Public Release Summary

Australian Pesticides & Veterinary Medicines Authority

Draxxin injectable solution

APVMA Product Number 59304

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The APVMA invites comments on this PRS until 6 July 2007. Submissions should be sent to:

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FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for assessing and approving agricultural and veterinary chemical products prior to their sale and use in Australia.

In undertaking this task, the APVMA works in close cooperation with advisory agencies, including the Office of Chemical Safety within the Department of Health and Ageing, Department of Environment and Water Resources (DEW), and State departments of agriculture or primary industries.

The APVMA has a policy of encouraging openness and transparency in its activities and of seeking community involvement in decision making. Part of that process is the publication of public release summaries for all products containing new active ingredients and for all proposed extensions of use for existing products.

The information and technical data required by the APVMA to assess the safety of new chemical products and the methods of assessment must be undertaken according to accepted scientific principles. Details are outlined in the APVMA's publication the *Manual of Requirements and Guidelines for Veterinary Chemicals* (Vet MORAG).

This Public Release Summary is intended as a brief overview of the assessment that has been completed by the APVMA and its advisory agencies. It has been deliberately presented in a manner that is likely to be informative to the widest possible audience thereby encouraging public comment.

The APVMA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to the Veterinary Medicines Program Manager, Australian Pesticides and Veterinary Medicines Authority, PO Box E240, Kingston ACT 2604.

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LIST OF ABBREVIATIONS AND ACRONYMS

ac	active constituent
ADI	Acceptable Daily Intake (for humans)
AchE	acetylcholinesterase
ALAT	alanine aminotransferase
AM	arithmetic mean
AP	alkaline phosphatase
ARfD	Acute Reference Dose
BCF	bioconcentration factor
bw	bodyweight
BZ	benzimadazole
CAS	Chemical Abstracts Service
ChE	cholinesterase
cm	centimetre
CXL	Codex maximum residue limits
d	day
DAT	days after treatment
DEW	Department Environment and Water Resources
DMW	dilute mineral water
E_bC_{50}	concentration at which the biomass of 50% of the test population is impacted
EC_{50}	concentration at which 50% of the test population are immobilised
EEC	estimated environmental concentration
E_rC_{50}	concentration at which the rate of growth of 50% of the test population is impacted
ESI	export slaughter interval
EUP	end use product
F_0	original parent generation
F_1	first generation
g	gram
GAP	Good Agricultural Practice
GCP	Good Clinical Practice
GC-MS	gas chromatography –mass spectometry
GLP	Good Laboratory Practice
GM	geometric mean
GMP	Good Manufacturing Practice
GVP	Good Veterinary Practice
h	hour
ha	hectare
Hb	haemoglobin
HDPE	high density polyethylene

HPLC	high pressure liquid chromatography *or* high performance liquid chromatography
id	intradermal
im	intramuscular
ip	intraperitoneal
IPM	Integrated Pest Management
iv	intravenous
in vitro	outside the living body and in an artificial environment
in vivo	inside the living body of a plant or animal
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_{ow}	octanol water partitioning coefficient
L	litre
LC_{50}	concentration that kills 50% of the test population of organisms
LD_{50}	dosage of chemical that kills 50% of the test population of organisms
LEV	levamisole
LOD	Limit of Detection – level at which residues can be detected
LOQ	Limit of Quantification – level at which residues can be quantified
meq	millequivalent
mg	milligram
mL	millilitre
ML	lega litre / macrocyclic lactone
MRL	Maximum Residue Limit
MSDS	Material Safety Data Sheet
NDPSC	National Drugs and Poisons Schedule Committee
NESTI	National Estimated Short Term Intake
ng	nanogram
NHMRC	National Health and Medical Research Council
NOEC/NOEL	no observable effect concentration level
OECD	Organisation for Economic Co-operation and Development
po	oral
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
Q-value	quotient-value
RBC	red blood cell count
s	second
sc	subcutaneous
SC	suspension concentrate
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
TGAC	technical grade active constituent

T-Value	a value used to determine the First Aid Instructions for chemical products that contain two or more poisons
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
µg	microgram
vmd	volume median diameter
WHP	withholding period

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1. INTRODUCTION

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has before it an application from Pfizer Animal Health, a division of Pfizer Australia Pty Ltd for registration of a new product containing the active constituent tulathromycin. The product is **Draxxin Injectable Solution**.

Tulathromycin is a semi-synthetic, broad spectrum, triamilide antimicrobial agent that belongs to the macrolide group of antibiotics. It acts by selectively binding to 23S ribosomal RNA, which leads to a disruption of bacterial protein synthesis.

Draxxin Injectable Solution is a sterile preparation for injection that contains 100 mg/mL tulathromycin. The product will be formulated and packaged in 50 and 100 mL containers in France.

The proposed use is for the treatment of bovine respiratory disease caused by *Mannheimia haemolytica* and *Pasteurella multocida* in cattle, and swine respiratory disease caused by *Mycoplasma hyopneumoniae* and *Pasteurella multocida* in pigs.

A dose rate of 2.5 mg tulathromycin/kg bodyweight is proposed as a single subcutaneous injection in cattle and as a single intramuscular injection in pigs.

Draxxin Injectable Solution is currently registered in 25 member states in the European Union, USA, Canada, Switzerland, Bulgaria, Croatia, Romania, Turkey, Ukraine, Ecuador, Mexico, Peru, Argentina, Chile, Venezuela, Korea, Vietnam and the Philippines. An application in Japan is being assessed.

This publication provides a summary of data reviewed and an outline of the regulatory considerations for the proposed registration of **Draxxin Injectable Solution**.

The APVMA seeks public comment on the product outlined in this document prior to the antibiotic being registered for use in Australia. The APVMA will consider all responses received during the public consultation period in deciding whether the product should be registered and in determining conditions of registration and product labelling.

Written comments should be received by the APVMA by 3 July 2007 and addressed to:

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2. CHEMISTRY AND MANUFACTURE

2.1. Active constituent

Tulathromycin and has the following properties:

Common name (ISO): Tulathromycin

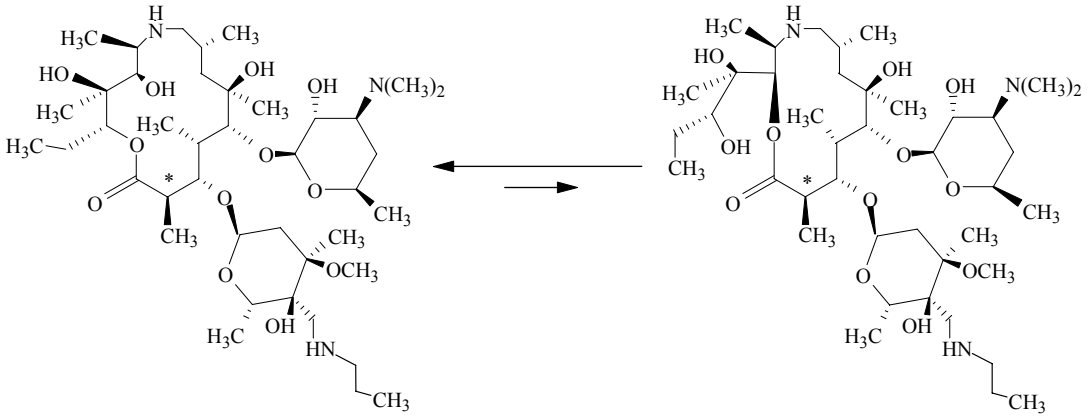
Chemical name: A combination of 2 isomers –

2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-dieoxy-3-C-methyl-3-O-methyl-4-C-[(propylamino)methyl]-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one (CAS) (A; CP472,295)

and

2R,3R,4R,5R,8R,10S,11S,12R)-11-[[2,6-dieoxy-3-C-methyl-3-O-methyl-4-C-[(propylamino)methyl]-α-L-ribo-hexopyranosyl]oxy]-2-[(1R,2R)-1,2-dihydroxyl-1-methylbutyl]-8-hydroxy-3,6,8,10,12-pentamethyl-9-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-4-azacyclotridecan-13-one (CAS) (B; CP547,272)

Product name:	Draxxin Injectable Solution
CAS Registry Number:	217500-96-4 and 280755-12-6
Empirical formula:	$C_{41}H_{79}N_3O_{12}$ for each isomer
Molecular weight:	806.23 for each isomer
Physical form:	White to off-white solid
Colour:	
Odour:	
Melting point:	190-192^{o}C
Density:	
Octanol/water partition:	
coefficient (K_{ow}):	
Vapour pressure at 25^{o}C:	
Structural formula:	See next page.



A (CP-472,295) B (CP-547,272)

The Chemistry and Residues Program (CRP) of the APVMA has evaluated the chemistry aspects of tulathromycin (manufacturing process, quality control procedures, batch analysis results and analytical methods).

Tulathromycin is a new active constituent and there is no compendial specification available. On the basis of the data provided, it is proposed to establish the following Active Constituent Standard for tulathromycin:

Constituent	Specification	Level
Tulathromycin	Tulathromycin	Not less than 940 g/kg (anhydrous, solvent free basis)

2.2. Chemistry of the product

Name: Draxxin Injectable Solution
Formulation type: Sterile solution for injection
Concentration of active constituents: 100 mg/mL tulathromycin

2.2.1. Physical and chemical properties of the product

Appearance: Clear, colourless to slightly yellow solution
Bulk density:
pH: 5.1 – 5.7

2.2.2. Storage and stability

The applicant provided the results of real time stability testing conducted using samples stored in the proposed commercial container. The results indicate that the formulated product is expected to be stable for three years when stored at room temperature in the proposed commercial packaging.

2.2.3. Packaging

Draxxin Injectable Antibiotic Solution will be packaged in glass vials. The packaging is not adversely affected by the product, nor is the product unstable in the packaging.

2.2.4. Recommendation

The APVMA has evaluated the chemistry and manufacturing aspects of Draxxin Injectable Antibiotic Solution and is satisfied that the data provided support the application of registration. CRP is satisfied that the chemistry requirements of Section 14 (5) of the Agricultural and Veterinary Code Act 1994 have been met.

3. TOXICOLOGICAL ASSESSMENT

3.1. Evaluation of Toxicity

Tulathromycin is a semi-synthetic macrolide antimicrobial. Tulathromycin and other macrolide antibiotics (eg. erythromycin, clarithromycin) inhibit bacterial protein synthesis resulting in bacterial death. The applicant submitted 45 studies to support the establishment of an Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD). A poison schedule for tulathromycin was also required.

3.1.1. Acute studies

Tulathromycin was of low acute oral toxicity in rats (LD50>2000 mg/kg bw) and dogs (LD50 >1000 mg/kg bw), and low dermal acute toxicity in rabbits (LD50>2000 mg/kg bw). It is a severe eye irritant in rabbits and a skin sensitiser in guinea pigs by the Maximisation test, but is not a skin irritant in rabbits.

The toxicity of Draxxin Injectable Solution was estimated based on the known toxicity of individual ingredients in the product. The oral LD50 and dermal LD50 toxicity of the product is estimated to be very low and low. The product is a mild skin irritant and a moderate eye irritant.

3.1.2. Short-term repeat-dose studies

Rats were treated once daily with tulathromycin at 55, 220 or 550 mg/kg bw/d by oral gavage for 10 consecutive days. There were no mortalities during the study. Treatment-related clinical signs were limited to the 550 mg/kg bw/d group and consisted of salivation in males and females, and abdominal distension in all rats. At necropsy, enlarged ceca was observed in both sexes at the 220 mg/kg bw/d dose and in all animals treated with 550 mg/kg bw/d. Mean food consumption was lower for male rats in the 550 mg/kg bw/d group with no apparent effect on bodyweight. Significant increases in mean neutrophil and monocyte counts at 550 mg/kg bw/d, and increased monocytes at 220 mg/kg bw/d were observed in both sexes. In females, monocyte counts increased significantly at 50 mg/kg bw/d, and mean eosinophil counts at the 220 and 550 mg/kg bw/d dose groups. Dose-related increases in serum chemistry parameters (ALT and AST) were noted at the two highest doses. All mean relative liver weights of treated animals were less than the controls, reaching significance in both sexes at doses ≥220 mg/kg bw/d and at doses ≥55 mg/kg bw/d in males. The NOEL for this study was 55 mg/kg bw/d.

