NRA Special Review of

(Dihydro) Streptomycin/ Penicillin Combination Products and (Dihydro) Streptomycin Products

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FOREWORD

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals.

The NRA's Special Review Program allows the NRA to immediately review chemicals if issues arise which may alter the terms of their registration. Chemicals for review are chosen when there is reason to believe the current conditions for registration are no longer met.

In undertaking reviews, the NRA works in close cooperation with advisory agencies including the Department of Health and Aged Care (Chemicals and Non-Prescription Drug Branch), Environment Australia (Risk Assessment and Policy Section), National Occupational Health & Safety Commission (Chemical Assessment Branch) and State Departments of Agriculture.

The NRA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of evaluation documents for all reviews is a part of that process.

The NRA also makes these reports available to the regulatory agencies of other countries as part of bilateral exchange agreements and the OECD *ad hoc* exchange program. Under this program it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

This report provides details of a Special Review of (dihydro) streptomycin and (dihydro) streptomycin /penicillin products for injectable use in food-producing animals that has been conducted by the NRA and its advisory agencies. The review's findings are based on information collected from a variety of sources, including data packages and information submitted by registrants, information submitted by members of the public, key user/industry groups and government organisations and literature searches.

The information and technical data required by the NRA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken. Details of required data for registration are outlined in various NRA publications which can be purchased or obtained by contacting the NRA. Relevant publications include: *Ag Manual: The Requirements Manual for Agricultural Chemicals; Vet Manual: The Requirements Manual for Veterinary Chemicals* and Volume II of *Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products.*

CONTENTS

ABBREVIATIONS AND ACRONYMS

AUC	Area under the curve	**ML**	Millilitre
bw	Body weight	**N**	Number
C_{max}	Maximum concentration	**Sc**	Subcutaneous
DHS	(Dihydro) streptomycin	**$t_{½}$**	Half life
g	Gram	**T_{max}**	Time to attain C_{max}
im	Intramuscular	**U**	Units of activity
IU	International units	**Vd**	Volume of distribution
kg	Kilogram	**µg**	Microgram
mg	Milligram		

AACV	Australian Association of Cattle Veterinarians
ABARE	Australian Bureau of Agricultural and Resource Economics
ADI	Acceptable daily intake (for humans)
AMLC	Australian Meat & Livestock Corporation
AVA	Australian Veterinary Association
AVCARE	National Association for Crop Protection & Animal Health
BRS	Bureau of Resource Sciences
Codex	Codex Alimentarius Commission
D.N.R.E.	Department of Natural Resources and Environment, Victoria, formerly known as the Victorian Department of Agriculture and Rural Affairs
DESI	Drug Efficacy Study Implementation
EU	European Union
FAO	Food & Agriculture Organisation of the United Nations
HPLC	High pressure liquid chromatography or high performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit of detection
LOQ	Limit of quantitation
MIC	Minimum inhibitory concentration
MIT	Microbial inhibition test
MRC	Meat Research Council (now Meat and Livestock Australia)
MRL	Maximum residue limit
MRT	Mean residence time
NOEL	No observable effect level
NRS	National Residue Survey
SCRAP	Subcommittee on Residues in Animal Products
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TLC	Thin layer chromatography
TMDI	Theoretical maximum daily intake
US FDA	United States Food and Drug Administration
VMDA	Veterinary Manufacturers & Distributors Association
WHO	World Health Organisation
WHP	Withholding period

GLOSSARY

Active constituent	The substance that is primarily responsible for the effect produced by a chemical product
Aerobic	Requiring air or free oxygen in order to live
Aetiological	Pertaining to the science or study of the causes of disease
Agalactia	Loss of milk production
Anaerobic	Growing best in an oxygen-free atmosphere
Antagonism	A drug combination produces a lesser effect than that of the most effective drug used alone
Anterior	Toward the front
Antimicrobial	Destroys or inhibits microbes
Bactericidal	An agent that destroys bacteria
Bioequivalent	When the active ingredient in two similar drug products are absorbed at the same rate and to the same extent
Bovine	Cattle
Carrier state	An animal that is infected and sheds bacteria but without clinical signs of infection
Clinical	Symptoms that are observable by a veterinarian or medical doctor
Cocci	Spherical shaped bacteria
Cystitis	Inflammation of the urinary bladder
Dermatitis	Inflammation of the skin
Efficacy	Production of the desired effect
Extrapolate	To make an assumption based on indirect information
Exudative	Exuding a substance that is usually high in protein
Formulations	A combination of both active and inactive constituents to form the end use product
Gram-negative	A bacterium that has been classified as Gram -ve on the basis that it does not retain the violet of Gram's stain
Gram-positive	A bacterium that has been classified as Gram +ve on the basis that it does retain the violet of Gram's stain
Hypotension	Low blood pressure
In vitro	Outside the living body and in an artificial environment
In vivo	In the living animal
Inflammation	Reaction of the tissues to injury, characterised by heat, swelling, redness and pain
Intramammary	Into a mammary (milk) gland eg. a cow's udder
Intramuscular	Into the muscle
Lesions	A structural change caused by disease
Mastitis	Inflammation of the udder usually due to bacterial infection
Metabolism	The conversion of food into energy by bodily processes
Metritis	Inflammation of the uterus
Necrosis	The death of cells in contact with living cells
Nephrotoxicity	Toxicity to the cells of the kidneys
Off-label	Contrary to the approved label directions
Orally	By mouth
Osmotic	Pertaining to osmosis, the passage of fluid through a membrane from a dilute solution into a more concentrated one
Osteomyelitis	Inflammation of the marrow and the bone
Ototoxicity	Toxicity to the ear
Ovine	Sheep
Parenteral	Introduced into the body by any route except via the mouth or any other part of the digestive system
Pathogen	Any living agent capable of causing disease
Peracute	Having a very rapid onset
Peritoneum	Lining of the mammalian abdominal cavity

Peritonitis	Inflammation of the peritoneum
Pharmacokinetics	The study of the movement of drugs through the body over time
Pharmacology	The study of the actions of drugs
Plasma	The fluid portion of blood
Polypharmacy	The prescription of many drugs at the one time
Preputial	Pertaining to the prepuce (foreskin)
Prophylaxis	Prevention of disease
Septicaemia	Severe bacterial infection of the blood
Subcutaneous	Under the skin
Synergy	A drug combination produces a greater effect than the sum of the effects of the two drugs used alone
Systemic	Pertaining to the body as a whole
Therapeutic	To cure or to provide therapy
Virulent	Highly infectious
Zoonotic	Diseases transmissible from animal to man

SUMMARY

The NRA has reviewed the registrations of products containing (dihydro) streptomycin and (dihydro) streptomycin/ penicillin for administration by injection to food-producing animals following advice from the Meat Research Corporation and the State agricultural authorities in NSW and Victoria. Concerns were raised about the therapeutic value of (dihydro) streptomycin/ penicillin combination products as well as the detection of streptomycin residues at injection sites and in liver and kidneys of animals after expiry of label withholding periods, posing a possible threat to Australian meat and offal exports. The objective of this review was to address the issues responsible for the residue violations and the therapeutic justification for the continued registration of the (dihydro) streptomycin/ penicillin combinations.

Key Outcomes

From this review, the NRA has concluded that there is no justification for continuation of the use of injectable (dihydro) streptomycin/ penicillin combination products in food-producing animals. There was limited evidence of efficacy of the combination products in control of virulent footrot and dermatophilosis in sheep, but the dose rates required are above recommended label rates and are likely to lead to residue violations. Other registered uses on food-producing animals were not justified, either by new data or by information in the literature. There was also evidence that use of combination products even at recommended label rates has the potential to lead to residue violations in produce from treated animals.

Similarly, it was not possible to set suitable withholding periods for products containing (dihydro) streptomycin alone and continued registration of the products cannot be supported on residue grounds. However, (dihydro)streptomycin products have several registered uses that lack suitable alternatives and the NRA considers that continued availability of (dihydro) streptomycin products for certain uses under permit is necessary on grounds of human health, animal welfare and trade. These uses include removal of the carrier state of leptospirosis in cattle, pigs and sheep, a disease that causes a significant human health risk, the treatment of campylobacteriosis in bulls and the treatment of live cattle for export to countries who require it.

The review recommends that combination (dihydro)streptomycin /penicillin products are withdrawn from sale for use by injection in food-producing animals and that labels of products for use in non-food-producing animals should be amended to prevent such use.

Products containing streptomycin alone will also be withdrawn from sale for use in food-producing animal species. These products will be available under NRA permits issued to veterinarians for control of leptospirosis in cattle, sheep and pigs, campylobacteriosis in bulls and for live cattle for export to countries which require (dihydro)streptomycin injection prior to shipment. Permits will specify that treated animals for slaughter in Australia are identified and that offal from treated animals must not be used for human consumption.

The review also recommends the withdrawal of MRLs for poultry commodities, which were not supported by residue data.

Detailed recommendations from the review are shown on page 17 of the report.

Technical Evaluations

This review includes (dihydro) streptomycin/ penicillin combination products and (dihydro) streptomycin alone products and is limited to those that are registered for injection in food-producing animals, though other uses may be discussed in the course of the review.
Detailed technical evaluations were carried out on the efficacy, residues and trade implications of continued use of the registered products.

Efficacy

(Dihydro) streptomycin/ penicillin products:

(Dihydro) streptomycin/ penicillin combination products that are formulated for injectable therapeutic use are currently used as broad-spectrum antibiotics for the treatment of infections due to a wide range of Gram-positive and Gram-negative bacteria. These include foot abscess, virulent footrot, osteomyelitis, peritonitis, septicaemia, scours, pneumonia, cystitis, peracute mastitis, pre- and post-operative prophylaxis, metritis, bacterial enteritis, leptospirosis, actinomycosis/actinobacillosis and respiratory, reproductive and urinary tract infections.

Combination products were not shown to be more effective than penicillin used alone. The dosage regime commonly used for virulent footrot and dermatophilosis is likely to compromise the existing MRLs and withholding periods. Effective alternative therapies are available for those disease entities treated currently by the combination products.

