summary of findings:

At 24 hours post-treatment, two birds in the 22 mg/kg dose group and 3 birds in the 44 and 66 mg/kg dose groups were found dead or died. At 48 hours, one bird at 22 mg/kg, and 1 bird in the 44 mg/kg dose groups were found dead. At 72 hours, one bird in the 66 mg/kg dose group died during sampling. There were no survivors in the 44 mg/kg dose group. In the 22 and 66 mg/kg treatment groups, birds appeared depressed through study termination. All treated groups displayed anorexia relative to the control group 24 hours post-treatment. Dose-related food consumption continued to decrease through 48 hours post-treatment in the 22 and 66 mg/kg treatment groups. At 72 hours, anorexia was reversed in the surviving birds. No treatment related effects were reported in body weights, food consumption, water consumption, hematological evaluations in either sex at any dose level. Treatment with SCH 14714-NMG resulted in increased in CPK levels at 22 mg/kg at 24 and 72 hours. Birds displayed elevated SGPT and LDH levels at 72 hours post-treatment. Organ weight changes were limited to the kidneys and liver, without dose-response effects. White filmy adhesions in the abdominal cavity and on the liver were the most notable changes noticed in gross pathology. White foci also were found on the abdominal fat, lungs, kidney, heart and pericardium. Lungs contained fluid, displayed congestion, edema, and pleuritis and were friable in texture. Kidneys were enlarged, discolored, friable, had accentuated tubules and displayed nephritis. Livers were enlarged and congested. Histopathology was characterized by inflammation, necrosis, and changes in the bone and marrow. Changes were similar for all groups and were most evident 24 hours after treatment.

Study conducted by: Schering Corporation	**Compliance with GLP:**
Author:	No
Slepetys, R.M. and D.G. Campbell	**If no, justification:**
Address:	Study for informational purposes
Schering Corporation, Schering Animal Health Animal Health Research Center Allentown, N.J.	

Appendix 14

Reference:

Smedley, J.W. 2013. Flunixin Transdermal Solution (SCH14714): An Acute Toxicity Study by Oral Gavage in Rats. Charles River Study No. 20030770; Intervet Reference No. S12206-00-FPO-SAF-OT, Charles River Laboratories, Spencerville, OH.

Title:

Flunixin Transdermal Solution (SCH14714):Acute Oral Toxicity of SCH 14714 in Rats

Name of Company:	**Study number:**	**Report date:**
Intervet Inc. (d/b/a Merck Animal Health)	Sponsor Ref. No. S12206-00-FPO-SAF-OT Testing Facility Study No. 20030770	February 13, 2013

Study type: Acute Toxicity by Oral Gavage in Rats (OECD 425)

Name of test material:	**Name of formulated product:**
Flunixin Transdermal Solution	Flunixin Transdermal Solution, tested at 5% FFA w/v

Test design:

The toxicity of a single oral dose of SCH 14714 to female rats was investigated. The study was initiated with a limit test at 2000 mg/kg body weight and followed by and an up/down study phase, initiated at 550 mg/kg body weight. The dose progression for the up/down phase was based on the OECD 425 Acute Oral Toxicity Statistical Program with an approximate 3.2 multiplier dose progression. Thus, the dose level selected for evaluation in the up/down phase was either 550 mg/kg or 2000 mg/kg. A single female rat was dosed at each evaluation point. The dose selection progression (increased/decreased/remained the same) for each evaluation point in the up/down phase was based on the outcome (death vs survival) of the previous dose evaluated. The up/down phase of the study was terminated when five reversals were observed among six tests. The following parameters and end points were evaluated in this study: mortality, moribundity, clinical signs, body weights, and gross necropsy.

Statistical analysis:

After each dose level was conducted, the short-term and long-term outcome/results were input into the AOT 425 StatPgm. The LD50 was estimated based on the 1 dose with a partial response.

Summary of findings:

In the limit test group, mortality occurred in 2/3 rats dosed at 2000 mg/kg. Clinical signs noted during the limit test included reduced fecal output, rough coat, unkempt appearance, urine stain, hunched posture, and piloerection. Necropsy findings in rats from the limit test group included abnormal content of the entire gastrointestinal tract and adhesions within the abdominal cavity involving the uterine horn and the intestine.

In the up/down phase, no mortality was observed in the three rats dosed at 550 mg/kg. Clinical signs observed in the 550 mg/kg rats from the up/down phase included piloerection, hypothermia, hunched posture, ocular discharge, and small feces. No significant gross necropsy findings were observed in the 550 mg/kg rats at study termination.