In a second study, tulathromycin was administered by oral gavage to rats at dose levels of 10, 50 or 200 mg/kg bw/d for 29-30 consecutive days. Treatment-related mortalities, clinical signs, and effects on bodyweights, food consumption or urinalysis endpoints were not noted during the study. Mean monocyte and eosinophil counts elevated in both sexes in the 200 mg/kg bw/d group. Monocytes and eosinophils increased in males at 50 mg/kg bw/d. Significant increases in mean ALT and AST values in males at 200 mg/kg bw/d and AST at 50.0 mg/kg bw/d were observed. AST levels increased significantly in females at 50 and 200 mg/kg bw/d. A significant decrease in mean relative liver weights

occurred in males at 200 mg/kg bw/d. A NOEL of 50 mg/kg bw/d was established in this study.

Beagle dogs were administered tulathromycin by oral gavage at dose levels of 5, 15 or 50 mg/kg bw/d for 1 month. Following treatment, treatment-related mortalities, and effects on bodyweights, food consumption, physical examinations, vital signs, blood pressure or ocular, haematology, urinalysis or electrocardiogram endpoints were not observed during the study. There was a treatment-related increase in the number of animals with loose stools at the high dose. Significant increases in mean ALT and AST levels were noted in males from the 50 mg/kg bw/d and elevated AST levels in males at 15 mg/kg bw/d. Mean kidney weights increased in females dosed with 50 mg/kg bw/d. The NOEL for this study was 15 mg/kg bw/d.

3.1.3. Subchronic studies

Rats were dosed with tulathromycin by oral gavage at 5, 15 or 100 mg/kg bw/d for 3 consecutive months. There were no treatment-related mortalities during the study. Treatment-related effects on bodyweights, food consumption, organ weights or urinalysis parameters were not observed. Mean haemoglobin, hematocrit and mean cell haemoglobin increased in males only at 100 mg/kg bw/d. In females, increases in neutrophils and monocytes were noted at 100 mg/kg bw/d. Levels of AST in males increased at 15.0 and 100 mg/kg bw/d. Decreased levels of bile acids, serum total protein, calcium, albumin and globulin were noted in males at 100 mg/kg bw/d. Similar decreases were noted at 15 mg/kg bw/d for total protein, calcium and globulin values. In females, increased levels of ALT, AST and sorbitol dehydrogenase were observed at 100 mg/kg bw/d. At the same dose, decreased levels of gamma-glutamyltransferase and globulin were noted compared. The NOEL in this study was 15 mg/kg bw/d.

Tulathromycin was administered by oral gavage to dogs at doses of 5.7, 17.0 and 56.7 mg/kg bw/d for 3 consecutive months. There were no treatment-related mortalities during the study. Treatment-related effects on bodyweights, food consumption, physical examinations, vital signs, blood or ocular pressure, haematology, urinalysis or electrocardiogram endpoints were not observed following treatment. There was a treatment-related increase in the number of animals with loose stools. Mean ALT and AST values significantly increased in both sexes at 56.7 mg/kg bw/d by day 29 and continued throughout treatment. In females, mean AST values were also elevated at 17 mg/kg bw/d. Multiple small, focal, unilateral, silver foci were noted ophthalmicroscopically near the tapetal junction of the retina in 2 dogs at 17 mg/kg bw/d, but not in the control, 5.7 or 56.7 mg/kg bw/d groups. Spontaneous retinal detachments are rare for dogs, thus it was not possible to discount this effect as unrelated to treatment. Based on the significant increases in ALT/AST levels in both sexes at 56.7 mg/kg bw/d, a NOEL of 17 mg/kg bw/d was established in this study.

3.1.4. Chronic studies

Dogs received tulathromycin by oral gavage at doses of 2, 5 or 25 mg/kg bw/d for 12 consecutive months. There were no treatment-related mortalities during the study. Increased salivation was seen in the 5 and 25 mg/kg bw/d treatment groups after day 110 and continued throughout the study, with greater incidences noted towards the end of the study. No treatment-related effects were noted on bodyweights, food consumption, ophthalmological alterations, physical examinations, vital signs, blood pressure, haematology, urinalysis and electrocardiogram endpoints. Mean ALT and AST levels increased in females only from the 25 mg/kg bw/d group from day 85 of treatment through to the end of the study. There was an increase in the mean absolute and relative testicular weights in the 25 mg/kg bw/d males. Possible treatment-related microscopic alterations were observed in both sexes at 5 and 25 mg/kg bw/d and included infiltration/periarteritis of the heart, testicular tubular hypoplasia and monocellular infiltration of the skeletal muscle.

Based upon the significant increased levels of AST and ALT in females, and in males increased relative testicular weight at 25 mg/kg bw/d, a NOEL of 5 mg/kg bw/d was established in this study.

3.1.5. Reproduction Studies

Tulathromycin was administered by oral gavage once daily at doses of 15, 50 or 100 mg/kg bw/d for 70 consecutive days prior to mating to three groups of F_0 and F_1 parental rats. There were no treatment-related mortalities or clinical findings observed in the F_0 and F_1 generations. Reproductive parameters (fertility, days between pairing and coitus, gestation, parturition and oestrous cyclicity) were unaffected by treatment. The mean live litter size, number of pups born, pup survival and pup sex ratio at birth were unaffected by treatment at all parental doses in the F_1 and F_2 generations.

Mean bodyweight gains in F_0 and F_1 males were significantly reduced in the 100 mg/kg bw/d group that correlated with a reduction in food consumption. Mean bodyweight gains were also reduced in F_1 females during gestation at the same dose. In F_0 males, the mean AST levels were significantly increased at 50 and 100 mg/kg bw/d. Reductions in mean urea nitrogen and total protein were noted at week 9 and 18 in both F_0 sexes at the 100 mg/kg bw/d, and some incidences at 50 mg/kg bw/d.

Mean absolute and relative liver weights were reduced in both F_0 sexes in all treatment groups. In F_1 rats, mean liver weights were significantly reduced in both sexes at 100 mg/kg bw/d, and in males at 50 mg/kg bw/d. Mean relative liver weights were also significantly reduced in males in the 15 mg/kg bw/d group and in females in the 50 mg/kg bw/d group, While mean absolute and relative adrenal gland weights were significantly increased in F_0 males only and in both F_1 sexes at 100 mg/kg bw/d. Absolute kidney weights were unaffected by treatment in F_0 males at all doses, but relative kidney weights were increased significantly at the 2 highest doses. In F_0 females, increased absolute and relative kidney weights were noted at 50 and 100 mg/kg bw/d compared to the control. Relative kidney weights were also increased significantly in both F_1 sexes at 100 mg/kg bw/d. The increases in mean kidney and adrenal gland weights were considered treatment-related, but did not correlate to any macroscopic or microscopic findings. In F_2 pups, mean organ weights were unaffected by parental treatment at all dose levels. A NOEL of 15 mg/kg bw/d was established based on the

changes in liver and kidney weights, and serum chemistry parameters at the two highest doses.

3.1.6. Developmental Studies

In a dose range-finding study, pregnant rats were administered tulathromycin by oral gavage once daily at doses of 50, 100, 200 or 500 mg/kg bw/d from either day 6 – 17 of gestation or from gestation day 6 (GD 6) through to lactation day 11 (LD 11). Seven females in the 500 mg/kg bw/d group died or were euthanised between GD 11 and LD 1. Clinical signs noted before death included red staining around nasal and buccal areas, or on forelimbs, hunched appearance, unkempt, rocking, lurching and hypoactivity. Mean maternal bodyweight gains during GD 12-20 in the 500 mg/kg bw/d group were significantly reduced. A mean bodyweight loss in the surviving dams in the 500 mg/kg bw/d group during LD 1-11 correlated to a reduction in food consumption during lactation. Mean bodyweights and bodyweight gains in the 50 and 200 mg/kg bw/d were similar to control values. Intrauterine parameters (post-implantation loss, mean foetal bodyweights, foetal sex ratios and mean numbers of implantation sites and corpora lutea) were unaffected by test article at any dose. Pup bodyweight gains in both sexes reduced in the 500 mg/kg bw/d group during LD 1-7. Mean offspring weights of both sexes in the 500 mg/kg bw/d group were reduced on LD 1, 4 and 7 and in males on LD 11. There were no external malformations or developmental variations in foetuses.

The NOEL for maternal toxicity was considered to be 200 mg/kg bw/d. Similarly, the developmental NOEL was 200 mg/kg bw/d.

Pregnant rats were administered tulathromycin by oral gavage once daily at doses of 15, 100 or 200 mg/kg bw/d from day 6 – 17 of gestation. There were no treatment-related mortalities or clinical signs noted during the study. Mean gestation bodyweight and bodyweight gains, net bodyweights, net bodyweight gains and gravid uterine weights, as well as food consumption were unaffected by treatment. At necropsy, there were no treatment-related internal findings at any dose level. Intrauterine growth and survival were unaffected by treatment. The mean number of viable foetuses in the 100 and 200 mg/kg bw/d groups was significantly reduced when compared to the concurrent control group. Increases in the mean litter proportions of early resorptions, total resorptions and post-implantation loss were noted in both treatment groups. Mean foetal bodyweights of both sexes were significantly lower for all treatment groups, but was considered to be unrelated to treatment as there was no apparent dose-response relationship. There were no treatment-related external, skeletal malformations or variations in this study. The NOEL for maternal toxicity and developmental toxicity was 200 mg/kg bw/d.

In a second dose-range finding study, artificially inseminated pregnant rabbits were administered, either vehicle (citric acid solution) or tulathromycin at doses of 15, 50 or 75 mg/kg bw/d from day 7 – 20 of gestation by oral gavage. One animal in the 75 mg/kg bw/d group was found dead one hour following dosing on GD 10. Two rabbits in the 50 mg/kg/d group and another in the 75 mg/kg/d group, were nongravid. One rabbit each from the 50 and 75 mg/kg bw/d groups aborted on GD 25 and 26, respectively. These animals had large bodyweight losses, reductions in food consumption and decreased defecation for up to two weeks prior to abortion. Clinical signs observed included decreased defecation by animals in the 50 and 75 mg/kg bw/d groups during GD 10-29, with the frequency of occurrence increasing in the 75 mg/kg bw/d group. Mean reductions in bodyweight losses or reductions in bodyweight gain in the 75

mg/kg/d group during GD 7-29 correlated with a significant reduction in food consumption and a significant mean net bodyweight loss (bodyweight – uterine content) in the same group. Mean bodyweight, net bodyweight, net bodyweight gain and gravid uterine weights in the 15 mg/kg bw/d and 50 mg/kg bw/d groups were unaffected by treatment. Intrauterine growth was significantly reduced in the 75 mg/kg bw/d group only, probably due to the reduced foetal bodyweights. Pup survival was unaffected by treatment at all dose levels. Other intrauterine parameters (mean numbers of corpora lutea) were similar to control values. There were no treatment-related external, skeletal or visceral malformations or variations noted. For this study, the NOEL for maternal toxicity and foetal toxicity was concluded to be 50 mg/kg bw/d.

Artificially inseminated rabbits were administered tulathromycin by oral gavage once daily at doses of 5, 15 or 50 mg/kg bw/d from day 7 – 20 of gestation. There were no treatment-related mortalities or clinical signs. Mean gestation bodyweight and bodyweight gains, net bodyweights, net bodyweight gains and gravid uterine weights were unaffected by treatment, as was food consumption. At necropsy, there were no treatment-related internal findings at any dose level. Intrauterine growth parameters and survival were unaffected by treatment. There were no treatment-related external, skeletal or visceral malformations or variations. The NOEL for maternal toxicity and developmental toxicity was concluded to be 50 mg/kg bw/d.

3.1.7. Genotoxicity Studies

Tulathromycin was tested for clastogenic activity *in vitro* in human lymphocyte cultures, with and without metabolic activation at concentrations ranging from 1810 to 2820 µg/ml and 812 to 1450 µg/mL, respectively. Chromosome damage was evaluated by metaphase analysis after 3 hours. Chromosome damage was also evaluated after 24-hour treatment without metabolic activation at concentrations ranging from 248 to 1084 µg/mL.

In the 3-hour test without metabolic activation, tulathromycin caused increasing mitotic suppression in the 812 to 1450 µg/mL concentration range. There were no changes to the percentage of abnormal cells with chromosome aberrations.