(Dihydro) streptomycin products:

Published information indicates that (dihydro) streptomycin alone is efficacious in the treatment of leptospirosis in cattle, pigs and sheep and campylobacteriosis in bulls. Leptospirosis is a zoonotic disease and (dihydro) streptomycin is an important tool to remove the carrier state and thus reduce the contamination of the working environment for butchers, abattoir workers, farmers and veterinarians, as well as controlling the disease in cattle, sheep and pigs. There do not appear to be effective alternatives for the elimination of carriers of leptospirosis and campylobacteriosis in cattle. On this basis, the continued availability of (dihydro)streptomycin products is desirable. These uses can be accommodated by off-label use under permits.

Residues and trade

(Dihydro) streptomycin/ penicillin products:

The residue depletion data which were submitted for some combination products did not support withholding periods appropriate for good veterinary practice for use on beef and dairy cattle, sheep and pigs. The data provided in the review did not allay the concerns raised about residues persisting at injection sites and in liver and kidney for longer than the existing label withholding periods. The continued availability of combination products was considered to pose an unacceptable risk to Australian meat and offal exports.

(Dihydro) streptomycin products:

No data on residues in tissues, milk or injection sites were provided to support the continued registration for the use of (dihydro) streptomycin alone products in cattle, sheep, pigs or poultry. In the absence of supporting data it is not possible to determine appropriate withholding periods for the products.

The continued registration of (dihydro)streptomycin products was considered to pose an unacceptable risk to Australian meat and offal exports. In the case of live cattle exports, the risk is minimised as the cattle are retained at the destination for long periods prior to slaughter. Use of (dihydro)streptomycin products under permit will require the prescribing veterinarian to ensure that animals are identified and that offal from treated animals is not used for human consumption.

1. OVERVIEW REPORT

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) has conducted a Special Review of products and associated labels containing the active constituents (dihydro) streptomycin alone and in combination with penicillin for injection in food-producing animals. The review was initiated by concerns raised by the Meat Research Corporation, the Victorian Department of Agriculture and NSW Agriculture about (dihydro) streptomycin residues in livestock.

The purpose of this document is to provide a summary of the data evaluated and the regulatory decisions.

1.1 Regulatory Information

Initiating a Special Review

The NRA has statutory powers to reconsider the approval of active constituents, the registration of chemical products or the approval of labels for containers at any time. The basis for a reconsideration is whether the NRA is satisfied that the requirements prescribed by the Agricultural and Veterinary Chemicals Code (scheduled to the *Agricultural and Veterinary Chemicals Act 1994*) for continued approval are being met. These requirements are that the use of an active constituent or product, in accordance with the recommendations for its use:

- would not be an undue hazard to the safety of people exposed to it during handling or people using/consuming anything containing its residues;
- would not be likely to have an effect that is harmful to human beings;
- would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment; and
- would not unduly prejudice trade or commerce between Australia and places outside Australia.

Obligations to submit data and other information on chemicals under Special Review

On initiating a review, the NRA has to notify relevant approval holders and registrants of the matters it intends to reconsider and its reasons for doing so, and to invite them to make written submissions on those matters. These parties are also requested to submit all existing information and data relating specifically to the areas to be reviewed (regardless of its age or confidentiality) on the chemical under review.

The NRA may also consult with the members of the community and persons, organisations or government agencies with relevant knowledge or interests for the purposes of obtaining information or advice relating to the review.

Once a review is under way, the NRA may request additional information from approval holders and registrants. If such a request is denied, the NRA may suspend or cancel the relevant approval or registration.

Outcomes of reviews

There are three possible outcomes to a Special Review:

1. The NRA is satisfied that the chemical under review continues to meet the prescribed requirements for the initial approval or registration and confirms the approval or registration.

2. The NRA is satisfied that the conditions to which the approval or registration is currently subject can be varied in such a way that the requirements for continued approval or registration will be complied with and varies the conditions of approval or registration.

3. The NRA is not satisfied that the conditions continue to be met and suspends or cancels the approval or registration.

The NRA must notify the approval holders, registrants and the community of the outcomes of these reviews.

1.2 Data Protection

To grant protection to providers of certain information relating to agricultural and veterinary chemicals, the NRA introduced a program of data protection. The objectives of this program are:

- to provide an incentive for the development of products and data applicable to Australian or local conditions;

- to encourage the availability of overseas products and data; and

- to provide reciprocal protection for Australian products and data under overseas data protection systems.

In general, the NRA designates information as ‘protected registration information’ for a ‘protection period’ of two to seven years if the information:

- is requested by the NRA for the purposes of reviewing a product;
- is relevant to the scope of the review; and

- relates to the interaction between the product and the environment of living organisms or naturally occurring populations in ecosystems, including human beings.

If the NRA proposes to use the same information to determine whether to register, or continue registration, of another chemical product, the NRA must not use the information until the parties come to an agreement as to terms for compensation, unless the protection period has expired or the NRA is satisfied that it is in the public interest to use the information.

1.3 Chemical and Product Details

Penicillin is the generic name for a large group of antibiotic substances derived from several species of *Penicillium*. Some are obtained from cultures of the fungus, while others are prepared by biosynthetic manipulation of 6-aminopenicillanic acid produced by the fungus. Penicillin G (benzyl penicillin) in the form of potassium, benzathine and procaine salts is the most widely used member of the group. Penicillin is a potent β-lactam with rapid bactericidal activity against most anaerobic and many Gram-positive aerobic pathogens in animals. The mode of action is the disruption of the cell wall structure and function by interference with cell wall synthesis. The result is that the bacteria are unable to withstand variable osmotic pressures causing them to absorb water, swell and rupture.

Streptomycin is an aminoglycoside antibiotic derived from *Streptomyces griseus* that was developed in the 1950s. Dihydrostreptomycin is a derivative of streptomycin. Streptomycin and dihydrostreptomycin have very similar properties and for the purposes of this review "(dihydro) streptomycin" refers to either or both antibiotics. (Dihydro) streptomycin is bactericidal and effective against Gram-negative aerobic bacteria and some Gram-positive cocci. The mode of action of streptomycin, as with all the aminoglycosides, is essentially by interference with peptide synthesis. (Dihydro) streptomycin is poorly absorbed when administered orally and is usually administered intramuscularly when systemic therapy is required.

Formulations containing combinations of procaine penicillin and (dihydro) streptomycin were developed during the 1960s because of potential synergy. Penicillin and (dihydro) streptomycin combinations have been popular since the first descriptions of potential synergy where penicillin increased the permeability of the cell membrane to aminoglycosides. This led to the development of fixed ratio (dihydro)streptomycin /penicillin combination products, which are one of the most commonly used parenteral therapeutic antibiotics used in veterinary medicine.

There are nine (dihydro) streptomycin/ procaine penicillin combination and four (dihydro) streptomycin injectable products currently registered for use in food-producing animals in Australia.

Products containing (dihydro) streptomycin/ penicillin in combination include injectable therapeutics and vaccines, while those containing (dihydro) streptomycin alone include

parenteral, oral and intramammary therapeutics. Vaccines may contain (dihydro)streptomycin /penicillin at low concentration to act as a preservative. (Dihydro) streptomycin on its own is generally only used when the diagnosis confirms that the pathogen involved is susceptible to this antibiotic. Its predominance is in the treatment of leptospirosis. (Dihydro)streptomycin-containing products are registered for use in poultry, cats, dogs, pigs, sheep, goats, cattle and horses.

1.4. Reasons for the Review

The NRA has undertaken this review following advice from the Meat Research Corporation, the Department of Natural Resources and Environment (Victoria) and NSW Agriculture.

In August 1996 the (then) Meat Research Corporation (MRC) proposed the withdrawal of all injectable (dihydro) streptomycin/ penicillin combination products for the following reasons:

- Doses of procaine penicillin in (dihydro) streptomycin/ penicillin combination products are two thirds that recommended for effective therapy when they are used alone. Thus it is common practice to use dosages two or more times the label directions. No data are available to determine withholding periods appropriate for such off-label use rates.

- Residues of (dihydro) streptomycin in kidney and liver following label use rates frequently exceed the Australian maximum residue limit (MRL) (0.2 mg/kg) even after the withholding period has expired. The European Union (EU), Australia's major offal market, has provisional tolerances of 1 mg/kg for (dihydro) streptomycin in cattle and sheep kidney and 0.5 mg/kg in liver. These European provisional MRLs are due to expire on 1 June 2000.

- There is evidence that (dihydro) streptomycin residues persist at variable but often high levels at injection sites for more than 60 days following treatment in accordance with the label.

- Approximately 60% of Australia's beef production is exported to Japan, Korea and North America. Japan, Korea and Canada have no tolerances listed for (dihydro) streptomycin. In 1991 a shipment of Australian grain-fed beef was rejected by Japan after 130 mg/kg of (dihydro) streptomycin residue was found. The problem meat originated from a feedlot in which a footrot outbreak was treated with (dihydro) streptomycin/ penicillin. Investigators reported that label WHPs had been observed meticulously.

The concerns about (dihydro) streptomycin residues are confirmed by several studies as follows.

In 1993, the Victorian Department of Agriculture and the Australian Meat and Livestock Commission (AMLC) sampled 3584 cull cows (predominantly dairy cows) at slaughter. Twelve were found to have antimicrobial residues exceeding the Australian MRL. (Dihydro) streptomycin was identified as the predominant residue despite the questionable sensitivity of the methods then in use for that analyte. Six were violative for (dihydro) streptomycin and four for penicillin G.

The Australian Animal Health Committee, through its Subcommittee on Residues in Animal Products (SCRAP), noted the withdrawal of (dihydro) streptomycin/ penicillin combination products in the USA in 1993 after applicants failed to demonstrate synergy in combination products and requested that corresponding Australian registrations be reviewed.