Three of the four rats dosed with the test article at 2000 mg/kg during the up/down phase had to be either euthanized moribund or died of acute toxicity by Day 6 of the study. Clinical signs observed in the 2000 mg/kg rats from the up/down phase included thin appearance, hunched posture, piloerection, hypothermia, partial palpebral closure, decreased activity, rough coat, urine stain, and small/few feces. Gross necropsy findings observed in the 2000 mg/kg animals that died or were euthanized moribund from the up/down phase included abnormal content of the gastrointestinal tract and adhesions within the abdominal cavity involving the uterine horn, colon, and small intestine.

An acute oral LD50 of 2000 mg/kg Flunixin Transdermal Solution in the female rat was estimated in the study.

The 95% confidence interval ranged between 614.6 to 5110 mg/kg.	
Study conducted by: Charles River Laboratories, Preclinical Services, Ohio **Author:** Smedley, J.W. **Address:** 640 North Elizabeth Street Spencerville, OH 45887	**Compliance with GLP:** Yes **If no, justification:**

Appendix 15

Reference:

Vincent, W.R. 1990. Environmental assessment report flunixin

Title:

Environmental Assessment Report Flunixin

Name of Company:	**Study number:**	**Report (Testing) date:**
Schering-Plough Corporation	Not Available	August 30, 1989 (dissociation constant) June 11, 1990 (water solubility) June 15, 1990 (partition coefficient) June 21, 1990 (UV Spectra) June 25, 1990 (melting temperature and density)

Study type: Environmental physico-chemical properties

Name of test material:	**Name of formulated product:**
SCH-14714 (Flunixin); CAS# 38677-85-9	Not applicable

Test design:

This report included summaries of assays for water solubility, dissociation constant, partition coefficient, UV spectra, melting point and density. The test material was SCH 14714 (Flunixin) and the batch number utilized for all assays was 17490-073 and the purity was >99%. The assay for water solubility was performed using the undersaturation/oversaturation method (Method 3.01.IV.B, Environmental Assessment Technical Assistance Handbook, March 1987) at 23±1ºC by HPLC Assay. The dissociation constant assay was performed using the UV/Visible spectrophotometry method (Method 3.04.IV.B, Environmental Assessment Technical Assistance Handbook, March 1987) at 23±1ºC. The partition coefficient assay was performed using the shake flask method (Method 3.02.IV, Environmental Assessment Technical Assistance Handbook, March 1987) at 23±1ºC by HPLC Assay. UV/Visible Absorption Spectra were determined by UV/Visible Spectrophotometry with cell pathlength 1cm at 23±1ºC in aqueous solutions containing 1% methanol (Method 3.05.IV.B, Environmental Assessment Technical Assistance Handbook, March 1987). Melting Temperature was assessed utilizing the Markley-Hershberg modified Thiele tube method (Method 3.06/III.B.8, Environmental Assessment Technical Assistance Handbook, March 1987). Density was assessed utilizing the wide-mouth, thermometer-stoppered pycnometer method at 23±1ºC (Method 3.07/III.B.3.b, Environmental Assessment Technical Assistance Handbook, March 1987).

Statistical analysis:

Least squares regression analysis was utilized for the dissociation constant assay.

Calculation of standard deviation was performed on all endpoints.

Summary of findings:

Water solubility:

pH 5: 0.0081 ± 0.0002 mg/mL
pH 7: 1.13 ± 0.02 mg/mL
pH 9: 16.5 ± 0.7 mg/mL

Dissociation constant:

$K_a = 2.50 \pm 0.15 \times 10^{-6}$
$Pk_a = 5.60 \pm 0.03$

Partition coefficient:

pH 5: 400 ± 100
pH 7: 22 ± 1
pH 9: 6.3 ± 0.4

Ultraviolet-Visible Absorption Spectra:

pH 2: Wavelength 252 nm; Average Molar Absorptivity = $1.46 \times 10^4 \pm 40$
pH 2: Wavelength 326 nm; Average Molar Absorptivity = $5.73 \times 10^4 \pm 6$
pH 7: Wavelength 282 nm; Average Molar Absorptivity = $1.32 \times 10^4 \pm 25$
pH 12: Wavelength 282 nm; Average Molar Absorptivity = $1.34 \times 10^4 \pm 40$

Melting Temperature:

226 to 228 ºC

Density:

1.48 ± 0.01 mg/cm^3

Study conducted by: Schering-Plough Corporation	**Compliance with GLP:**
Author:	No
Vincent, W.R.	**If no, justification:**
Address:	Summary of individual studies
Schering-Plough Corporation Kenilworth, N.J.	