Tulathromycin was tested for the induction of micronuclei in male and female rat bone marrow cells. Rats were administered either vehicle as the negative control or tulathromycin at dose levels of 500, 1000 or 2000 mg/kg bw by oral gavage, once daily for 3 days. A third group received Mitomycin C as positive control. There were no clinical signs or mortality observed during the treatment period. Bodyweight gain in males decreased at 2000 mg/kg bw/d, but weight gain in female was unaffected by treatment. Neither sex showed a treatment-related reduction in mean % polychromatic erythrocytes (PCE). An increase in the numbers of PCE with micronuclei was not observed. Positive control group showed elevations in micronucleated PCE in accordance with historical controls.

Tulathromycin, spiked with 1% CP-60,300, was tested for gene mutational activity in Chinese hamster ovary cells assay. The definitive test was conducted in the absence or presence of Aroclor-induced rat liver S9 metabolic activation over concentrations ranging from either 500 to 5000 µg/mL or 500 to 6000 µg/ml, respectively. Moderate to substantial cytotoxicity was observed at concentrations of ≥2000 µg/mL in the test

conducted without metabolic activation. Mean mutants per 10^6 survivors for the treated cultures ranged from 0.0 to 3.5. There was no evidence of a treatment-related increase in mutant frequency.

The ability of tulathromycin to induce forward mutations at the thymidine kinase locus was assessed in the mouse lymphoma L5178Y cell line. Results from a preliminary solubility and cytotoxicity study showed that tulathromycin was noncytotoxic in the presence and absence of metabolic activation at concentration ranges of 9.85 to 78.5 µg/mL (without S9 activation) and 9.85 to 625 µg/mL (with S9 activation), respectively, after a treatment period of 4 hours. After a 24-hour treatment period, weak cytotoxicity was noted from 9.85 to 157 µg/mL in the absence of activation. In the two test assays without metabolic activation, tulathromycin in the concentration range of 100 to 300 (or 550) µg/mL did not induce mutant frequency after a 24- hour incubation period. Similarly, for the two test assays with metabolic activation, tulathromycin in the concentration range of 200 (or 400) to 1000 µg/mL did not induce mutant frequency after a 24-hour incubation period.

Tulathromycin was tested against *Salmonella typhimurium* strains TA 1535, TA 1537, TA98, TA100 or *Escherichia coli* strain WP2 uvrA pKM101 in the presence and absence of metabolic activation. With each of the *Salmonella* strains, concentrations ranged from 0.15 to 15 µg/plate and 0.05 to 5.0 µg/plate with and without metabolic activation, respectively. With *E. coli*, concentrations ranged from 0.50 to 50 µg/plate and 0.15 to 15 µg/plate with and without metabolic activation, respectively. In the absence of metabolic activation, dose-related cytotoxicity was observed at 5.0 µg/plate with each of the *Salmonella* strains and at 15 µg/plate with *E. coli*. In the presence of metabolic activation, dose-related cytotoxicity was observed at concentrations ≥ 5.0 µg/plate with TA 1535 and TA 100, and at concentrations ≥ 15 µg/plate with TA 1537, TA 98, and *E. coli*. There was no evidence of significant dose-related increases in the number of revertant colonies in any of the strains tested in either the absence or presence of metabolic activation.

3.1.8. Other Studies

The Minimum Inhibitory Concentration (MIC) of tulathromycin against 100 bacteria of human gut origin, comprising 10 isolates from each of 10 genera regarded as dominant in human faecal microbiota were screened. No strain was sensitive to tulathromycin at concentrations below 0.5 µg/mL (lower inoculum) or 1 µg/mL (higher inoculum). The most sensitive genus was *Bifidobacterium spp*. under anaerobic conditions, with a MIC of 0.5 µg/mL.

The MIC of tulathromycin against four *Escherichia coli*, four *Enterococcus* and four *Bifidobacterium* strains was determined under four sets of environmental conditions: i) or ii) laboratory culture media at either pH 7.2 or 6.5, respectively and iii) or iv) culture media plus faecal material at either pH 7.2 or 6.5, respectively. Results obtained indicate that tulathromycin activity can be markedly reduced by low pH and that the compound is extensively bound to faeces.

Under conditions simulating those of the human gastrointestinal tract (GIT), the activity of tulathromycin at either 2 or 8 µg/mL concentrations against two *Bifidobacterium* and two *Fusobacterium* strains of human gut origin were determined. Viable counts recorded in control gut model preparations (free from tulathromycin) demonstrated that the bacterial strains were able to survive and multiply.

Final viable counts obtained for each test strain in the presence of tulathromycin were comparable or higher with those obtained in the absence of antimicrobial compound, except for *Bifidobacterium longum DWC 4554.* Viable *Bifidobacterium longum DWC 4554* counts were reduced by 50-70% compared to the control values for the tulathromycin treated groups.

Another study incorporating similar methodology as the one detailed above, with the exception that the concentration of tulathromycin employed against two *Bifidobacterium* and two *Fusobacterium* strains of human gut origin were 10, 15 or 20 µg/mL. Final viable counts obtained for each test strain in the presence of tulathromycin were comparable or higher with those obtained for the control, except for *Fusobacterium necrophorum DWC 1460* at the top concentration. Exposure to 20 µg/mL tulathromycin caused a 48% reduction in viable *Fusobacterium necrophorum DWC 1460* counts.

The MIC of tulathromycin against 10 *Fusobacterium* strains was determined using broth microdilution methodology under either pH 7.0 or 6.6. The pH shift from 7.0 to 6.6 had little effect on bacterial growth, but increased the activity of tulathromycin and the MIC from 4 to 8 µg/mL for *Fusobacterium* strains.

3.2. Discussion of the toxicity studies

Oral toxicity of tulathromycin was low and the adverse effects (increase in liver transaminases, vomiting and diarrhoea) are reversible. Intravenous toxicity of tulathromycin was high, with transient ataxia/decreased activity observed in rats, and in dogs, diarrhoea, erythema and apnoea was noted. Acute dermal toxicity in rabbits is low; clinical signs included slight oedema, erythema and decreased food consumption and defecation. Tulathromycin is a severe eye irritant in rabbits and a skin sensitiser in guinea pigs, but is non-irritating to rabbit skin.

In all oral repeat-dose toxicity studies performed in rats (10 days, 1- and 3-month) and dogs (1- and 3-month and 1 year), the main toxicological effect noted in all studies across both species was an increase in serum liver enzymes (ALT, AST and SDH). The adverse effect on liver enzymes progressively increased with time and dosing, and was associated with decrease in liver weights in 2 studies. There were no histopathological changes in the liver. The observation that the area under the curve value after chronic administration) was significantly greater than the single dose AUC suggested inhibition of the detoxification system.

In developmental studies in rats and rabbits, no teratogenic effects were noted in the offspring, however foetal weight was decreased significantly at maternotoxic doses. Maternotoxicity presented as decreased mean bodyweight gains and food consumption at the highest dose tested in rats (500 mg/kg bw/d).

Increased testicular weights were noted in the 1-year chronic dog study, but not in the 2-generation reproductive study in F_0 and F_1 rats. The effect of tulathromycin on spermatogenesis and/or reproduction remains unclear.

Tulathromycin was examined for its mutagenic potential in the absence and presence of metabolic activation in *in vitro* bacterial (*Salmonella, E. coli*) and mammalian systems, as well as *in vivo* mammalian systems. No mutagenic activity was noted in bacterial systems. Tulathromycin did not induce chromosomal aberrations in human lymphocytes or mouse lymphoma cells, and did not induce gene mutations at the HPRT locus in Chinese hamster ovary cells. In the mouse micronucleus test, an increase in the frequency of micronuclei of erythrocytes was not observed. Thus tulathromycin is classified as being non-genotoxic.

Tulathromycin is unlikely to be carcinogenic when factors such as, the absence of a chemical structural relationship to known carcinogens, the negative results of genotoxic assays and the lack of carcinogenic potential of similar macrolide antibiotics are considered.

Increased kidney weights were noted in dogs in the 1-month oral gavage study and rats in the 2-generation reproduction study, but not in longer duration studies, i.e. in the 90-day rat or 1-year dog studies using similar dosing levels. Given that abnormal histopathology was not observed in the kidneys of animals treated with tulathromycin and that no effects were noted in the longer dosing studies, tulathromycin is unlikely to be nephrotoxic.

A series of studies measured the effects of tulathromycin on the human intestinal flora. The standard MIC study demonstrated that the most sensitive genus was *Bifidobacterium spp* with a MIC_{50} of 1 µg/mL (higher inoculum). A second MIC study revealed that tulathromycin activity can be reduced by low pH (6.5 versus 7.2) and that it is extensively bound to faecal matter. The relevance of these parameters to *in vivo* intestinal conditions and the activity of tulathromycin in the human GIT was difficult to ascertain. Two other studies employing simulated gut models showed that digestive enzymes did not inactivate tulathromycin.

In vitro data indicated that tulathromycin was acid labile and subsequently devoid of any microbiological activity. *In vivo* data in dogs suggested that tulathromycin remained microbiologically active on entering the colon following oral administration. Dogs had diarrhoea, a clinical sign, frequently associated with retention of microbiological activity in the colon following oral antibiotic administration. The likelihood that tulathromycin retains its microbiological activity is supported by the fact that less than 10% of the chemical was absorbed from the GI tract and most of it was excreted in the faeces unchanged in all tested species.

3.3. Public health standards

3.3.1. Poison scheduling

Tulathromycin is a semi-synthetic macrolide antimicrobial that is comprised of an equilibrated mixture of 2 isomers CP-472,295 (~90%) and CP-547,272 (~10%) in aqueous solutions. The National Drugs and Poisons Schedule Committee (NDPSC) considered the toxicity of tulathromycin and its use pattern. On the basis of its low toxicity and its intended use as a therapeutic agent under the supervision of a veterinarian, the NDPSC agreed to include tulathromycin in Schedule 4 of the Standard for the Uniform Scheduling of Drugs and Poisons.

3.3.2. NOEL/ADI

Toxicological ADI

The Acceptable Daily Intake (ADI) is that quantity of a veterinary chemical that can safely be consumed on a daily basis for a lifetime and is based on the lowest NOEL obtained in the most sensitive species. This NOEL is then divided by a safety factor, which reflects the quality of the toxicology database and takes into account the variability in responses between species and individuals.

There was no evidence of genotoxicity, teratogenicity or acute reproductive effects in the toxicological database for tulathromycin, thus the 12-month oral gavage study in dogs was considered the most appropriate study to derive the toxicological ADI. The toxicological ADI for tulathromycin was established at 0.005 mg/kg bw/d based on a NOEL of 5 mg/kg bw/d and increased levels of serum transaminases (ALT and AST) in females and increased testicular weights in males at the next highest dose.

The safety factor of 1000 incorporates 10-fold each for inter- and intra-species variability, and an additional 10-fold safety factor for the lack of a chronic study in rats.

Microbiological ADI

The microbiological ADI was estimated using information from studies that measured tulathromycin activity (Minimum Inhibitory Concentration) against bacteria normally found in human colonic microflora. The JECFA equation used to evaluate the upper limit of the ADI when based on a microbiological endpoint is:

$$\text{Microbiological ADI} = \frac{MIC_{50}\ (\text{mg/mL}) \times MCC\ (\text{g})}{FA \times SF \times BW\ (\text{kg})}$$

Where: MIC_{50} = the minimum concentration of an antibiotic that inhibits 50% of the growth of cultures of a given micro-organism, as judged by the naked eye, after a period of incubation. For tulathromycin, the lowest MIC_{50} value was used for the most sensitive species.

MCC = Mass of colonic content; a value of 220 g is used

FA = Fraction of oral dose available to act upon micro-organisms in the colon. For tulathromycin, <23% of the total radioactivity in rats and dogs was

represented by metabolites in the liver, bile, urine and/or faeces and thus systemically absorbed, it was concluded that up to 77% (rounded up to 80%) of the total radioactivity may not have been systemically absorbed and is potentially available to act on GIT bacteria.

SF = The safety factor used to account for uncertainty about the amount and relevance of data for MIC values available for review, may range from 1 – 10. For Tulathromycin, a value of 1 was used due to the extensive relevant microbiological data available.