NSW Agriculture recommended that a review of (dihydro) streptomycin/ penicillin combination products be undertaken for similar reasons. They particularly had concerns for the residue implications of high doses of these products when used to treat footrot in sheep. They noted that the usual dose for this purpose was some six times the label dose, administered as a once only injection (compared to repeated injections for label doses). This practice was allowed in NSW and in most other states under the veterinarian's "right to prescribe". Sheep so treated were then returned to their paddocks for six weeks after which they were yarded for inspection. Any sheep that had not responded to the treatment were usually culled for immediate slaughter. This meant an effective six week withholding period was in place, but this was considered unlikely to be adequate to ensure compliance with MRLs. It was considered that such a use pattern could be virtually guaranteed to produce persistent liver, kidney and injection site residues.

In 1995, the National Residue Survey (NRS) screened 802 beef kidney samples and detected three with residues of (dihydro)streptomycin in excess of the Australian MRL[1].

In 1995, the (then) Victorian Department of Agriculture asked the NRA to review the withholding periods for all (dihydro) streptomycin containing products in the light of a commissioned sheep study by Centaur International Pty Ltd[2]. which showed that withholding periods were inadequate for compliance with domestic MRLs.

A 1995 NRA report of a working party on residues of long-acting antibiotics at injection sites considered that injection site residues (including (dihydro) streptomycin) pose a threat to Australian trade in meat.

In view of the above information, the NRA decided to review the registration of the (dihydro) streptomycin/ penicillin combination products as well as products containing (dihydro) streptomycin alone. The purpose of this review is to address the concerns that the use of these products might unduly prejudice trade or commerce between Australia and places outside Australia, or that they might not be effective as claimed.

[1] See Table 1 in this report, NRS randomised monitoring in cattle (July 1993-June 1997).
[2] Webber et al. 1995. Dihydrostreptomycin residues after parenteral administration to sheep of Aquacaine S. Centaur International Pty Ltd., Hamilton, Victoria.

1.5 Scope of the Review

The NRA has therefore reconsidered, under Division 4, Part 2 of the AgVet Code:

- the approval of the active constituents streptomycin and dihydrostreptomycin
- all current registrations for injectable therapeutic products containing (dihydro) streptomycin/ penicillin in combination and (dihydro) streptomycin alone for use in food-producing animals
- associated label approvals

All injectable preparations containing (dihydro) streptomycin alone or in combination with procaine penicillin when used on food-producing animals are within the scope of this review while injectable formulations of procaine penicillin as the sole active ingredient and oral preparations of (dihydro) streptomycin are not. Similarly, intramammary, intrauterine and topically applied products containing (dihydro) streptomycin do not fall within the scope of this review. Uses on companion animals are also excluded.

Injectable vaccines containing (dihydro) streptomycin/ penicillin have not been included in this review because the quantities of antibiotic added to maintain the integrity of vaccines are too small to be considered a risk in terms of residues.

The following data were requested from applicants and assessed during the review process:

- Residue depletion studies at injection sites, kidney and liver, including withholding periods;
- Pharmacokinetics and general efficacy data on both (dihydro) streptomycin alone and in combination with penicillin.

A literature review of the efficacy for the (dihydro) streptomycin/ penicillin combination products and products containing (dihydro) streptomycin alone was also conducted because the therapeutic value of the combination products had been challenged. The therapeutic value of (dihydro) streptomycin for use in modern veterinary medicine also needed to be critically assessed.

1.6. Overseas Registration / Regulatory Status

European Union (EU)

(Dihydro) streptomycin/ penicillin combination products and (dihydro) streptomycin products are currently registered for use in the EU. This situation may change when the temporary MRLs due to expire on 1 January 2000 are reviewed.

Codex Alimentarius Commission (Codex)

(Dihydro) streptomycin has been evaluated and reported in the Forty-third Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Based on an assessment of toxicological and microbiological data, the committee established a temporary acceptable daily intake (ADI) of 0-30 μg/kg bw for the combined residues of (dihydro) streptomycin.

Temporary MRLs for (dihydro) streptomycin were adopted by Codex at the 43rd session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). MRLs recommended were 0.5 mg/kg for muscle, liver and fat of cattle, pigs, chickens and sheep and 1 mg/kg for kidney in the same species. The committee recommended that further information from experiments and expert opinion be sought on farm animal metabolism of (dihydro) streptomycin as well as residue data for eggs. Information on the relationship between antimicrobial activity of the residues and the measurement of residues by specific chemical methods was also requested.

United States of America (USA)

(Dihydro) streptomycin/ penicillin combination products were marketed on an interim basis from 1971 to 1993, following a National Academy of Sciences – National Research Council report which concluded that the combination of penicillin and (dihydro) streptomycin was not more effective than either antimicrobial used alone. As a result of this report, registrants were asked to submit data to support combination synergistic effects. No data were submitted to show that combining the drugs in a single product was more effective than the use of ether antibiotic alone. Subsequently, all combination products containing penicillin and (dihydro) streptomycin were voluntarily withdrawn from the US market by their respective manufacturers in 1993.

1.7 Australian Registration / Regulatory Status

Registered products

The ten injectable (dihydro) streptomycin/ penicillin combination products currently registered for therapeutic use in Australia are included in Table 1.

Table 1 – Injectable (dihydro) streptomycin/ penicillin combination products currently registered for therapeutic use.

Registrant	Product name	Species
Boehringer Ingelheim P/L	Aquacaine S Injectable Suspension	Cats, cattle, dogs, goats, horses, pigs & sheep
Bomac Laboratories P/L	Hydropen Penicillin/Dihydrostreptomycin Injection	Cats, cattle, dogs, pigs & sheep
Dopharma Australia P/L	Dopharma Penicillin/Streptomycin	Cattle, horses, pigs & sheep
Novartis Animal Health Australasia P/L (syn. Norbrook, nee Youngs, Heriot).	Penicillin & Streptomycin Injection	Cats, cattle, dogs, horses, pigs & sheep
Intervet (Australia) P/L	Biodexamine Sterile Suspension for IM Injection	Cats, cattle, dogs, horses, pigs & sheep
	Depomycin Procaine Penicillin & Dihydrostreptomycin Injection	Cats, cattle, dogs, horses, pigs & sheep
Jurox P/L	Streptopen Injection	Cats, cattle, dogs, goats, horses, pigs & sheep
	Vetstrep Injection Aqueous Suspension	Cats, cattle, dogs, goats, horses, pigs & sheep
Troy Laboratories P/L	Ilium Penstrep Sterile Injection	Cats, cattle, dogs, horses, pigs & sheep
	Ilium Penstrep Injection	Dogs, cattle, horses, pigs & sheep

There are also four injectable (dihydro) streptomycin products currently registered for therapeutic use in Australia including one product registered only for use in poultry. These are listed in Table 2.

Table 2 – Injectable (dihydro) streptomycin products currently registered for therapeutic use.

Registrant	Product name	Species
CCD Animal Health	C.C.D. Formula 4 Dihydrostreptomycin Sulphate	Poultry
Hart Ray Kent	Leptostrep	Cattle
Jurox P/L	VR Vibrostrep Injection	Cattle (Bulls)
	Strepolin SD Injection	Cattle, dogs, horses, pigs & sheep

All injectable (dihydro) streptomycin/ penicillin combination products and (dihydro) streptomycin products registered for therapeutic use in Australia are classified as Schedule 4 in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). These products are restricted for use by veterinarians and by others under the direct control of a veterinarian.

Current permits

There are no NRA permits current for injectable (dihydro) streptomycin/ penicillin combination products or injectable products containing only (dihydro) streptomycin for therapeutic use.

1.8 Notification of the Review

All registrants of injectable veterinary products containing (dihydro) streptomycin including those in combination with penicillin, used in food-producing animals were notified of the review, the reasons for review and the specific data required for that review in accordance with Division 4 of Part 2 of the Agricultural and Veterinary Chemicals Code Act 1994 (Agvet Code).

The NRA also notified all members of the Registration Liaison Committee (RLC), the Australian Veterinary Association, Australian Meat, Pork, Goat and Dairy Industry Councils, National Residue Survey, Australian Meat and Livestock Corporation, AVCARE, VMDA and others.

1.9 Stakeholder Responses

During the review, the NRA consulted with several interested parties including veterinary associations, government bodies and registrants. Details of key issues are presented below. While all views expressed were considered in the review, these remain views of the authors and not those of the NRA.

Veterinary Associations

The Australian Association of Cattle Veterinarians (AACV) reported that they actively discourage the use of (dihydro) streptomycin/ penicillin combination products due to the poor disease spectrum of (dihydro) streptomycin, the prolonged WHP and the potential for residue problems. The AACV recognised (dihydro) streptomycin as the drug of choice for campylobacteriosis, leptospirosis and actinobacillosis and supported continued availability for these uses. They consider the use of (dihydro) streptomycin/ penicillin combination products to constitute polypharmacy, which is not considered to constitute "good veterinary practice".

The Australian Veterinary Association **(**AVA) supported the withdrawal from sale in Australia of (dihydro) streptomycin/ penicillin combination products. The Association noted that it is important that veterinarians retain access to an injectable (dihydro) streptomycin product that they can use for specific disease conditions for which the drug may offer particular therapeutic advantages.

Government bodies

NSW Agriculture recommended that residue data on (dihydro) streptomycin be reviewed to reduce the likelihood of persistent residues occurring in the liver and kidneys or at injection sites of treated food animals. They also noted the importance of (dihydro) streptomycin in the treatment of leptospirosis and campylobacteriosis (vibriosis), both of which are widespread in NSW. Loss of (dihydro) streptomycin would severely

compromise treatment of both these infections and may have animal welfare implications. While there are leptospirosis vaccines available, these do not cure infected animals and because "cure" is presently impossible without (dihydro) streptomycin treatment, many overseas countries to whom Australia exports live cattle and deer require a pre-shipment course of treatment with (dihydro) streptomycin.

The Bureau of Resource Sciences (BRS), which conducts the National Residue Survey, supported the de-registration of (dihydro) streptomycin/ penicillin combination products. BRS considers that the withholding periods for these products are inadequate as demonstrated by the number of residue violations detected. BRS believes that the appropriate withholding period is likely to be in excess of 50 days, and questions whether it is "good veterinary practice" to use a product with such a long withholding period.

Results from the NRS Randomised Monitoring Program indicate that (dihydro) streptomycin is the most common of the antimicrobial residues detected in slaughtered cattle and, as such, poses a significant and unacceptable risk to Australia's export beef markets.

Registrants

Intervet (Aust.) advised that in their view, (dihydro) streptomycin was the treatment of choice for leptospirosis and campylobacteriosis. They stated that it had been shown that leptospirosis can be eliminated from piggeries with a single injection given to all pigs. Intervet argued that there is still a place for (dihydro) streptomycin/ penicillin combination products because the combination products exhibit synergy in the treatment of footrot and fleece rot (dermatophilosis). They suggested that combination products were the treatment of choice for anthrax, ovine infectious/virulent footrot and fleece rot and that the combination broadens the spectrum because penicillin is mainly active against Gram-positive bacteria while (dihydro) streptomycin is mainly active against Gram-negative bacteria. Intervet suggested that it was in Australia's trade interests that we do not export products containing pathogens such as those responsible for anthrax, infectious/virulent footrot and fleece rot (dermatophilosis) in sheep.

Norbrook Laboratories Limited advised that they consider (dihydro) streptomycin/ penicillin injection an established antimicrobial treatment which is both safe and effective. With respect to residues, they argue that if the Australian authorities were to adopt the MRLs which have been recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA), then the scale of the alleged (dihydro) streptomycin residue problem in Australia would be dramatically reduced. Norbrook Laboratories are opposed to the proposal that (dihydro) streptomycin/ penicillin combination products be withdrawn from the Australian food animal market, as they believe there is no sound scientific basis for such a proposal.

Jurox Pty Limited argued that injection site residues and residues in general are unlikely to be an issue with their product, which is used for the treatment of bovine campylobacteriosis in breeding bulls. They argued that the animals are unlikely to be slaughtered for a significant period of time after treatment.

1.10 Evaluation of Submissions

The detailed efficacy assessment is included as Section 2 of this report and the residues and trade assessment is included as Section 3.

The main findings of the efficacy and residues reviews are summarised below.

(Dihydro) streptomycin/ penicillin combination products

Beef and Dairy Cattle

Efficacy and pharmacokinetic data were provided by Norbrook, Intervet and Dopharma for their (dihydro) streptomycin/ penicillin combination products, but the data presented did not demonstrate synergy *in vivo*. Other registrants of combination products failed to provide data. In the absence of synergism, there can be no justification for using (dihydro) streptomycin/ penicillin combination formulations in preference to penicillin alone.

Residue data were submitted for six combination products but the submitted tissue residue data did not support continued registration for use on beef or dairy cattle.

Sheep

There is sufficient research data available to demonstrate synergy when (dihydro) streptomycin/ penicillin combination formulations are used (at greater than label rates) to treat both virulent footrot and dermatophilosis in sheep. There was no evidence to demonstrate synergy in clinical cases of virulent footrot or dermatophilosis when treated at label dose rates. It should be noted that the use pattern commonly used to treat both these diseases is not included on the approved labels of these products. This represents an "off-label" use and as such renders the label withholding period invalid. It is then the responsibility of the prescribing veterinarian to advise the producer of an appropriate withholding period without having access to scientific data to determine this.

Residue data were submitted for four products but these data did not support continued registration of these products for use in sheep. No data were provided in support of the other five products.

Pigs

The efficacy and pharmacokinetic data presented by Norbrook, Intervet and Dopharma did not demonstrate synergy *in vivo*. Other registrants did not provide supporting data. Therefore the use of (dihydro) streptomycin/ penicillin combination formulations cannot be justified on the grounds of synergism.

Residue data were submitted for four products but the residue data for three of these did not allow a withholding period to be set. Although tissue residue data for the fourth product (Depomycin) demonstrated that residues were below the MRL within 35 days,

the proposed withholding period of 35 day is not considered to be compatible with good veterinary practice.

Data deficiencies

There were three products for which no data addressing residues, withholding periods, pharmacokinetics or efficacy were submitted. If continued registration of these products were to be proposed, data would be required to address the following areas of concern:

- Residue depletion studies at injection sites, kidney and liver, including withholding periods

- Pharmacokinetics and general efficacy data on (dihydro) streptomycin in combination with penicillin

(Dihydro) streptomycin products

No data were submitted for any of the (dihydro) streptomycin products.

(Dihydro) streptomycin on its own is generally only used when the diagnosis confirms that the pathogen involved is susceptible to this antibiotic. It is used to treat the zoonotic disease leptospirosis in cattle, pigs, sheep and dogs. The ability of (dihydro) streptomycin to remove the carrier state makes it an important tool in reducing the contamination of the working environment for butchers, abattoir workers, farmers and veterinarians as well as controlling the disease in cattle, sheep, pigs and dogs. The use of (dihydro)streptomycin for these uses should continue, but as an off-label use under permit with careful identification of treated animals to prevent residue violations.

The use of (dihydro) streptomycin products to meet the requirements of the live cattle export trade is not considered to pose a threat to trade as the cattle would be retained for fattening in the country of destination for more than 12 months. The use of (dihydro) streptomycin for the treatment of cattle for live export can be accommodated by the issuing of NRA permits.

A balance between the special considerations relating to human health, efficacy against animal pathogens, and the risks to trade posed by residues of (dihydro) streptomycin must be established. Most of the diseases for which (dihydro) streptomycin/ penicillin combination products are used can be controlled effectively by penicillin alone. If combination products are being used where penicillin would suffice, then the removal of the (dihydro) streptomycin/ penicillin combination products together will reduce the total quantity of (dihydro) streptomycin used in food-producing animals. For the two diseases (leptospirosis and campylobacteriosis) where (dihydro) streptomycin is the most effective treatment, the off-label use of the single antibiotic (dihydro)streptomycin under permit in place of combination products will also reduce the antibiotic load in food-producing animals by reducing the amount of penicillin used.

The use of (dihydro) streptomycin in poultry appears to be in decline because there are many alternatives that are easier to administer. The literature indicates that (dihydro) streptomycin is no longer the drug of choice for chronic respiratory disease and coryza in poultry. Whilst the use of (dihydro) streptomycin in poultry is not considered to pose a risk to trade, the use of (dihydro) streptomycin in poultry intended for slaughter for human consumption cannot be supported as there is currently no Australian MRL in the *MRL Standard* for (dihydro) streptomycin in poultry meat. The only product currently registered for use in poultry is not registered for use on hens producing eggs for human consumption.

1.11 Conclusions

In the absence of proven synergy at label dose rates, there can be no justification for using (dihydro) streptomycin/ penicillin combination formulations in preference to penicillin alone. Single intramuscular injections of (dihydro) streptomycin/ penicillin at a dose rate of 70,000 U/kg procaine penicillin and 70 mg/kg dihydrostreptomycin are effective and have been a widely used treatment for both virulent footrot and dermatophilosis in sheep. This dosage regime is higher than the registered label dose rate and existing withholding periods are therefore invalid. Apart from these conditions, the use of (dihydro) streptomycin/ penicillin combination formulations cannot be justified on the grounds of synergy.

The residue depletion data submitted by some registrants of (dihydro) streptomycin/ penicillin combination products do not allow the setting of appropriate withholding periods when considered in conjunction with good veterinary practice. The data provided do not address the concerns about residues persisting at injection sites and in liver and kidney for longer than the withholding period. This is considered to pose an unacceptable risk to Australian meat and offal exports.

(Dihydro) streptomycin alone is considered the treatment of choice in the treatment of leptospirosis in cattle, pigs, sheep and dogs, and campylobacteriosis in bulls. Leptospirosis is a zoonotic disease and (dihydro) streptomycin is an important tool to remove the carrier state and thus reduce the contamination of the working environment for butchers, abattoir workers, farmers and veterinarians, as well as controlling the disease in cattle, sheep, pigs and dogs. The off-label use of (dihydro)streptomycin under permit will allow the continued use, where necessary, for these conditions.

1.12 Review Outcomes

On the basis of the reconsideration of (dihydro)streptomycin products and (dihydro)streptomycin /penicillin combination products for injectable use in food producing animals, the NRA has decided that:

1. For all injectable therapeutic products containing penicillin in combination with (dihydro) streptomycin:

- The Australian manufacturers and the importers must cease the sale and distribution of product labelled for use in food-producing animals by **30 June 1999**.

- The retail sale of current stocks of product currently labelled for use in food-producing animals may continue until **30 June 2000.**

- The registrants of product currently approved for use in non-food-producing animals must submit draft labels for approval by the NRA before **30 June 1999** amended as follows:

    a) Remove any reference to use in food-producing animals (not including horses) from the claims, directions for use and withholding period statements;

    b) Add the following restraint statement "**NOT TO BE USED in food-producing animals**";

    c) Amend the horse meat withholding period to "**NOT TO BE USED in horses that may be slaughtered for human consumption**";

    d) Pack sizes are to be limited to 50 mL.

- All future applicants for new products must submit draft labels consistent with the amendments required for currently registered products.

2. For all injectable therapeutic products containing only (dihydro) streptomycin:

- The Australian manufacturers and the importers must cease the sale and distribution of product labelled for use in food-producing animals by **30 June 1999**.

- The retail sale of current stocks of product currently labelled for use in food-producing animals may continue until **30 June 2000.**

- The registrants of product currently approved for use in non-food-producing animals must submit draft labels for approval by the NRA before **30 June 1999** amended as follows:

    a) Remove any reference to use in food-producing animals (not including horses) from the claims, directions for use and withholding period statements;

    b) Add the following restraint statement "**NOT TO BE USED in food-producing animals except under NRA permit**";

c) Amend the horse meat withholding period to "**NOT TO BE USED in horses that may be slaughtered for human consumption**".

- The NRA will issue permits to veterinarians to allow use in the control of leptospirosis in cattle, sheep and pigs and the control of campylobacteriosis in bulls.

- The NRA will issue permits for treatment of live cattle destined for export.

- The above permits will specify that treated animals for slaughter in Australia are identified and that offal from treated animals must not be used for human consumption and that injection volumes must be limited to 10mL at any one site.