BW = Bodyweight (a value of 60 kg is used)

Using this formula to establish an ADI based on a microbiological endpoint gives a value of 0.005 mg/kg bw/d, i.e.

$$\text{Microbiological ADI} = \frac{0.001^{*} \times 220}{0.8 \times 1 \times 60}$$

* Against a range of bacterial strains that are representative of human colonic microflora, with *Bifidobacterium spp* as the most sensitive to tulathromycin. The mean MIC_{50} of 1 µg/mL for the higher inoculum (i.e. $2x10^7$ cfu/mL, for the most sensitive genus, *Bifidobacterium*) was thought to simulate conditions in the colon, compared to the lower inoculum value of 0.5 µg/mL thus the higher mean MIC_{50} value was used in the derivation of a microbiological ADI.

The microbiological ADI is supported by the toxicological ADI of 0.005 mg/kg bw/d.

3.3.3. ARfD

The Acute Reference Dose (ARfD) is the maximum quantity of a veterinary chemical that can safely be consumed as a single, isolated event. The ARfD is derived from the lowest single or short-term dose, which causes no effect in the most sensitive species and individuals. The highest acute dose of a compound at which no evidence of toxicity was detected is the starting point for the estimation of the ARfD.

In rats, the oral LD_{50} was >2000 mg/kg bw/d. Diarrhoea was noted in all animals receiving a single gavage dose of 2000 mg/kg bw/d. Relative to controls, mean hepatic AST concentrations were increased in both sexes receiving 1000 or 2000 mg/kg bw dose, and in females, ALT concentrations were increased significantly in animals receiving the same doses. Based upon the above effects, a NOEL of 300 mg/kg bw was established.

In dogs, the oral LD_{50} was > 1000 mg/kg bw/d, with emesis and/or loose stools noted after single oral doses of 100, 300 or 1000 mg/kg bw/d. One female dosed with 100 mg/kg exhibited elevated bile acids and AST levels. A NOEL was not set in this study due to the effects noted at the lowest dose. Given that the same toxicity effects were noted in both rats and dogs, with the dog being the more sensitive species, a LOEL of 100 mg/kg bw in the dog oral acute study was used as the basis for setting an ARfD.

The ARfD was established at 0.1 mg/kg bw on the basis of this LOEL using a 1000-fold safety factor that incorporates 10-fold each for inter- and intra-species variability, and an additional 10-fold for the use of a LOEL.

4. METABOLISM AND TOXICOKINETICS ASSESSMENT

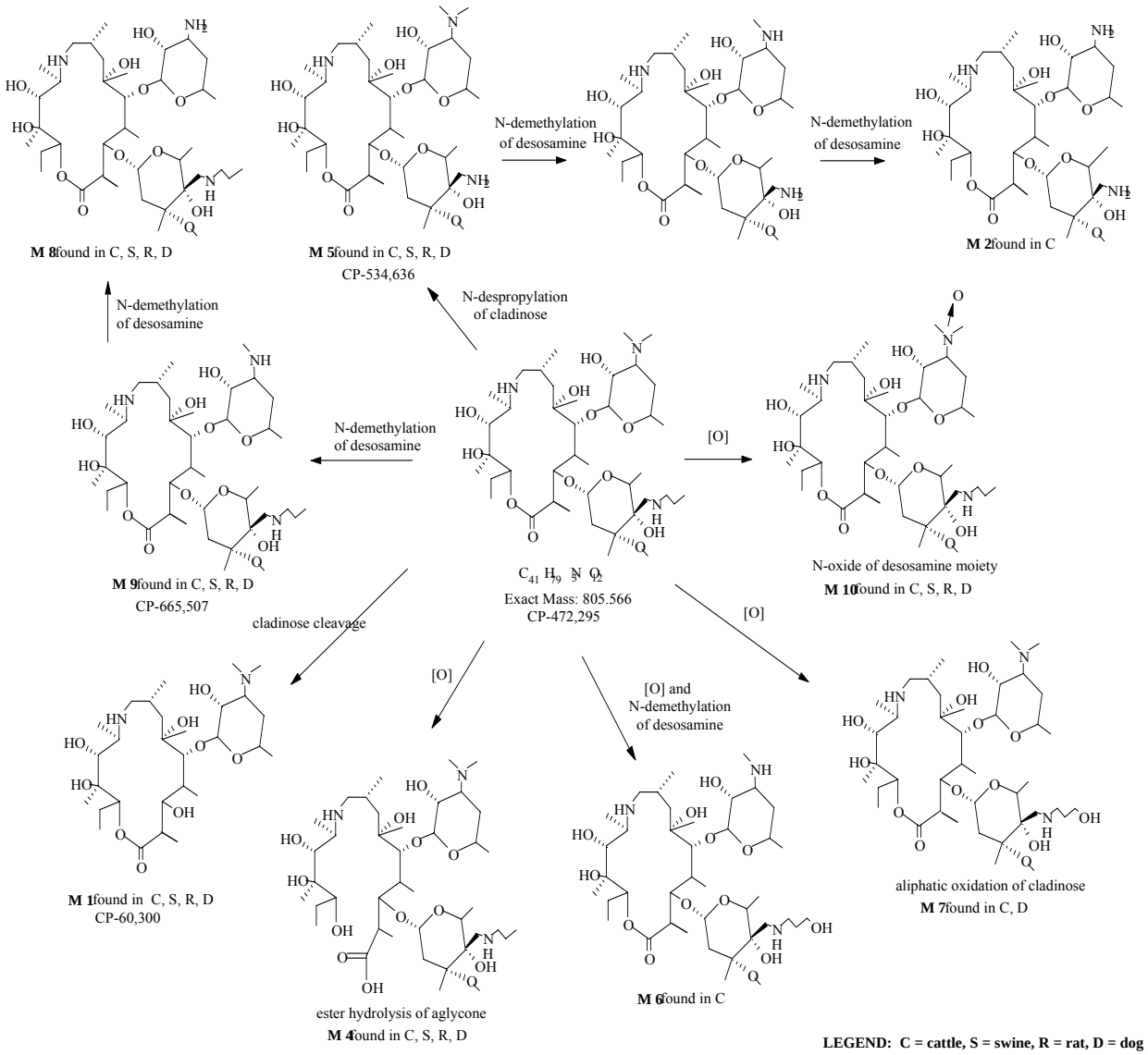
Tulathromycin exists as either one isomer in crystallised form, CP-472,295 or as an equilibrated mixture of two structural isomers(CP-472,295 and CP-547,272) in aqueous solutions. The equilibrated mixture is referred to as CP-472,295(e).

Following oral administration of a radio-labelled tulathromycin (CP-472,295(e)) dose in repeat-dose studies, plasma drug concentration in dogs generally increased with increasing dose, with peak levels reached at 0.5-2 hours. Peak plasma drug concentration did not increase with time after dosing with 56.7-57.3 mg/kg bw/d for 28 days despite continual dosing for 60 days. After daily doses of 17 mg/kg bw/d, peak plasma drug concentration increased for up to 90 days. For doses <5mg/kg bw/d, systemic concentrations were below the limit of quantification. Similar results were observed in rats.

In cattle, pigs, dogs and rats, the majority of the radio-labelled tulathromycin dose is eliminated in faeces as unchanged parent drug. Less than 8%, 3%, 3.2 and 8.8% of total metabolites are found in liver, bile, urine and faeces, respectively. Two metabolites, the N-demethylation of the desoamine and the N-oxide of the same moiety, were observed consistently. One metabolite was identified as the N-despropylation of the cladinose moiety in cattle bile and another as the desoamine N-oxide in cattle skin/fat. Other metabolic pathways that were identified for radio-labelled CP-472,295(e) represented multiple steps of the oxidative N-dealkylation on the desosamine, modified cladinose moieties, aliphatic oxidation of the N-propyl moiety, ester hydrolysis of the aglycone and cleavage of the entire modified cladinose moiety (see Figure 1).

Given that in rats and dogs, <23% of the total radioactivity was represented by metabolites in the liver, bile, urine and/or faeces and thus systemically absorbed, it was concluded that up to 77% of the total radioactivity may not be systemically absorbed. The total radioactivity of parent compound – as a % of the dose – excreted in urine and faeces was not reported.

Figure 1: Metabolic Pathways of Tulathromycin in Rats, Dogs, Swine and Cattle



5. RESIDUES ASSESSMENT

5.1. Introduction

Draxxin Injectable solution contains 100 mg/mL tulathromycin. The antibiotic has a long duration of action and achieves high concentrations in lung tissue, affording effective single dose treatment for respiratory infections of cattle and pigs. The maximum nominal dose rate in cattle and pigs is 2.5 mg/tulathromycin/kg bw. As part of the residues assessment, animal metabolism studies, residues trials, analytical methodology and trade aspects were considered.

5.2. Metabolism

The metabolism of tulathromycin in cattle, pigs, dogs and rats is similar, with the parent isomers being the major components in edible tissues, urine, faeces and bile from treated animals. Metabolism is limited and involves:

(i) N-depropylation of the modified cladinose moiety (M5);
(ii) primary and secondary demethylation of the desosamine moiety (M9 and M8);
(iii) N-oxide formation on the desosamine moiety (M10);
(iv) loss of the modified cladinose moiety (M1, CP-60,300); and
(v) combinations of oxidation and/or dealkylation processes (M2, M3, M4, M6 and M7).

Analysis of the radiolabelled residues in urine, faeces, bile, liver, kidney, muscle, fat, skin/fat and injection site samples revealed that the residues in matrices from pigs and cattle were predominantly made up of unchanged parent tulathromycin. Other major metabolites were identified as the desosamine N-oxide in pig skin/fat (~20 % of Total Radioactive Residues (TRRs)) and N-depropylation product of the cladinose moiety in cattle bile (~16% of TRRs). All other pig and cattle tissues contained a number of metabolites, in addition to parent tulathromycin, but none of the metabolites exceeded 10 % of the total radioactivity for the matrix.

The relative rank order of tulathromycin residues in edible cattle tissues is: liver > kidney >> muscle > fat, and in pig tissues: kidney> liver>>muscle>fat.

5.3. Analytical methods

Parent tulathromycin isomers are extracted from homogenised tissue samples with phosphoric acid, cleaned up on SPE cartridges, and analysed using an HPLC/MS/MS method with electrospray ionisation in the positive ion mode. The concentration of tulathromycin residues is determined using a standard calibration curve of peak area ratios of parent compound to internal standard (the hepta-deuterated form of the main tulathromycin isomer) against the concentration of parent tulathromycin.

A second method measures tulathromycin residue components that can be converted to an acid digest hydrolytic common fragment CP-60,300. Homogenised tissue samples are hydrolysed (2 M HCl, 60°C, 1 hour); the hydrolysate is centrifuged, then cleaned up on SPE cartridges, and analysed for drug hydrolytic common fragment CP-60,300 using an HPLC/MS/MS method with electrospray ionisation in the positive ion mode. The concentration of CP-60,300 residues is determined using a standard calibration curve of peak area ratios of CP-60,300 to internal standard (CP-66,458, desosaminyl azithromycin, which differs from CP-60,300 only by the presence of a N-methyl group on the aglycone ring) against the concentration of CP-60,300.

The LODs and LOQs for the method to quantify residues of the tulathromycin hydrolytic common fragment CP-60,300 in edible tissues are tabulated below (Table 1).

Table 1: LODs and LOQs of CP-60,3000

Tissue matrix	LOD (mg/kg)		LOQ (mg/kg)	
	Cattle	Pigs	Cattle	Pigs
Liver	0.009	0.004	0.3	0.05
Kidney	0.004	0.027	0.2	0.1
Muscle	0.011	0.001	0.03	0.02
Fat (Skin/fat)	0.003	0.003	0.06	0.02
Injection site	0.002	0.001	0.3	0.1

5.4. Residue definition

The acid digest fragment analytical method accounts for a higher percentage of total residues than the method for measuring parent tulathromycin. Since the residue decline profile of CP-472,295 and CP-60,300 parallel the depletion curves of total residues in most edible tissues, both analytes are acceptable as the residue definition of tulathromycin. For monitoring and surveillance purposes, the residue definition for tulathromycin is set as the sum of tulathromycin and its metabolites that are converted by acid hydrolysis to CP-60,300, expressed as tulathromycin equivalents.