3. Amend the *MRL Standard* as follows:

Delete:	Table 1	PE 0112	Eggs *0.2

4. Recommend to ANZFA to amend Standard A14 of the Food Code as follows:

Delete:	Eggs	*0.2
	Poultry, edible offal of	0.2
	Poultry meat	0.2

5. The NRA consider a separate review of all aminoglycoside products used on the major food animal species.

2 EFFICACY ASSESSMENT

2.1 Introduction

This efficacy review is a review of the scientific literature, published studies and data provided by the registrants and other interested groups. The purpose of this efficacy review is to provide an objective overview of the current scientific information available on the efficacy of (dihydro) streptomycin/ penicillin combination formulations and also (dihydro) streptomycin when used as the sole antibiotic. The focus of the review is on injectable products used parenterally for food-producing animals.

2.2 (Dihydro) streptomycin /Penicillin Combination Products

2.2.1 Background

In Australia, (dihydro) streptomycin injectable products, and those in combination with procaine penicillin, find many uses in veterinary medicine. Products containing (dihydro) streptomycin are used to treat a variety of conditions particularly in cattle, sheep and pigs.

The major reasons for this popularity appear to be cost, broad-spectrum activity and the frequently stated view that the combination exhibits synergism against most pathogenic organisms encountered in veterinary practice. This perception of synergistic activity has been promoted by both veterinary textbooks and in university veterinary schools for many years. Synergy is the notion that the (dihydro) streptomycin/penicillin combination produces a greater effect than the sum of the effects of the two antibiotics used alone[(1)]. Synergy has been demonstrated on numerous occasions *in vitro*, however no assumption can be made that the effect occurs *in vivo*[(1)]. Jawetz[(2)] stated that "It is incorrect and misleading to speak of a synergistic drug combination, without specifying a specific microbial strain".

The notion of synergy was challenged by the Food and Drug Administration of the USA (USFDA) in 1971. A study report indicated that the combination of penicillin and (dihydro) streptomycin did not provide greater efficacy than either drug administered alone[(3)]. Similarly, the veterinary literature provides no evidence that (dihydro) streptomycin/ penicillin combination formulations provide better therapy for any bovine infections than can be achieved by single drug therapy with either drug used alone.

Furthermore, when the United States Food and Drug Administration (US FDA) mandated a requirement to produce such evidence, no evidence was forthcoming over 22 years[(4)]. The combination formulations were subsequently and voluntarily withdrawn from the market in 1993[(5)].

2.2.2 Current Usage

Table 3 summarises the currently approved uses of (dihydro) streptomycin/ penicillin combination products for food-producing animal species.

Table 3 Registered (dihydro)streptomycin /penicillin combination products

Product name	Species	Label dose rate of product	Concentration of penicillin	Concentration of (dihydro) streptomycin
Aquacaine S Injectable Suspension	Cattle, pigs Goats, sheep	5 mL/100 kg im/day 1 mL/10 kg im/day	200 mg/mL	250 mg/mL
Hydropen (dihydro) streptomycin/ penicillin injection	Cattle Pigs & sheep	10 to 20 mL im/day 3 to 5 mL im/day	113.5 mg/mL	200 mg/mL
Dopharma Penicillin/ Streptomycin	Cattle Pigs & sheep	1 mL/20 kg im/day (3-5days) 1 mL/20 kg im/day (3-5days)	200 mg/mL	200 mg/mL
Penicillin & Streptomycin Injection	Cattle Pigs & sheep	16 mL/400 kg im or sc/day 2 mL/50 kg im or sc/day	200 mg/mL	250 mg/mL
Biodexamine Sterile Suspension for IM Injection	Cattle, pigs Sheep	15 to 20 mL im/day 8 mL im/day	200 mg/mL	250 mg/mL
Depomycin Procaine Penicillin & Dihydrostreptomycin Injection	Cattle, pig Sheep	16 mL/400 kg im/ day 2 mL/50 kg im/day	200 mg/mL	250 mg/mL
Streptopen Injection	Cattle, pigs Sheep	15 to 20 mL im/day 3 to 5 mL im/day	250 mg/mL	250 mg/mL
Vetstrep Injection Aqueous Suspension	Cattle, pigs Goats, sheep	15 to 20 mL im/day 3 to 5 mL im/day	250 mg/mL	250 mg/mL
Ilium Penstrep Sterile Injection	Cattle, pigs Sheep	10 mL/250 kg 2 mL/50 kg	250 mg/mL	250 mg/mL

(Dihydro) streptomycin/ penicillin combination products that are formulated for injection are currently used as broad-spectrum antibiotics for the treatment of infections due to a wide range of Gram-positive and Gram-negative bacteria. These include foot abscess, osteomyelitis, peritonitis, septicaemia, scours, pneumonia, cystitis, peracute mastitis, pre- and post-operative prophylaxis, metritis, bacterial enteritis, leptospirosis, actinomycosis/actinobacillosis and respiratory, reproductive and urinary tract infections. They are also widely used in footrot eradication and control programs.

2.2.3 Cattle

Use pattern

(Dihydro) streptomycin/ penicillin combination products are used to treat both leptospirosis in cattle and campylobacteriosis in bulls. *Campylobacter fetus* subspecies *venerealis* (also known as campylobacteriosis or vibriosis) is one cause of infertility and abortion in cattle. Whilst (dihydro) streptomycin/ penicillin combination products are not the first choice for control of leptospirosis and campylobacteriosis, they are frequently used due to their ready availability and low cost.

Actinobacillosis (*Actinobacillus lignieresii*) is a specific infectious disease of cattle characterised by inflammation of the tongue leading to the common name of wooden tongue. Veterinary textbooks recommend (dihydro) streptomycin/ penicillin combination formulations for the treatment of actinobacillosis [11, 12] and bovine dermatophilosis[9, 11, 13].

One registrant indicated that (dihydro) streptomycin/ penicillin combination formulations were the products of choice for anthrax (*Bacillus anthracis*). Lincoln *et al* [17] recommend penicillin or (dihydro) streptomycin/ penicillin for the treatment of septicaemic anthrax in most animals. This appears to be the only study that indicates that synergy may exist for penicillin and (dihydro) streptomycin in the treatment of anthrax. Unfortunately the number of mice treated in this study with penicillin and (dihydro) streptomycin was too small to be confident that synergy had been demonstrated. Other literature suggests that penicillin is the drug of choice for the treatment of anthrax, and also states that synergism may occur when streptomycin is combined with penicillin[18, 19], but it is considered that limited and unconvincing evidence of synergy between penicillin and (dihydro) streptomycin has been reported in the treatment of anthrax in mice[17].

Efficacy

Exhaustive literature searches have identified no reports of evidence for *in vitro* synergism of (dihydro) streptomycin/ penicillin combination formulations in cattle[9, 11, 12, 13, 14]. Although (dihydro) streptomycin/ penicillin combination products are used to treat both leptospirosis in cattle and campylobacteriosis (bulls), there is no evidence to suggest that these are more effective than (dihydro) streptomycin administered alone. The Australian Association of Cattle Veterinarians (AACV) is not in favour of mixed antibiotic products and actively discourages the use of (dihydro) streptomycin/ penicillin combination products by its members because of the prolonged withholding period and the residue problems associated with (dihydro) streptomycin[10].

The registrants of (dihydro) streptomycin/ penicillin combination formulations were asked to provide pharmacokinetics and general efficacy data to support these products. There were no published or unpublished data supplied by the registrants to demonstrate synergy between penicillin and (dihydro) streptomycin *in vivo.* Some of the studies did show synergy for bovine mastitis pathogens *in vitro* but did not confirm synergy in

clinical cases[1]. While there was some evidence of synergy for ovine dermatophilosis, synergy has not been demonstrated for bovine dermatophilosis.

Argument from the registrants that (dihydro) streptomycin/ penicillin combination formulations provide synergism in the treatment of bovine infections is not supported by evidence from the literature. Proof of synergy requires the conduct of blinded clinical trials with positive controls. Reports of such trials for (dihydro) streptomycin/ penicillin combination formulations have not been published[3].

Dopharma, Intervet and Norbrook submitted a number of unpublished studies. Most of these were *in vitro* studies to determine the minimum inhibitory concentration (MIC) of both penicillin and (dihydro) streptomycin in combination and alone against a range of pathogens responsible for veterinary diseases. The *in vitro* studies demonstrated synergy between penicillin and (dihydro) streptomycin against a number of veterinary pathogens. Some *in vivo* studies were submitted to determine the blood plasma concentrations of both penicillin and dihydrostreptomycin in cattle, pigs, sheep and horses. The *in vivo* studies demonstrate that adequate blood levels of both penicillin and (dihydro) streptomycin are achieved following administration of specific (dihydro) streptomycin/ penicillin combination formulations.

Another claimed advantage of (dihydro) streptomycin/ penicillin combination formulations is their broad-spectrum activity. This is based on the known activity of penicillin against most anaerobic and many Gram-positive aerobic pathogens and (dihydro) streptomycin against Gram-negative aerobic bacteria and some Gram-positive cocci. However, with the exception of leptospirosis and campylobacteriosis, the spectrum of activity of (dihydro) streptomycin against bovine pathogens is small[3]. In the absence of synergy, there is little justification for using (dihydro) streptomycin/ penicillin combination formulations in preference to either penicillin or (dihydro) streptomycin alone.

Alternatives[3]

Table 4 Alternatives to (dihydro) streptomycin/ penicillin in cattle

Species	Disease	Alternatives to (dihydro) streptomycin/ penicillin
Cattle	Actinobacillosis	Sodium and potassium iodide, penicillin, tetracycline and (dihydro) streptomycin
	Anthrax	Penicillin, erythromycin and tetracycline
	Campylobacteriosis	(Dihydro) streptomycin
	Dermatophilosis	Tetracycline
	Leptospirosis	(Dihydro) streptomycin

There are many alternatives for the treatment of actinobacillosis in cattle, including sodium and potassium iodides[14, 22], penicillin[9, 23], tetracycline[14, 23, 24] and (dihydro) streptomycin[9, 14, 23, 24].

[3] The residue status of these alternatives has not been investigated in this review.

Penicillin is the drug of choice for the treatment of anthrax, although erythromycin and tetracycline are also effective[18, 19].