5.5. Residues trials

Two trials were conducted in which one subcutaneous dose of 2.5 mg radiolabelled tulathromycin/kg bw was administered to beef cattle. A similar dose was administered in pigs intramuscularly in two other trials. Samples of liver, kidney, muscle, skin/fat or perirenal fat and injection site tissues from pigs and cattle were collected up to 36 and 48 days after treatment (DAT), respectively. All samples were stored frozen until analysed for the concentrations of unchanged parent tulathromycin, the acid digest fragment and total radioactive residues.

Highest residues level observed in all cattle tissues were 10 mg tulathromycin equivalents/kg in liver, 6.06 mg tulathromycin equivalents/kg in kidney and 0.52 mg tulathromycin equivalents/kg in perirenal fat 5 DAT, and 1.49 mg tulathromycin equivalents/kg in muscle 0.5 DAT. Corresponding observations in pig tissues were 2.8 mg tulathromycin equivalents/kg in liver and 6.2 mg tulathromycin equivalents/kg in kidney 4 DAT, 0.3 mg tulathromycin equivalents/kg in skin/fat and 0.63 mg tulathromycin equivalents/kg in muscle 5 DAT. As liver samples of cattle and kidney samples of pigs contained the highest concentration of tulathromycin residues and displayed the longest residues decline profile, these tissues determined the duration of the withholding periods (WHPs) for the product.

Statistical analysis of the combined residues data from the cattle trials showed that periods of 33, 19 and 6 DAT would be required for residues in liver, kidney and fat, respectively, to decline below proposed MRLs of 3 mg/kg for liver and kidney, and 0.5 mg/kg for fat of cattle. A similar analysis of the residues data from the pig trials indicated that periods of 14, 8 and 7 days would be required for tulathromycin residues in kidney, skin/fat and liver to decline below their proposed MRLs of 3 mg/kg for kidney and liver, and 0.5 mg/kg for skin/fat. At all times after treatment, residues in cattle and pig muscles were likely to decline below an MRL of 3 mg/kg.

Based on these analyses, the APVMA has recommended a 35-day WHP for the product when used in cattle and 14 days when used in pigs. When the 35-day WHP is observed, the occurrence of tulathromycin residues in cattle is covered by MRLs of 3 mg/kg in liver, 0.1 mg/kg in fat, 1 mg/kg in kidney and 0.1 mg/kg in muscle of cattle. The occurrence of tulathromycin residues in pigs is covered by MRLs of 3 mg/kg in kidney, 0.3 mg/kg in skin/fat, 2 mg/kg in liver and 0.5 mg/kg in muscle when the 14-day WHP is observed. These MRLs are recommended for compliance and monitoring purposes.

Tulathromycin residues decline profile in milk has not been determined. Therefore, the following milk WHP is recommended:

DO NOT USE *in cows which are producing or may in the future produce milk or milk products for human consumption*.

5.6. Re-treatment interval

Draxxin Injectable Solution is intended as a single injection treatment of cattle and pigs showing signs of bovine or swine respiratory disease. There is no information of a minimum re-treatment interval should the target animals experience a subsequent bout of disease. Since residues in edible tissues from treated cattle and pigs are above the method LOQ after the recommended withholding periods have been observed, there is potential for accumulation of residues and violation of the recommended MRLs if the re-treatment interval is too short.

The APVMA has estimated that minimum re-treatment intervals of 8 and 12 weeks are required for the use of the product in pigs and cattle, respectively. This estimate is based on calculations of tulathromycin residues in pig kidney and cattle liver at the upper 95% confidence limit after the recommended withholding periods have been observed and then statistically analysing the residues decline data.

5.7. Estimated dietary intakes

Most of the identified tulathromycin metabolites retain the intact macrocyclic ring moiety (aglycone ring), and are expected to retain some degree of antimicrobial activity. In the absence of any toxicological data to demonstrate that the tulathromycin metabolites are not biologically active, the APVMA's dietary exposure assessment for tulathromycin has encompassed all residue components.

For chronic dietary exposure estimates, the recommended tulathromycin MRLs (based on the common hydrolytic fragment CP-60,300) were divided by the ratios of 'marker residues' to total residues that were determined in studies conducted with ^{14}C-labelled tulathromycin. For acute dietary exposure estimates, the values for the highest total residue concentrations at the recommended WHP were interpolated from the residues decline curves; the highest residues values were then corrected to total tulathromycin residues. Details are tabulated in Table 2.

Table 2: Determination of total tulathromycin residues for dietary exposure calculations

Species	Tissue	Recommended MRL (mg/kg)	Ratio of 'marker residue' to total residues	Total residue concentration for chronic dietary exposure calculations (mg/kg)	Highest residue concentration for acute dietary exposure calculations (mg/kg)
Cattle	Liver	3	0.61	4.9	‡(2.728) 4.47
	Kidney	1	0.78	1.3	(0.917) 1.18
	Muscle	0.1	0.79	0.15	(0.0521) 0.066
	Fat	0.1	0.51	0.20	(0.043) 0.084
	Injection site	--	0.90	--	(3.35) 3.72
Pigs	Liver	2	0.95	2.1	(1.644) 1.73
	Kidney	3	0.83	3.6	(2.913) 3.51
	Muscle	0.5	0.86	0.6	(0.329) 0.38
	Skin/fat	0.3	0.32	0.9	(0.254) 0.79
	Injection site	--	0.89	--	(4.40) 4.94

‡Values in parenthesis are the estimated highest residues (CP-60,300) at the recommended WHP.

The chronic dietary exposure to tulathromycin is estimated by the National Estimated Daily Intake (NEDI) calculation encompassing all registered/temporary uses of the chemical and the mean daily dietary consumption data derived from the 1995 National Nutrition Survey of Australia. The NEDI calculation is made in accordance with WHO Guidelines and is a conservative estimate of dietary exposure to chemical residues in food. The NEDI for total tulathromycin residues is equivalent to 8.5% of the ADI.

The acute dietary exposure is estimated by the National Estimated Short Term Intake (NESTI) calculation. The NESTI calculations are made in accordance with the deterministic method used by JMPR with 97.5th percentile food consumption data derived from the 1995 National Nutrition Survey of Australia. NESTI calculations are conservative estimates of acute exposure (24 hour period) to chemical residues in food.

The highest acute exposure to tulathromycin through the consumption of 300 g of injection site tissues from treated cattle and pigs is 16.7 and 22.1% of the ARfD, respectively, for the general population, but 59 and 78% for infants (2-6 years). The

acute exposure to tulathromycin from consuming meal-sized portions of non-injection site tissues such as meat, fat and offal does not exceed 15% of the ARfD.

The NESTIs for edible commodities are summarised in Table 3.

Table 3: Acute Dietary Exposure (NESTI) Calculations for Tulathromycin

Commodity	NESTI Calculation (% of the ARfD)	
	Children (2-6 years of age)	Whole population (2 years and over)
Cattle fat	0.02	0.02
Cattle meat	0.83	0.46
Cattle liver	<0.1	11.2
Cattle kidney	1.7	1.6
Cattle injection site	58.7	16.7
Pig skin/fat	0.20	0.24
Pig meat	3.6	1.8
Pig kidney	<0.1	<0.1
Pig liver	0.11	3.1
Pig injection site	78.0	22.1

5.8. Bioaccumulation potential

The report of the Thirty-eighth Session of the Codex Committee on Pesticide Residues (April 2006) revisited the issue of fat-soluble pesticides in meat and fat. The meeting decided to revise the empirical limits recommended by the 1991 JMPR when considering the octanol/water partition coefficient (log P_{ow}), so that when no evidence is available to the contrary and log P_{ow} exceeds **3**, the compound would be designated fat-soluble, and when log P_{ow} is less than 3 it would not be so designated.

The log P_{ow} for tulathromycin is estimated to be 3.33, which indicates that this drug has the propensity to preferentially partition into fat tissues. Residues decline data have shown that tulathromycin residues are highest in liver and kidney of treated animals, and that those residues that are present in fat decline readily. On this basis, tulathromycin is not considered to be a bioaccumulatory/bioretentive compound.

5.9. Recommendations

The following amendments to the MRL Standard are recommended:

Amendments to Table 1 of The MRL Standard

Compound	Food		MRL (mg/kg)
Tulathromycin			
ADD:			
	MF 0812	Cattle fat	0.1
	MO 1280	Cattle kidney	1
	MO 1281	Cattle liver	3
	MM 0812	Cattle meat	0.1
		Pig skin/fat	0.3
	MO 1284	Pig kidney	3
	MO 1285	Pig liver	2
	MM 0818	Pig meat	0.5

Amendments to Table 3 of The MRL Standard

Compound	Residue
ADD:	
Tulathromycin	Sum of tulathromycin and its metabolites that are converted by acid hydrolysis to (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-ß-D-xylohexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one, expressed as tulathromycin equivalents.

6. ASSESSMENT OF OVERSEAS TRADE ASPECTS OF RESIDUES IN FOOD

6.1. Commodities exported

Beef, pork and live cattle are the commodities exported.

6.2. Destination and value of exports

In 2004, Australia exported ~914 kt of beef and veal valued at $AUS 4.4 billion, and over 600,000 head of live cattle valued at approximately $AUS 328 million. During the 10 month period from July 2005 to April 2006, Australia exported ~40.2 kt of pork, which was valued at $AUS 123.6 million. The major export markets for Australian beef/veal and pork are provided in Tables 4, 5 and 6.

Table 4: Beef and veal exports in 2004 (Source ABARE 2005)

Rank (by $ value)	Importing country	Quantity (kt)	Value ($AUS million)
1	Japan	393.5	2189.8
2	USA	347.2	1374.4
3	Korea, Rep. of	93.3	434.4
4	Chinese Taipei	25.5	119.5
5	Malaysia-Singapore	6.9	74.6
6	European Union†	6.7	62.7

†Regarded as 15 countries to May 2004, then 25 countries from June 2004.

Table 5: Live cattle exports in 2004 (Source: ABARE 2005)

Rank (by $ value)	Importing country	Quantity ('000 of animals)	Value ($AUS million)
1	Indonesia	356.8	207.3
2	Philippines	46.7	30.1
3	Malaysia	47.4	25.2
4	Jordan	34.2	15.3
5	Japan	17.1	14.6
6	Israel	20.9	11.9

Table 6: Pork exports for the period July 2005 to April 2006 (Source: APL)

Rank (by $ value)	Importing country	Quantity (kt)	Value ($AUS million)
1	Singapore	18.285	62.29
2	New Zealand	9.575	33.64
3	Japan	2.545	12.51
4	Korea	2.223	5.18
5	Hong Kong	2.102	3.74
6	Philippines	2.130	2.04
7	Thailand	1.729	1.79
8	Papua New Guinea	0.855	1.33
9	Malaysia	0.450	0.63
10	Taiwan	0.279	0.40
Total		**40.173**	**123.6**

6.3. Overseas registrations

The applicant indicated that the product is currently registered in the European Union (all 25 Member States), the USA, Canada, Switzerland, Bulgaria, Croatia, Romania, Turkey, Ukraine, Ecuador, Mexico, Peru, Argentina, Chile, Venezuela, Korea, Vietnam and the Philippines. The use-pattern worldwide is 2.5 mg tulathromycin/kg bw by subcutaneous injection in cattle and intramuscular injection in pigs. A summary of the overseas withholding periods is in Table 7.

Table 7: WHPs established for Draxxin Injectable Antibiotic Solution in overseas countries

Country	Overseas WHPs	
	Cattle	Pigs
Australia (recommended)	35 days	14 days
European Union[§]	49 days	33 days
USA	18 days	5 days
South Korea	49 days	33 days
Malaysia[†]	49 days	33 days

[§] The European WHPs are based on depletion of injection site residues to the point at which total consumption will not exceed the microbiological ADI for tulathromycin (660 µg/60 kg person/day).

[†] Registration of Draxxin is expected to occur in the first quarter of 2007; Documentation from the Malaysian authority supports Pfizer's proposal that EU withdrawal periods will be adopted by Malaysia.

6.4. Comparison of Australian MRLs with Codex and overseas MRLs

The Codex Alimentarius Commission (Codex) is responsible for establishing Codex Maximum Residue Limits (CXLs) for pesticides. Codex CXLs are primarily intended to facilitate international trade, and accommodate differences in Good Agricultural Practice employed by various countries. Some countries may accept Codex CXLs when importing foods. Tulathromycin has not been considered by Codex. However, tulathromycin MRLs/tolerances have been established by a number of overseas countries, and these are presented in Table 8 along with the proposed Australian MRLs.