(Dihydro) streptomycin is the drug of choice for the treatment of *Campylobacter fetus* subspecies *venerealis* carrier state in bulls[21] and also provides an effective alternative for the elimination of carriers of leptospirosis in cattle.

Tetracycline provides an effective alternative for the treatment of dermatophilosis in both sheep and cattle. It has been demonstrated that high doses of (dihydro) streptomycin/ penicillin combination formulations failed to cure dermatophilosis, while a single dose of tetracycline (20 mg/kg) was effective[25].

Leptospirosis in cattle can be treated effectively by (dihydro) streptomycin. Recent literature suggests that tetracyclines and amoxycillin may be effective alternatives to (dihydro) streptomycin for treating cattle with *Leptospira borgpetersenii* serovar *hardjo*[60]. Their efficacy has not been established for other *Leptospira* isolates.

2.2.4 Sheep

Use pattern

Virulent footrot (*Dichelobacter nodosa*) is a sheep disease of major economic importance in some areas of Australia. Eradication programs are generally based on controlling the disease (which includes the use of antibiotics) during periods when it is spreading followed by repeated inspection of sheep when it is not spreading. Sheep that are identified as infected on inspection may be either culled or treated to eliminate infection. In such cases (dihydro) streptomycin/ penicillin combination products are used under veterinary advice as a single dose at 6 to 7 times the recommended label rate.

Ovine dermatophilosis (lumpy wool) is an important disease of sheep caused by *Dermatophilus congolensis.* This is an exudative dermatitis causing hard scabs on the skin and wool of sheep. Dermatophilosis can be an important cause of financial loss[6,7] because of reduced wool production, culling of affected sheep, treatment costs, reduced value of wool, hide damage and increased risk of flystrike. Most lesions in sheep resolve within a few weeks, but some take many months to heal[8]. The main indication for treatment has been to permit shearing of infected sheep. Systemic antibiotics are the preferred treatment. (Dihydro) streptomycin/ penicillin combination products (at greater than label rates) are widely used in the treatment of ovine dermatophilosis[9].

Actinobacillosis (*Actinobacillus lignieresii*) is an infectious disease of sheep, causing lesions in the lips and cheeks. Affected sheep have difficulty in eating and may die of starvation. Treatment is with antibiotics or iodine.

Efficacy

There is field evidence that (dihydro) streptomycin/ penicillin combination formulations at very high dose rates are effective in the cure of dermatophilosis in experimentally and

naturally infected sheep, despite an inability to show synergy *in vitro*[15]. Similarly, a single intramuscular injection of (dihydro) streptomycin/ penicillin at a dose rate of 70,000 U/kg procaine penicillin and 70 mg/kg (dihydro) streptomycin is an effective and widely used treatment for virulent foot-rot[16]. In these cases it has been contended that synergy has been demonstrated between the (dihydro)streptomycin and penicillin. It should be noted however, that synergy has only been demonstrated in the treatment of virulent footrot and dermatophilosis in sheep following the use of dose rates considerably greater than the registered label rates.

Support for the use of (dihydro) streptomycin/ penicillin combination formulations or the existence of any synergism for the treatment of bovine or ovine actinobacillosis was not found in the literature.

In May 1994 the AVA Therapeutics Advisory Committee published a discussion paper on injectable antibiotics for cattle and sheep. Two respondents drew attention to scientific studies that (dihydro) streptomycin/ penicillin combination formulations may be superior to penicillin alone for the treatment of virulent infectious foot-rot and dermatophilosis in sheep. Synergy between penicillin and (dihydro) streptomycin was demonstrated in the treatment of dermatophilosis in sheep following use of dose rates considerably greater than the registered label rates. With these two exceptions, no livestock diseases were identified where (dihydro) streptomycin/ penicillin combination formulations were considered superior to other antibiotics[20].

The published studies[8, 15, 16] demonstrating synergy of penicillin and (dihydro) streptomycin in combination *in vivo* for virulent foot-rot and dermatophilosis of sheep only achieved this at doses that were seven times greater than the label rate.

The absence of *in vivo* studies demonstrating synergy of penicillin and (dihydro) streptomycin in combination against veterinary pathogens does not support claims of synergy when these products are used at label rates.

Alternatives

Table 5 Alternatives to (dihydro) streptomycin/ penicillin in sheep

Species	Disease	Alternatives to (dihydro) streptomycin/ penicillin
Sheep	Actinobacillosis	Sodium and potassium iodide, penicillin, tetracycline and (dihydro) streptomycin
	Anthrax	Penicillin, erythromycin and tetracycline
	Dermatophilosis	Tetracycline
	Virulent footrot	Erythromycin, tetracycline, lincomycin[4] and lincomycin/ spectinomycin[5]

As for cattle, there are many alternatives for the treatment of actinobacillosis in sheep, including sodium and potassium iodides[14, 22], penicillin[9, 23], tetracycline[14, 23, 24] and (dihydro) streptomycin[9, 14, 23, 24]. Penicillin is the drug of choice for the treatment of anthrax, although erythromycin and tetracycline are also effective[18, 19]. Long-acting tetracycline (20 mg/kg) is reported to be effective in the treatment of ovine dermatophilosis[28, 29].

A single intramuscular injection of erythromycin (10mg/kg) or long-acting oxytetracycline (24 mg/kg) is at least as effective as (dihydro) streptomycin/ penicillin combination formulations for the treatment of all stages of virulent footrot in sheep[26, 27]. Lincomycin and lincomycin-spectinomycin combinations (used off-label) provide another alternative[29].

2.2.5 Pigs

Use pattern

(Dihydro) streptomycin/ penicillin combination products are used to treat leptospirosis and mastitis-metritis-agalactia syndrome in pigs. The latter is a disease complex that occurs in sows within 48 hours of farrowing and the clinical signs can include a vaginal discharge, agalactia, mastitis, metritis, fever, inappetence and weak dehydrated piglets. The aetiology of this disease has not been established, however a number of organisms are considered to be involved and management practices also appear to contribute. Treatment involves a combination of antibiotics, oxytocin and corticosteroids[35, 36]. Broad spectrum antibiotics are indicated for this disease because of the involvement of a variety of organisms.

[4]involves off-label use
[5]involves off-label use

Efficacy

No data were provided to support clinical efficacy of the combined (dihydro) streptomycin/ penicillin combination products for the treatment of mastitis-metritis-agalactia syndrome or other diseases in pigs.

Alternatives

Table 6 Alternatives[6] to (dihydro) streptomycin/ penicillin in pigs

Species	Disease	Alternatives to (dihydro) streptomycin/ penicillin
Pigs	Mastitis-metritis-agalactia syndrome	Long-acting penicillin and oxytetracycline
	Leptospirosis	(Dihydro) streptomycin

Recommended alternative antibiotics for the treatment of mastitis-metritis-agalactia syndrome include long-acting penicillin and oxytetracycline(36).

Leptospirosis in pigs can be treated effectively by (dihydro) streptomycin.

2.2.6 Other uses

Goats and horses are included on the labels of some of the combination products, but no data or published information has been provided to support efficacy in these species.

2.2.7 Resistance

(Dihydro) streptomycin is commonly used in combination with penicillin as a broad-spectrum antibiotic in the belief that synergism occurs. However, due to widespread resistance in veterinary pathogens to streptomycin, clinical responses to combination products are likely to be attributable only to penicillin. There is evidence that its use against most respiratory tract pathogens and in Gram-negative bacterial infections does not result in synergism because of widespread resistance in cattle pathogens to (dihydro) streptomycin(21).

2.2.8 Discussion

It is evident from both textbooks and the literature that (dihydro) streptomycin/ penicillin combination formulations continue to be used for many diseases of food-producing animals in Australia. The preference for this combination over other antibiotics appears to have largely arisen from the overstated significance of synergy. With the exception of therapy for virulent footrot and dermatophilosis in sheep at rates exceeding the label

[6] Residue status of the alternatives has not been investigated.

rates, the use of (dihydro) streptomycin/ penicillin combination formulations cannot be justified on the grounds of demonstrable synergism.

2.3 (Dihydro) streptomycin Products

2.3.1 Background

(Dihydro) streptomycin has a narrow spectrum of activity that includes Gram-negative aerobic bacteria and some Gram-positive cocci. (Dihydro) streptomycin is rarely used alone for infections in animals because of widespread resistance, particularly in Gram-negative bacteria[21]. Also, these products have been reported to cause varying degrees of ototoxicity and nephrotoxicity and in this respect, dihydrostreptomycin is more likely to cause deafness than streptomycin (particularly in cats).

2.3.2 Current usage

There are four (dihydro) streptomycin products currently registered for injection in food-producing animals in Australia. The use of one of these products, CCD Formula 4, is limited to poultry. The registered use rates of (dihydro) streptomycin products are summarised in Table 7.

Table 7 Injectable (dihydro) streptomycin products registered

Product name	Food-producing species	(Dihydro) streptomycin concentration in formulation	Label dose rates
C.C.D. Formula 4 Dihydrostreptomycin Sulphate	Poultry	770 mg/g	100 mg/kg body weight (im or sc) up to a maximum of 250 mg/bird
Leptostrep	Cattle	720 mg/g	25 mg/kg body weight im
VR Vibrostrep Injection	Cattle (Bulls)	500 mg/mL	10 mL infused into preputial cavity. Repeat 3 times at 24 or 48 hour intervals. Additionally, give 20 mL by sc injection in loose skin of neck at 1st and 3rd treatment
Strepolin SD Injection	Horses, cattle,	333.4 g/L	10-15 mL
	Calves, foals		5-8 mL
	Sheep, pigs & dogs		1-3 mL

2.3.3 Cattle

Use pattern

(Dihydro) streptomycin alone has traditionally been used for the treatment of leptospirosis in cattle and pigs, campylobacteriosis in bulls and actinobacillosis in cattle and sheep, at the label rates as shown in Table 7.

The major use for (dihydro) streptomycin is the treatment of leptospirosis, which is a zoonotic disease with *Leptospira interrogans* serovar *pomona* and *Leptospira interrogans* serovar *hardjo* being responsible for economic losses in cattle, pigs and sheep in Australia. In bovine leptospirosis, the mortality rate is low and the morbidity rate is generally high. The disease is of significant concern in dairy cattle. A high rate of abortions and a loss of milk production are the major causes of economic loss, while mortality in calves may also be significant.

A single dose of (dihydro) streptomycin (15 mg/kg im) is used in conjunction with herd vaccination to prevent abortions during outbreaks of leptospirosis. A single dose of 25 mg/kg im in cattle and pigs has been used to remove *Leptospira interrogans* serovar *pomona* from the kidney of carrier animals, however this may not be effective for *Leptospira interrogans* serovar *hardjo*[30].

(Dihydro) streptomycin is used for the elimination of campylobacteriosis carrier state from bulls[21]. Treatment involves infusing 5 g of (dihydro) streptomycin into the preputial cavity and massaging the exterior for five minutes. This is done on three consecutive days. In conjunction with this, 10 g of (dihydro) streptomycin is given by subcutaneous injection under the loose skin of the neck at the first and third preputial infusions.

Efficacy

Whilst no data were provided by registrants, the literature supports the efficacy of (dihydro) streptomycin in the treatment of the three abovementioned conditions in cattle.

(Dihydro) streptomycin alone was shown to be an effective antibiotic for the treatment of clinical leptospirosis and the removal of the carrier state for *Leptospira interrogans* serovar *pomona* in cattle and pigs[9, 14, 23, 24]. However, its use to remove the carrier state for *Leptospira interrogans* serovar *hardjo* is not reliable[30, 32, 33]. The main aim of leptospirosis therapy is to control the infection before irreparable damage to the liver and kidneys occurs. The most effective treatment of the clinical disease is with either (dihydro) streptomycin or tetracycline as soon as signs appear[9]. An important part of treatment is the removal of the carrier state to prevent the shedding of bacteria and the transmission of the disease to both humans and other farm animals. This is achieved by a single injection of (dihydro) streptomycin at 25 mg/kg body weight.

Similarly, (dihydro) streptomycin appears to be effective for the elimination of campylobacteriosis from bulls[21].

The treatment of leptospirosis and campylobacteriosis with (dihydro) streptomycin is usually carried out in conjunction with a vaccination program. Vaccines represent an important means of control, but the removal of carrier animals is also necessary.

(Dihydro)streptomycin is also very effective in the treatment of clinical actinobacillosis in cattle and sheep[9, 14, 23, 24].

Alternatives

Tetracycline is an effective alternative for the control of clinical cases of leptospirosis in cattle and pigs but this treatment does not remove the carrier state of the disease. There do not appear to be effective alternatives for the elimination of carriers of leptospirosis in cattle.

Similarly, there do not appear to be effective alternatives to (dihydro) streptomycin for the elimination of the carrier state of campylobacteriosis in bulls.

There are many alternatives for the treatment of actinobacillosis in sheep and cattle including sodium and potassium iodide[14, 22], penicillin[9, 23] , tetracycline[14, 23, 24] and possibly erythromycin[9]. This disease has been discussed earlier in relation to (dihydro) streptomycin/ penicillin combination products. Whilst some authors[3, 21] have indicated that (dihydro) streptomycin may be the drug of choice for actinobacillosis, a closer examination of the literature indicates that the alternatives are more effective[9].

2.3.4 Sheep

The economic significance of leptospirosis in Australian sheep is unclear, however the disease may be important in the wetter sheep-producing areas especially when sheep are run in conjunction with cattle. Vaccines are available for the control of this disease in sheep, but (dihydro) streptomycin may be required for removal of the carrier state as is the case with cattle.

(Dihydro) streptomycin is very effective in the treatment of clinical actinobacillosis in sheep[9, 14, 23, 24]. However, there are many alternatives including sodium and potassium iodide[14, 22], penicillin[9, 23] , tetracycline[14, 23, 24] and possibly erythromycin[9].

2.3.5 Pigs and poultry

(Dihydro) streptomycin on its own has traditionally been used for the treatment of leptospirosis in pigs. In porcine leptospirosis, economic loss is due mainly to abortions and the deaths of weak and unthrifty newborn piglets. Condemnation of kidneys at slaughter is also a cause of economic loss. Treatment programs involve the use of oxytetracycline in the feed and vaccinating young pigs at weaning and again at 16 weeks of age. Vaccination prevents abortion and (dihydro) streptomycin is used to treat clinical infections and to remove the carrier state.

There is limited information on the efficacy of (dihydro) streptomycin in the treatment of respiratory diseases of poultry and supporting data were not provided by registrants.

Although the product CCD Formula 4 is registered for use on poultry only, (dihydro) streptomycin appears not to be used currently in significant quantities in the commercial poultry industries[31]. It was used in conjunction with tylosin for the treatment of chronic respiratory disease (*Mycoplasma gallisepticum*) and alone for the treatment of coryza (*Hemophilus paragallinarum*). Its use in the treatment of chronic respiratory disease has diminished with the advent of effective vaccines and the establishment of biosecurity programs on commercial poultry farms.

Chronic respiratory disease (*Mycoplasma gallisepticum*) and coryza (*Hemophilus paragallinarum*) in poultry can be treated effectively with a number of antibiotics. These include tylosin, tiamulin fumarate, erythromycin, tetracycline and lincomycin/spectinomycin combinations. These offer the further practical advantage of being administered either in feed or in water. There is currently no Australian MRL for (dihydro) streptomycin in poultry meat and no data were provided to support MRLs in poultry meat or eggs. Therefore continued use cannot be supported in poultry intended for human consumption.

2.3.6 Resistance

Many veterinary pathogens have acquired resistance to (dihydro) streptomycin[21]. Most clinically important resistance is caused by plasmid-encoded enzymes, certain of which specifically deactivate (dihydro) streptomycin. Apart from the specific uses discussed above, (dihydro) streptomycin is rarely used alone for infections in animals because of widespread resistance, particularly in Gram-negative bacteria.

2.3.7 Human health effects

Leptospirosis is transmissible to humans and it represents an occupational hazard to butchers, abattoir workers, farmers and veterinarians[34]. Human infection is most likely to be a result of contamination with infected urine or uterine discharges. Veterinarians may become infected following the handling of the tissues and urine of cows or sows that have aborted from infection with *Leptospira* spp. Dairy farmers are at risk from cows shedding bacteria during milking.

Leptospirosis poses a significant human health risk to those exposed to infected animals, with 148 cases of leptospirosis in humans being notified in Australia in 1995 and 227 cases in 1996, the majority of them in Victoria, NSW and Queensland[7]. Symptoms vary in severity, with many minor cases probably not being notified.

2.4 Conclusions

2.4.1 (Dihydro) streptomycin/ penicillin combination products

With the exception of ovine dermatophilosis and ovine virulent footrot, no evidence has been provided to prove synergy of (dihydro) streptomycin/ penicillin combinations

[7] Communicable Diseases Network – Australia New Zealand – National Notifiable Diseases Surveillance System, personal communication.

against major veterinary pathogens. Dermatophilosis and virulent footrot control require use of the products at greater than (up to 7 times) label rates. Effective control alternatives are available. Therefore the continued use of (dihydro) streptomycin/ penicillin combination products in food-producing animals cannot be justified.

The combination products are often used because of an apparent need to administer a broad-spectrum antibiotic where there is thought to be multiple pathogens involved or when there is difficulty in establishing the identity of the pathogen. This rationale has little merit as the spectrum of activity of (dihydro) streptomycin includes only a few pathogens of veterinary importance. Thus it is considered that procaine penicillin alone would achieve similar results. In most instances, several truly broad-spectrum alternatives are available. Good veterinary practice includes sensitivity testing of the organisms suspected of being involved and should be used in deciding the most appropriate antibiotic(s) to be used for the treatment of the disease.

The rapid development of resistance to (dihydro) streptomycin has resulted in the loss of effectiveness of that component causing the combination products to be no more effective than penicillin alone.

2.4.2 (Dihydro) streptomycin products

(Dihydro) streptomycin alone is generally only used when sensitivity testing confirms susceptibility of the pathogen. Although there is doubt as to the value of using (dihydro) streptomycin to treat actinobacillosis in cattle and sheep, there is no such doubt about its value to treat leptospirosis in cattle and pigs and campylobacteriosis in bulls. The importance of leptospirosis as a zoonotic disease should not be overlooked. The ability of (dihydro) streptomycin to remove the carrier state is an important tool in reducing the contamination of the working environment for butchers, abattoir workers, farmers and veterinarians as well as controlling the disease in cattle and pigs.

There are many alternatives for the treatment and control of chronic respiratory disease and coryza in poultry and it is noted that in recent times the use of (dihydro) streptomycin in poultry has declined. The latter may be attributed to factors such as cost and that alternative products are equally effective and more easily administered via feed and water medication. The literature indicates effective alternatives for the treatment of these two poultry diseases.

3 RESIDUES AND TRADE ASSESSMENT

3.1 Introduction

(Dihydro) streptomycin is effective against a narrow range of Gram-negative aerobic bacteria and some Gram-positive cocci. As there is negligible passage of (dihydro) streptomycin across the intestinal mucosa, systemic therapy can only be achieved by parenteral injection. Most injections are given intramuscularly and deliver peak plasma levels within one hour. The absorption after subcutaneous injection is much slower and this route of administration is not often used.

There are only scant details on the metabolism of (dihydro) streptomycin in animals and humans. (Dihydro) streptomycin is excreted, as are other aminoglycosides, via glomerular filtration. For example, after parenteral administration of (dihydro) streptomycin to humans, approximately 80% of the dose is recovered in the urine. It is recognised that (dihydro) streptomycin accumulates in the kidney. Because most of the glomeruli are located in the renal cortex, the concentration of (dihydro) streptomycin is higher in this region than in the medulla [37, 38, 39]. No studies with radiolabelled (dihydro) streptomycin have been carried out to identify metabolites. It is generally assumed there is little metabolism of (dihydro) streptomycin in animals.