Table 8: Comparison of Australian and overseas tulathromycin MRLs/tolerances

Commodity	Overseas MRLs/tolerances (mg/kg)			Proposed Australian MRLs (mg/kg)
	Europe	USA	†Japan	
Residue definition	Tulathromycin and metabolites hydrolysed to CP-60,300, expressed as tulathromycin equivalents	Tulathromycin and metabolites hydrolysed to CP-60,300, expressed as CP-60,300	Unknown	Tulathromycin and metabolites hydrolysed to CP-60,300, expressed as tulathromycin equivalents
Cattle muscle	--	--	0.3	0.1
Cattle fat	0.1	--	0.2	0.1
Cattle liver	3	5.5 (7.7)‡	5	3
Cattle kidney	3	--	3	1
Cattle, edible offal	--	--	3	--
Pig muscle	--	--	2	0.5
Pig fat	--	--	0.3	--
Pig skin/fat	0.1	--	--	0.3
Pig liver	3	--	4	2
Pig kidney	3	15 (21)‡	9	3
Pig, edible offal	--	--	5	--

† Source: USDA Foreign Agricultural Service GAIN Report No. JA6020. Japan Food and Agricultural Import Regulations and Standards: Japan Considering Changes to MRL for Tulathromycin, a Veterinary Drug. 18 April 2006.

‡Values in parenthesis are the corresponding MRLs when the residues are expressed as tulathromycin equivalents (rather than CP-60,300).

6.5. Potential risk to trade

Export of treated produce containing finite (measurable) residues of tulathromycin may pose a risk to Australian trade in situations where (i) no residue tolerance (import tolerance) is established in the importing country, or (ii) where residues in Australian produce are likely to exceed a residue tolerance (import tolerance) established in the importing country.

Singapore: Singapore Agri-Food and Veterinary Authority (AVA) has reviewed the toxicological and safety data for tulathromycin, including the assessment reports and recommendations by regulatory agencies such as the US FDA, EMEA and the Australian Therapeutic Goods and Administration, along with the analytical methodology used for residues analyses in pork and beef tissues. The Singaporean AVA advised that they would adopt the EU's MRLs and withdrawal periods for tulathromycin for their regulatory purposes.

New Zealand: Draxxin Antibiotic Solution is not registered for use in New Zealand. Under the Trans Tasman Mutual Recognition Arrangement, food imported from Australia may be legally sold in New Zealand, provided it complies with Australian requirements/MRLs.

Japan: Japan has established tulathromycin MRLs for edible pig tissues. Japanese MRLs for pig muscle, liver and kidney are higher than the corresponding Australian MRLs, and the MRL for pig fat is identical to the proposed Australian MRL of 0.3 mg/kg for pig skin/fat.

Other Asian countries: Korea, Thailand and Malaysia are likely to adopt the European MRLs and withdrawal periods when Draxxin Antibiotic Solution is registered for use in these markets.

EU: The EU MRLs for tulathromycin residues in pig liver and kidney are the same as, or higher than, the proposed Australian MRLs. However, the EU MRL for tulathromycin in pig skin/fat (0.1 mg/kg) is significantly lower than the proposed Australian MRL of 0.3 mg/kg. The EU has not recommended an MRL for tulathromycin residues in pig muscle, which implies that residues in muscle are lower than those in skin/fat at any specified slaughter time. The APVMA has considered the EU MRLs as the endpoints for determining an ESI for Draxxin Antibiotic Solution.

Statistical analysis of the residue decline data indicated that an Export Slaughter Interval (ESI) of 26 days would be required, to enable tulathromycin residues in pig skin/fat and muscle to decline to below the EU MRL of 0.1 mg/kg. Therefore, the APVMA has recommended that an ESI of 26 days be assigned to the use of Draxxin Antibiotic Solution in pigs.

Australian MRLs for tulathromycin in edible cattle tissues are either the same as or lower than the corresponding MRLs/tolerances established by the main importers of Australian beef. Therefore observance of the domestic WHP of 35 days will enable tulathromycin residues in edible cattle tissues to decline to below the standards of Australia's main export beef markets.

7. OCCUPATIONAL HEALTH AND SAFETY ASSESSMENT

7.1. Hazard classification

Tulathromycin (CAS: 217500-96-4) is not listed on the NOHSC Hazardous Substances Information System (HSIS) database (NOHSC, 2005). Based on the product toxicology information and concentrations of tulathromycin and other ingredients in the product (90%), the OCS has classified Draxxin Injectable Solution is determined to be a hazardous substance in accordance with NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 2004) with the following risk phrase:

R10 Flammable.

7.2. Use and exposure

Draxxin Injectable Solution is intended as a single intramuscular or subcutaneous treatment of bacterial respiratory diseases in cattle and pigs. The product will be manufactured and formulated overseas in 20, 50 and 100 mL sealed glass vials with serum stopper closure and aluminium overseal with a flip-off button. Therefore, occupational exposure is unlikely during manufacture and formulation.

Veterinarians will administer the end-use product. Their exposure while administering the product is limited to accidental self-injection or through ocular contact with the product when air bubbles are being cleared from the syringe. Post-application exposure is not applicable. The likelihood of repeated exposure when using Draxxin Injectable Solution is low, given that the product is self-contained and administered by injection only. Moreover, any potential for repeated exposure will be restricted to trained professionals who will be aware of the possible toxicity associated with the product.

7.3. Recommendations for safe use

Draxxin Injectable Solution will be administered parenterally under veterinary supervision. Users should heed the warning statements on the label and follow the instructions to manage the effects in the event of ocular or dermal contact with the product. Instructions on the labels include wearing face shield or goggles and seeking medical advice.

7.4. Conclusion

Draxxin Injectable Solution is likely to be a skin sensitiser and is a moderate eye irritant. The product is self-contained and can be safely administered by veterinarians in accordance with the label instructions.

8. ENVIRONMENTAL ASSESSMENT

8.1. Introduction

The Department of Environment and Water Resources (DEW) conducted a VICH Phase 1 Preliminary Assessment of tulathromycin. DEW considered the applicant's calculations for a Phase 1 Environmental Impact Assessment and proposal that Draxxin Injectable Solution has met the criteria in the Decision tree in accordance with the VICH GL6 (Ecotoxicity Phase 1) and that no environmental data are required.

8.2. Environmental fate and toxicity

Tulathromycin is a white to off-white solid that exists in anhydrous, monohydrate and sesquihydrate forms. It is soluble in most organic solvents and is highly soluble in water below pH of 8 (400 g/L) and still at pH 9 (19 g/L). The pKa values of 8.49, 9.28 and 9.80 indicate that, except in alkaline solution or soil, tulathromycin is likely to be ionised and to be relatively mobile.

The Material Safety Data Sheet (http://www.draxxin.com/PAHimages/msds_us/DR.pdf) for tulathromycin indicates that the 1 h EC_{50} for Daphnia magna, 48 h LC_{50} for mysid shrimp, 48 h LC_{50} for sheepshead minnow and 168 h IC_{50} for red algae are >20 mg/L at tulathromycin's maximum water solubility. These results should be treated with caution as the EC_{50}s for Daphnia magna (l h), mysid shrimp (48 h) and sheepshead minnow (48 h) were reported at non-standard exposure times. The toxicological end point will be considered as >20 mg/L.

Metabolism studies of tulathromycin in cattle indicate that approximately 48% of the dose was excreted in the urine and faeces in the first five days after subcutaneous dosing. Approximately 19% of the dose was excreted at slower rate over the next 42 days. The overall distribution of residues corresponds to 31.1 % of the dose in the faeces and 35.7% in the urine. Analysis of the urine collected in the first five days after dosing indicated that unchanged drug represented 89% of the profiled TRR and only 8% accounted for other metabolites. Analysis of the faeces in a similar fashion indicated that unchanged drug represented only 49% of the TRR and 17.5% of the TRR accounts for other metabolites.

Data from a pig metabolism study indicate that approximately 71 % of the dose was excreted in the first six days after intramuscular injection. An average of 43.5% and 27.5% of the administered dose was excreted in faeces and urine, respectively, within first six days administration. Approximately 26% of the dose was excreted at a slower rate over the next 29 days. The total % of the dose excreted in the urine and faeces was 32.8% and 63%, respectively. Unchanged drug represented approximately 92% and 95% of the profiled TRR in faeces and urine, respectively.

8.3. Environmental risk assessment

The main environmental risk will occur from the application of treated manure to cropland or from run-off following excretion to land, for example open pasture.

8.3.1. Cattle - feedlots

The Department of Environment and Water Resources estimated that 1.88 kg tulathromycin could be used in a feedlot each cycle. Manure from the feedlot is estimated to contain 0.34 mg/kg tulathromycin. When this manure is applied to the top 15 cm of cropland soil, the concentration of tulathromycin in soil is estimated to be 6 µg/kg. These estimations do not take into account the metabolic profile for tulathromycin (~67% of the administered dose in excreta over 47 days) and the possible biodegradation of tulathromycin in manure-amended soil. When these factors are taken into account, the PEC_{soil} of 6 µg/kg would be further reduced.

8.3.2. Run-off

Assuming that 5% of tulathromycin in manure from a feedlot runs off to a hectare pond 15 cm deep, the concentration of tulathromycin in run-off water is estimated to be 0.063 mg/L. The risk quotient at the toxicological end point of >20 mg/L is <0.003, indicating an acceptable risk. It is more than likely that any surface run-off would be captured before it reaches the aquatic compartment. Therefore, run-off from cattle feedlots will present minimal environmental risk for aquatic organisms. Should run-off occur from treated fertilised cropland, the concentration of tulathromycin would be reduced further even when assuming 10% run-off.

8.3.3. Open pastures

In feeding pastures, urine containing tulathromycin residues is likely to be distributed via treated animals in discrete deposits. Urination overlapping the same area could increase both the concentration and the depth of contamination. The likelihood of this overlapping occurring is minimal due to the low stocking rate as compared to a feedlot situation. In a worse case scenario, 625 mg of tulathromycin could be distributed in 3L urine over 0.5 m^2 of soil of 10 cm deep within the 120 h period post treatment. On average, each urination could result in a concentration of tulathromycin in soil of 39 µg/kg. The PEC_{soil} can be further reduced as faeces, which comprises half of the excreta, is included in the calculation and biodegradation of tulathromycin could potentially occur in soil.

Under the proposed use pattern, the product is less likely to be used on pasture as it is prohibited for use on dairy cows, and for beef cattle on pasture individual animal treatment is most likely. Therefore, taking into account the frequency of use and the expected stocking rate for beef cattle on pasture, the proposed use is unlikely to result in a significant environmental impact on soil organisms.

In the case of excreta that may be discharged from cattle into 1 m wide x 100 m long x 0.3 m deep stream that flows alongside a 1 ha pasture, DEW calculated 0.54 mg of tulathromycin could be excreted into the stream, resulting in the concentration of tulathromycin in the stream being 0.018 µg/L. When comparing this concentration with

the toxicological end point of >20 mg/L, a Q value « 0.1 is obtained indicating an acceptable aquatic risk. Considering the low frequency of use of the proposed product in a pasture scenario and the greater likelihood of cattle excreting on land than water, there is unlikely to be an environmental risk.

8.3.4. Pigs

In most animal sheds in Australia, pig wastes are flushed into channels beneath slatted floors and then combined with wastes from all sheds in a central treatment system. In intensive piggeries, wastes from all pigs are combined and treated in anaerobic and aerobic ponds. The effluent requires suitable treatment in a properly constructed and maintained treatment system prior to its utilisation. Effluent from these ponds is used to irrigate and fertilise pastures and croplands.

DEW estimated 90 mg tulathromycin could be administered per piglet per year in a 4500 intensive piggery. Assuming no degradation or metabolism occurs, 405 g of tulathromycin could be excreted in $4.2X10^7$L of effluent. Thus, on a yearly basis, the concentration of active in effluent is 9.6 µg/L. At a spread rate of 5 cm deep effluent per hectare of fertilised land, 4.8g tulathromycin could be ploughed or soaked into a hectare of soil 15 cm deep, resulting in the concentration of tulathromycin in fertilised soil being 2.1 µg/kg soil.

In comparison with the environmental end-point of >20 mg/L, the Q value of «0.1 indicates that there is unlikely to be an environmental risk in the aquatic environment should the effluent be discharged directly to water where low dilution rates occur despite the concerns about the aquatic toxicity end points.
Considering that most of the effluent will be contained in ponds and used for irrigation purposes, this scenario is not really a consideration. In rare occasion of a flood or breach in the dam wall, there may be an environmental concern as a result of over-flows from ponds containing tulathromycin to aquatic organisms. However, in this circumstance, a dilution of 10 would normally be incorporated into the Q value, resulting in environmental risk being reduced further.

Run-off Assuming a 10% run-off from the treated cropland, the concentration of tulathromycin would be reduced to 0.21 µg/L. Tulathromycin concentration at this level, when comparing to an environmental end-point of >2 mg/L for *Daphnia magna*, is unlikely to pose an environmental risk to aquatic organisms (Q «0.1).

8.4. Conclusions

DEW has evaluated the applicant's PEC_{soil} calculations for pig and cattle treatment and considered that those calculations were not appropriate under the Australian conditions since they were based on the EU guidelines for assessing the environmental risk of veterinary medicines.

Revised calculations by DEW concur that the PEC_{soil} calculated for cattle and pigs have met the VICH Phase I criteria. On the basis of these calculations, DEW has concluded that the proposed use of Draxxin Injectable Solution on cattle and pigs will not pose an unacceptable aquatic risk and the VICH trigger value of 100 µg/kg soil is unlikely to be exceeded.

9. EFFICACY AND SAFETY ASSESSMENT

9.1. Justification and use pattern

Draxxin Injectable Solution is proposed as a treatment of respiratory infections caused by *Mannheimia haemolytica* and *Pasteurella multocida* in cattle, and *Mycoplasma hyopneumoniae* and *Pasteurella multocida* in pigs. The product is to be administered as a single injection that delivers 2.5 mg tulathromycin/kg body weight subcutaneously in cattle and intramuscularly in pigs.

Macrolide antibiotics, which have been marketed globally for many years, are already available with similar spectra of antimicrobial activity and with similar indications for bovine and porcine respiratory diseases. Draxxin Injectable Solution contains 100 mg/mL tulathromycin, a new macrolide antibiotic that has a long duration of action and achieves high concentrations in lung tissues. This property allows an effective single treatment for bacterial respiratory infections of cattle and pigs. Tulathromycin inhibits bacterial growth by binding to the 50S ribosome. This is similar to currently available veterinary macrolide alternatives such as tilmicosin and erythromycin.

Bacterial respiratory infections in cattle and pigs are common in Australian farms. Bovine and porcine respiratory diseases are multifactorial in aetiology and antibiotic treatment remains an important component of managing disease outbreaks for both welfare and economic reasons.

9.2. Evaluation of efficacy data

9.2.1. MIC and pharmacokinetic studies

In vitro MIC testing was performed in six studies on overseas and Australian bacterial isolates, including *Mannheimia haemolytica* from cattle and *Pasteurella multocida* from cattle and pigs. MIC_{90} data for the Australian isolates of *P. multocida* and *M. haemolytica* from cattle were 2 µg/mL and 8 µg/mL respectively. Corresponding MIC_{90} data for overseas isolates were 1 and 2 µg/mL. The MIC_{90} result from Australian and overseas isolates of *P. multocida* from pigs was 2 µg/mL.

Seven studies evaluated the pharmacokinetic distribution of tulathromycin in cattle and pigs. Tulathromycin accumulated in lung tissue, with lung AUCs being 70x and 60x greater than plasma AUCs of cattle and pigs. Peak lung levels of 4.1 µg/mL and 3.5 µg/mL were attained in cattle and pigs, respectively, 24 hours after a single injection of Draxxin Injectable Solution. Ten days post-injection, mean lung tissue concentrations in cattle were sustained above the MIC_{90} values for *Mannheimia haemolytica* (2.0 µg/mL) and *P. multocida* (1.0 µg/mL) for approximately 9 and 15 days, respectively. Lung concentrations in pigs remained above the MIC_{90} for *P. multocida* (2.0 µg/mL) and *Mycoplasma hyopneumonia* (0.5 µg/mL) for approximately 5-15 days, based on EU data.

9.2.2. Clinical Efficacy Studies

Cattle

In two dose determination studies, subcutaneous doses of 1.25 mg/kg and 2.5 mg/kg tulathromycin were studied in field outbreaks of respiratory disease in cattle. The higher dose proved statistically superior (P=0.007). Three pivotal studies conducted during natural outbreaks of BRD confirmed the efficacy of the 2.5mg/kg dose.

The efficacy of tulathromycin was further confirmed in six controlled blinded studies in which the commercial formulation was used under typical commercial conditions. These studies represented a wide variety of geographic areas, management practices and cattle breeds. Efficacy was assessed on the basis of rectal temperature and respiratory scores. Results were compared with tilmicosin (10 mg/kg bw s/c once) in the EU trials and oxytetracycline (10 mg/kg bw s/c twice at 48 hour intervals) in an Australian feedlot trial. There were no significant difference between tulathromycin and either reference product. In the feedlot trial in which 26.8% of isolates was *M. haemolytica* and 29.9% *P. multocida*, a cure rate of 90.7% was attained, compared to 76.6% for oxytetracycline.

Pigs

Two proof of concept studies and 5 controlled, blinded field efficacy studies were conducted in pigs in the EU. One exploratory study compared doses of 2.5 mg/kg and 5 mg/kg bodyweight, administered once intramuscularly, against an induced infection of *Actinobacillus pleuropneumoniae*. There was no significant difference in efficacy between either dose therefore 2.5 mg/kg was selected as the optimal dose. A second model study supported the efficacy of 2.5 mg/kg in an induced infection of *Mycoplasma hyopneumoniae*.

The five clinical trials represented a variety of geographic areas, management practices and typical field outbreaks of swine respiratory disease from which *M. hyopneumoniae* and *Pasteurella multocida* were identified. Efficacy was assessed on the basis of reduction of rectal temperature, improvement in clinical signs of respiratory disease, mortality rate related to respiratory disease, average daily weight gain and successful completion of the study. Comparisons were made with tiamulin and florfenicol as reference products. Tulathromycin was equivalent to florfenicol and showed significant advantages over tiamulin (P=0.001) in the number of animals able to complete the studies without being withdrawn due to the severity of disease.

9.2.3. Evaluation of safety data

Eight studies examined the tolerance of calves and pigs to tulathromycin at 4x, 5x, 6x and 10x the recommended label dose (2.5 mg/kg bw), margin of safety of the active at 1x, 3x and 5x dose rate, and injection site reactions to the test product.

Minimal to mild histopathological changes were observed in the myocardium of cattle from the 5x and 6x groups treated with a developmental formulation, but not in cattle and calves in the 1x, 3x and 10x groups treated with the final formulation. Excessive vocalisation, restlessness and tremors were observed in pigs treated with the 3x, 5x and 10x injection. These signs were observed up to 2 hours post treatment. Hind leg lameness occurred in pigs that were injected in the hind limb and persisted for 21 days after injection. Mild injection site reactions were observed in cattle and pigs. Swellings in both species persisted up to 28 days after injection, but they completely resolved by day 35 post-injection. Of the cattle treated at the label recommended dose rate under field conditions, 15% displayed injection site swellings. Adverse reactions were not reported in pigs treated at the recommended dose rate and in a further population treated at 2x the dose rate during clinical studies.

9.2.4. Conclusions

Pharmacokinetic studies combined with MIC data support the efficacy of the proposed label dose rate of 2.5 mg/kg bodyweight, administered as a single parenteral dose in cattle and pigs. Clinical field studies demonstrated that tulathromycin was effective for treatment of respiratory infections caused by *Mannheimia haemolytica* and *P. multocida* in cattle, *Mycoplasma hyopneumoniae* and *P. multocida* in pigs.

Results from target animal safety studies and field studies support that the label dose of tulathromycin is well tolerated by cattle and pigs, but it is likely that a proportion of both species will experience localised injection site reactions.

10. SPECIAL DATA ASSESSMENT

Tulathromycin is a semi-synthetic, broad spectrum antimicrobial agent that belongs to the triamilide subclass of macrolide antibiotics. It acts by selectively binding to 23S ribosomal RNA, which leads to a disruption of bacterial protein synthesis. The binding site for tulathromycin is however different from the binding site of the streptogramin combination of quinupristin/dalfopristin. Tulathromycin stimulates the dissociation of peptidyl-tRNA from the ribosome during the translocation process. Tulathromycin has a long duration of action that is in part due to its three amine groups in its chemical structure.

Three mechanisms of resistance in macrolides are known: (1) modification of target site by either methylation of ribosomal RNA or sequence change in the ribosome as a result of a mutational event; (2) inactivation of drug by phosphorylation of the 2-hydroxy group of the amino sugar or hydroxylation of the macrolide lactose ring; and (3) efflux of drug.

The applicant contends that although tulathromycin is active *in vitro* against *Campylobacter*, *Escherichia coli*, *Salmonella* and *Enterococcus*, the microbiological activity is substantially diminished in neutral to acidic pH environment of the intestines. *In vitro* studies show that tulathromycin exposure to *Fusobacterium* and *Bifidobacterium* strains did not inhibit their growth under conditions simulating those of the human gastro intestinal tract. However, *in vivo* data show that dogs had diarrhoea following either acute or repeat dosing with tulathromycin.

10.1. Hazard

The emergence of macrolide-resistant *Campylobacter* due to the use of Draxxin Injectable Solution in cattle and pigs has been identified as the hazard. Based on the data provided, the applicant considers the hazard to be low to negligible because: macrolide resistance in *Campylobacter* occurs by mutation rather than by acquisition of macrolide-resistance genes, the observed frequency of mutation to tulathromycin was below the detection limits expected for spontaneous mutation, and tulathromycin is a relatively poor inducer of macrolide resistance compared with erythromycin.

10.2. Exposure

Susceptible humans could be exposed to macrolide-resistant *Campylobacter* through the consumption of beef or pork from animals treated with tulathromycin. Exposure is considered to be low based on the following: the product is not indicated for use in poultry and dairy cows (poultry commodities and raw milk are common sources of *Campylobacter* infections), about 0.5% of all slaughtered cattle and 2.8% of all slaughtered pigs are expected to be treated with tulathromycin, *Campylobacter* contamination of carcases is substantially lowered after the physical and chemical treatment used in processing beef and pork, and the level of macrolide resistance among human isolates of *Campylobacter* is low (<5%) and have not shown significant increases despite macrolides have been used in animals many years.

10.3. Impact

The impact of macrolide-resistant *Campylobacter* on susceptible humans is considered to be medium. The most vulnerable members of the population are the elderly, the very young and the immunosuppressed individuals. Macrolides are not indicated for enteric diseases caused by *Salmonella*, *Shigella* and *Escherichia coli*. Alternatives such as tetracyclines and fluoroquinolones for therapy of macrolide-resistant *Campylobacter* are available, but in some populations, there are limitations to their use, which make them less acceptable than a macrolide.

10.4. Consequences

Overall, the applicant concludes that the risk of adverse consequences to health as a result of the use of tulathromycin in beef cattle and pigs is estimated to be low.

Uncertainty of data used in risk assessment

A number of uncertainties have been identified and are summarised below.

10.4.1. Uncertainty due to inherent variability and measurement error

Local selection pressures and spontaneous mutations can cause variations in growth and microbial resistance patterns of the bacterial isolates used in the *in vitro* studies. These variations could result in different levels of susceptibility to tulathromycin being detected than those seen in the MIC studies.

In the estimation of disease incidence a number of sources of error exist. These include, variability in the reporting practices of different jurisdictions, differences in the definition of cases of campylobacteriosis, and cultural and behavioural factors such as cooking practices, proximity of the population to poultry and consumption of raw milk.

10.4.2. Uncertainty due to lack of information or understanding

The microbiological activities of tulathromycin are expected to be similar to other macrolides. Until tulathromycin has been utilised extensively *in vivo*, the extrapolations from *in vitro* and *in vivo* studies will be qualified.

Levels of *Campylobacter* contamination on animal and carcasses are affected by: the time of year that samples are taken, age of the animals, size of the herd and the number of locations from which samples are taken. The extent and mechanisms by which some of these factors affect contamination rates are unclear.

Estimates of beef and pork contamination with *Campylobacter* at the retail level are based on relatively few studies, which were designed to evaluate the risk to the consumer rather than identify the source of contamination. There are also limited data on the levels of macrolide resistance of *Campylobacter* still viable on retail meat.

Epidemiological data generally indicates that beef and pork meat are not major sources of *Campylobacter* in humans. Questions still remain about the extent of their contribution, and how much the methods of food preparation contribute to the incidence of campylobacteriosis. Information is lacking on the source of the macrolide resistance

found in the few cases of macrolide-resistant *Campylobacter* that emerge each year. Data on the incidence of human campylobacteriosis reflects cases reported to surveillance systems or included in specific studies, but do not include cases of patients not seeking medical attention.

The impact of improvements in meat processing and handling techniques, and of consumer education campaigns has not been evaluated. It would be expected that they would result in a reduction in the levels of *Campylobacter* and other faecal contaminants on meat prepared for human consumption.

10.5. Health advice

The National Health and Medical Research Council (NHMRC) considered the antimicrobial resistance risk assessment and endorsed the registration of Draxxin Injectable Solution, but recommended that the pack size be limited so as to prevent overuse and increased selection for resistance. NHMRC further recommended that the APVMA give consideration to the half life of tulathromycin, how this characteristic of tulathromycin contributes to its ability to induce resistance, and the relevance to human health given that Draxxin Injectable Solution is in the same class as azithromycin. NHMRC supported the inclusion of a statement on the label that restricts the use of Draxxin Injectable Solution to treatment of the respiratory infections indicated on the label.

11. LABELLING REQUIREMENTS

PRESCRIPTION ANIMAL REMEDY
KEEP OUT OF REACH OF CHILDREN
FOR ANIMAL TREATMENT ONLY

Draxxin
Injectable Solution

100mg/mL TULATHROMYCIN

For the treatment of respiratory infections in cattle and pigs

20/50/100mL

Pfizer [logo]

READ LEAFLET BEFORE USING THIS PRODUCT

DIRECTIONS FOR USE:
Restraints
DO NOT USE in cows which are producing or may in the future produce milk or milk products for human consumption.
Do not use in bobby calves.
USE ONLY in respiratory infections

Dosage: 1 mL per 40kg. Single injection. Cattle S/C. Pigs I/M

WITHHOLDING PERIODS:

Cattle Meat: DO NOT USE less than 35 days before slaughter for human consumption.

Pigs: DO NOT USE less than 14 days before slaughter for human consumption.

Retreatment Interval: Cattle 12 weeks, Pigs 8 weeks

Export Slaughter Interval (ESI): Cattle 35 days, Pigs 26 days.

APVMA: 60018/(20mL)(50mL)100mL/0507____ PM____

Made in France

Pfizer Animal Health
A division of Pfizer Australia Pty Ltd
Wharf Road, West Ryde. NSW 2114

Discard date:
Store below 30°C (Room Temperature).
Batch: Expiry:

LEAFLET/INSERT

PRESCRIPTION ANIMAL REMEDY
KEEP OUT OF REACH OF CHILDREN
FOR ANIMAL TREATMENT ONLY

Draxxin*
Injectable Solution
100 mg/mL tulathromycin

For subcutaneous injection in cattle and intramuscular injection in pigs.

DESCRIPTION

DRAXXIN* Injectable Solution is a ready-to-use sterile parenteral preparation containing tulathromycin, a semi-synthetic macrolide antimicrobial agent. Each mL of DRAXXIN contains 100 mg of tulathromycin as the free base in a 50% propylene glycol vehicle with citric and hydrochloric acids added to adjust pH.

INDICATIONS

Cattle

DRAXXIN Injectable Solution is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica* and *Pasteurella multocida* sensitive to tulathromycin.

Pigs

DRAXXIN Injectable Solution is indicated for the treatment of swine respiratory disease (SRD) associated with, *Pasteurella multocida* and *Mycoplasma hyopneumoniae* sensitive to tulathromycin.

DIRECTIONS FOR USE

Restraints

DO NOT USE in cows which are producing or may in the future produce milk or milk products for human consumption.
DO NOT USE in bobby calves.
USE ONLY in respiratory infections of cattle and swine.

Prudent Use:

Indiscriminate use of tulathromycin can contribute to the development of antibiotic resistance. Culture and sensitivity tests should be performed when appropriate to determine susceptibility of the causative organism(s). Empirical therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

Precautions

Any variation by the prescribing veterinarian to the approved dose, frequency, duration, route, disease or target species may result in the need to extend the approved withholding period.

Avoid using the product simultaneously with other macrolides or lincosamides. Laboratory studies in rats and rabbits have not produced any evidence of teratogenic, foetotoxic or maternotoxic effects. However, the effects of DRAXXIN on bovine and porcine reproductive performance, pregnancy and lactation have not been determined.

Side Effects
Subcutaneous administration of DRAXXIN to cattle frequently causes transient pain reactions, and local swellings at the injection site that can persist for up to 30 days. Pain reactions have not been observed in pigs after intramuscular administration, but local swellings at the injection site can persist for up to 30 days. These swellings may result in trim loss of edible tissue at slaughter.
In one pig field study, one out of 40 pigs exhibited mild salivation that resolved in less than four hours.

Warning: Tulathromycin is irritating to eyes. If accidental eye exposure occurs, flush the eyes immediately with clean water. Users should wear a face shield or goggles when administering Draxxin, to prevent exposure from syringe or needle breakage. Tulathromycin may cause sensitisation by skin contact. If accidental skin exposure occurs, wash the skin immediately with soap and water.
In case of accidental self injection, seek medical advice immediately and show the package insert or the label to the physician.

Dosage and Administration
In use shelf life: Use within 28 days of broaching the vial
Cattle: 1 mL/40 kg body weight (2.5 mg tulathromycin/kg) by a single subcutaneous injection high on the neck. For cattle over 400 kg body weight, divide the dose so that no more than 10.0 mL are injected at one site.
Pigs: 1 mL/40 kg body weight (2.5 mg tulathromycin/kg) by a single intramuscular injection in the neck. For pigs over 100 kg body weight, divide the dose so that no more than 2.5 mL are injected at one site.

WITHHOLDING PERIODS:

Cattle: Meat: DO NOT USE less than 35 days before slaughter for human consumption.

Milk: DO NOT USE in cows which are producing or may in the future produce milk or milk products for human consumption
DO NOT USE in bobby calves.

Pigs: DO NOT USE less than 14 days before slaughter for human consumption.

RE-TREATMENT INTERVALS:
Cattle: DO NOT RE-TREAT cattle for 12 weeks after last treatment.
Pigs: DO NOT RE-TREAT pigs for 8 weeks after last treatment.

EXPORT SLAUGHTER INTERVALS:

Cattle: DO NOT USE less than 35 days before slaughter for export.
Pigs: DO NOT USE less than 26 days before slaughter for export

The ESIs on this label were correct at the time of label approval. Before using this product, confirm the current ESI from the manufacturer on 1800 814 883 or the APVMA website (www.apvma.gov.au/residues/ESI.shtml).

PHARMACOLOGY

Tulathromycin is a semi-synthetic macrolide antimicrobial agent, which originates from a fermentation product. It differs from many other macrolides in that it has a long duration of action that is, in part, due to its three amine groups; therefore it has been given the chemical subclass designation of triamilide. Macrolides are bacteriostatic acting antibiotics and inhibit essential protein biosynthesis by virtue of their selective binding to bacterial ribosomal RNA. They act by stimulating the dissociation of peptidyl-tRNA from the ribosome during the translocation process.

Tulathromycin possesses *in vitro* activity against *Mannheimia (Pasteurella) haemolytica*, *Pasteurella multocida* and *Haemophilus somnus*, and *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Mycoplasma hyopneumoniae*, the bacterial pathogens most commonly associated with bovine and swine respiratory disease, respectively. Increased MIC values have been found in some isolates of *Haemophilus somnus* and *Actinobacillus pleuropneumoniae*.

Resistance to macrolides can develop by mutations in genes encoding ribosomal RNA (rRNA) or some ribosomal proteins; by enzymatic modification (methylation) of the 23S rRNA target site, generally giving rise to cross-resistance with lincosamides and group B streptogramins (MLSB resistance); by enzymatic inactivation; or by macrolide efflux. MLSB resistance may be constitutive or inducible. Resistance may be chromosomal or plasmid encoded and may be transferable if associated with transposons or plasmids.

In cattle, the pharmacokinetic profile of tulathromycin when administered as a single subcutaneous dose of 2.5 mg/kg body weight, was characterised by rapid and extensive absorption followed by high distribution and slow elimination. The maximum concentration (C_{max}) in plasma was approximately 0.5 µg/mL; this was achieved approximately 30 minutes post-dosing (T_{max}). Tulathromycin concentrations in lung homogenate were considerably higher than those in plasma. There is strong evidence of substantial accumulation of tulathromycin in neutrophils and alveolar macrophages. However, the *in vivo* concentration of tulathromycin at the infection site of the lung is not known. Peak concentrations were followed by a slow decline in systemic exposure with an apparent elimination half-life ($t_{1/2}$) of 90 hours in plasma. Plasma protein binding was low, approximately 40%. The volume of distribution at steady-state (V_{SS}) determined after intravenous administration was 11 L/kg. The bioavailability of tulathromycin after subcutaneous administration in cattle was approximately 90%.

In pigs, the pharmacokinetic profile of tulathromycin when administered as a single intramuscular dose of 2.5 mg/kg body weight, was also characterised by rapid and extensive absorption followed by high distribution and slow elimination. The maximum concentration (C_{max}) in plasma was approximately 0.6 µg/mL; this was achieved approximately 30 minutes post-dosing (T_{max}). Tulathromycin concentrations in lung homogenate were considerably higher than those in plasma. There is strong evidence of substantial accumulation of tulathromycin in neutrophils and alveolar macrophages. However, the *in vivo* concentration of tulathromycin at the infection site of the lung is not known. Peak concentrations were followed by a slow decline in systemic exposure with an apparent elimination half-life ($t_{1/2}$) of approximately 91 hours in plasma. Plasma protein binding was low, approximately 40%. The volume of distribution at steady-state (V_{SS}) determined after intravenous administration was 13.2 L/kg. The bioavailability of tulathromycin after intramuscular administration in pigs was approximately 88%.

FIRST AID
If poisoning occurs, contact a doctor or Poisons Information Centre. Phone *131126*.

STORAGE
Store below 30°C (Room temperature).

In use shelf life: Use within 28 days of broaching the vial.

PRESENTATION
DRAXXIN Injectable Solution is available in 50 and 100 mL vials.

For additional DRAXXIN product information call 1800 814 883

DISPOSAL:
Dispose of empty containers by wrapping with paper and putting in garbage.

APVMA Approval No. 60018/0507
Store below 30°C (Room Temperature).
Batch: Expiry:

Made in France

Pfizer Animal Health
A division of Pfizer Australia Pty Ltd
Wharf Road
West Ryde. NSW 2114

PM____

12. GLOSSARY

Active constituent	The substance that is primarily responsible for the effect produced by a chemical product.
Acute	Having rapid onset and of a short duration.
Carcinogenicity	The ability to cause cancer.
Chronic	Of long duration.
Codex MRL	Internationally published standard maximum residue limit.
Consequence	A measurement of the outcome of an adverse event.
Efficacy	Production of the desired effect.
Exposure	The interaction over a period of time between a living subject or the environment with a hazard, which can possibly result in an adverse event.
Formulation	A combination of both active and inactive constituents to form the end use product.
Genotoxicity	The ability to damage genetic material.
Hazard	An agent with the potential to cause an adverse effect on human health.
Histopathological	Findings from examination of tissues under a microscope.
Impact	The positive or negative event that occurs when a living subject is exposed to a hazard.
Intramuscular	Within the muscles
Log P_{ow}	Log to base 10 of octanol water partition co-efficient.
Metabolism	The conversion of food into energy
Subcutaneous	Under the skin
Toxicokinetics	The study of the movement of toxins through the body.
Toxicology	The study of the nature and effects of poisons.

13. REFERENCES

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Footnote:
Updated versions of these documents are available on the APVMA website http://www.apvma.gov.au.