Concern over persistent (dihydro) streptomycin residues first arose in the early 90's. In 1991, Japan detected a violation in a consignment of Australian grain-fed beef with residues of (dihydro) streptomycin exceeding 130 mg/kg. Although the residue was probably from an injection site, the consignment was rejected. In a trace-back, the problem was identified as occurring in a feedlot that had been treating footrot with a (dihydro) streptomycin/ penicillin formulation and in compliance with the label WHP. In Australia, results from the National Residue Survey's *Randomised Monitoring Program* indicated that (dihydro) streptomycin was the most common antimicrobial residue detected in slaughtered cattle.

Table 6 NRS Randomised Monitoring Program in cattle (July 1993 to June 1997)

	Total (from 2898 samples)	(Dihydro) streptomycin	Other antimicrobials
No. of samples with residues detected	18	8	10
No. of samples with residues >MRL	8 (<0.3%)	5 (<0.2%)	3

These violations may be the result of inadequate slaughter WHPs for the registered products. Additionally, it is recognised that aminoglycoside drugs have a propensity to leave high residues at the injection site and in kidney at times well past the slaughter WHP. Although the risk posed to human health by such residues is small, the presence of injection site residues may nevertheless present an appreciable risk to Australian trade in animal commodities as demonstrated by the violative detection in Japan.

3.1.1 Current Australian MRLs

Table 7. Australian MRLs for (dihydro) streptomycin

Food Commodity		A14 Food Code (mg/kg)	MRL Standard (mg/kg)
MO 0105	Edible offal (mammalian)	0.3	*0.3
PE 0112	Eggs	0.2	*0.2
MM 0095	Meat [mammalian]	0.3	*0.3
	Poultry, edible offal of	0.3	
	Poultry meat	0.3	
ML 0106	Milks	0.2	*0.2

*at or about the limit of analytical quantitation

The ADI and NOEL of (dihydro) streptomycin are 0.03 mg/kg/day and 5 mg/kg/day respectively.

The residue definition for (dihydro) streptomycin is:

Streptomycin and dihydrostreptomycin	**Inhibitory substance, identified as streptomycin**

3.2 Registered Products and Maximum Treatment Regimes

Products listed in the 1997 IVS Annual or on the NRA registration database.

Table 8. Injectable (dihydro) streptomycin products

Product	Company	(Dihydro) streptomycin	Indications	Route	No. of Treatments	Target Animal	Dose (dihydro) streptomycin (mg/kg bw)	WHP meat (days)	WHP milk, sd (h)	WHP milk, md (h)
C.C.D Formula 4	CCD Animal Health	770mg/g	CRD and Coryza	im or sc	Not specified	poultry	100.0	30	na	na
Leptostrep	Hart Kay Kent	720mg/g	Infections due to susceptible organisms	Im	Not specified	cattle	25.0	30	36	72
Strepolin Injection	Jurox Pty Ltd	333.4mg/mL	Infections due to susceptible Gram-ve organisms	Im	Not specified	cattle, horse calf, foal sheep, pig	10-15 mL 5-8 mL 1-3 mL	30 30 30	36	72
Vibrostrep	Jurox Pty Ltd	500mg/mL	Bovine vibriosis	1st, 3rd sc, 2nd infusion into preputial cavity	3× at 24-48 hour intervals	bull	20 mL	30	na	na

na = not applicable; im = intramuscular injection; sc = subcutaneous injection; sd = single dose; md = multiple dose; CRD = chronic respiratory disease.

Table 9. Injectable (dihydro) streptomycin/ penicillin combination products and label recommendations

Product	Company	(Dihydro) streptomycin (mg/mL)	Procaine penicillin (mg/mL)	Indications	Route	Number of Treatments	Target animals	Dose DHS	WHP meat (days)	WHP milk, sd (h)	WHP milk, md (h)
Aquacaine S Injectable Suspension	Boehringer Ingelheim	250	200	Infections due to susceptible organisms & where more than one organism is involved	im	3	Cattle, horses Ccalves, foals, sheep, pigs	10.0mg/kg bw 10.0mg/kg bw	30 30	36 36	72 72
Biodexamine	Intervet	250	200	Infections due to susceptible organisms, infectious disorders with toxic symptoms, inflammations or allergic conditions	im	2	Cattle, horses Calves, foals, sheep Pigs Piglets	15-20mL 8 mL 8-10 mL 1-2 mL	30 30 30 30	36 36 na na	72 72 na na
Depomycin®	Intervet	250	200	Infections due to susceptible organisms and respiratory, reproductive and urinary tract infections, esp enzootic pneumonia in pigs	im	ns	Horses Cattle Sheep, pigs	10.0mg/kg bw 10.0mg/kg bw 12.5mg/kg bw	30 30 30	na 36 36	na 72 72
Dopharma penicillin/ Streptomycin	Dopharma Australia P/L	200	200	Infections due to susceptible organisms	im	daily for 3 to 5 days	Cattle, horses, Sheep, pigs	10.0mg/kg bw 10.0mg/kg bw	30 30	36 36	72 72
Hydropen	Bomac	250	200	Systemic infections due to susceptible organisms	im	twice daily	Cattle, horses Calves, foals Sheep, goats, pigs	10-20 mL 5-10 mL 3-5 mL	30 30 30	72 na na	72 na na
Pen & Strep	Heriot/ Norbrook	250	200	Infections due to susceptible organisms	im	ns	Horses Cows Sheep Pigs	10.0mg/kg bw 10.0mg/kg bw 10.0mg/kg bw 10.0mg/kg bw	30 30 30 30	na 72 72 na	na 72 72 na
Penstrep*	Troy	250	250	Infections due to susceptible organisms, respiratory, reproductive and urinary tract infections, pre- and post- operative prophylaxis, mycotic dermatitis, foot abscess, foot rot control/eradication	im	twice daily	Cattle, horses Sheep, pigs, calves, foals	10.0mg/kg bw 10.0mg/kg bw	30 30	36 36	72 72
Streptopen	Jurox Pty Ltd	250	250	Bacterial infections susceptible to penicillin and/or streptomycin	im	ns	Cattle, horses Calves, foals Sheep, goats, pigs	15-20 mL 5-8 mL 3-5 mL	30 30 30	36 na 36	72 na 72
Vetstrep Injection	Jurox Pty Ltd	250	250	Infections susceptible to penicillin and/or streptomycin	im	ns	Cattle, horses Calves, foals Sheep, goats, pigs	15-20 mL 5-8 mL 3-5 mL	30 30 30	36 na 36	72 na 72

im = intramuscular injection; sc = subcutaneous injection; ns = not specified; sd = single dose; md = multiple dose
*special dosage regimes are necessary for footrot eradication, mycotic dermatitis and foot abscess
DHS = (dihydro) streptomycin

Table 10 Summary of residue data for injectable (dihydro) streptomycin/ penicillin combination products

Product	Submitted Target Animal	Body weight (kg)	Sample size per time point	Dose (mg/kg bw)&[no. T_X days]	Tissue/ milk	Sampling times after last injection	Comments	Evaluation report reference; Company study number
Aquacaine S	Sheep	17-25	2	25.0 [1]	edible[¥]	18 days	kidney >MRL; no LOD/LOQ	5.13 (no study number)
Suspension	Sheep	17-25	2, 2	25.0 [3]	edible	18, 36 days	kidney, ISR >MRL at 36 days; no LOD/LOQ	5.13 (no study number)
Depomycin®	Cattle	450-600	4	10.0 [3]	milk	am/pm for 6 days	<LOD at 60 h for penicillin; <LOD by 36 h for DHS	5.2; Exp No. 89/3712
	Cattle	82-140	5, 4	10.0 [3]	edible	14, 21 days	>MRL kidney, liver, ISR at 21 days; WHP not addressed	5.8; Ref 870316
	Sheep	50-60	5, 4	10.0 [3]	edible	14, 21 days	>MRL kidney, liver, ISR at 21 days; WHP not addressed	5.8; Ref 870316
	Sheep	36-43	4, 4, 4	12.5 [3]	edible	28, 35, 42 days	>MRL kidney, liver, ISR at 35 days; ISR > MRL at 42 days	5.9; Ref 90363-3
	Pigs	50-120	4, 5, 5	10.0 [3]	edible	3, 14, 21 days	>MRL kidney, liver, ISR at 21 days; WHP not addressed	5.8; Ref 870316
	Pigs	38-64	4, 4, 4	12.5 [3]	edible	28, 35, 42 days	> MRL for ISR at all time points	5.10; Ref 90353-3
Dopharma	Cattle	610-800	8	10.0 [5]	milk	8, 21, 32, 44, 56, 68, 80, 92 h	< MRL by 92 h for DHS	5.3; Rep No. 042/95/1093
penicillin/	Cattle	143-270	4, 4, 4, 4	10.0 [5]	edible	7, 14, 28, 36 days	kidney, liver, ISR >MRL at 36 days	5.16; Rep No. 042/95/1186
Streptomycin	Pigs	46-60	4, 4, 4,4	10.0 [5]	edible	7, 14, 28, 36 days	kidney, liver, ISR > MRL at 36 days	5.15; Rep No. 042/95/1265
Pen & Strep	Cattle	450-525	6	10.0 [3]	milk	8, 24, 32, 48, 56 h	<MRL penicillin by 48 h; <MRL DHS by 32 h	5.1; Study No. 043/88
	Cattle	135-205	4, 4, 4	10.0 [3]	edible	14, 18, 21 days	LOQ>MRL, WHP not addressed	5.4; Protocol No. 079/95; 072/95; 014/96
	Sheep	58.5-85.5	4, 4, 4	10.0 [3]	edible	14, 18, 28 days	LOQ>MRL, WHP not addressed	5.4; Protocol No. 085/95; 074/95; 015/96
	Sheep	17-25	2, 2, 2	10.0 [3]	edible	30, 46, 64 days	>MRL at 30 days; ISR > MRL at 64 days; no LOD/LOQ	5.12; MRC DAV.085
	Pigs	35.5-43.0	4, 4	10.0 [3]	edible	14, 18 days	LOQ>MRL, WHP not addressed	5.6; Protocol No 087/95; 076/95
Penstrep	Cattle	279-348	1, 1, 1	10.0 [1*]	edible	5, 15, 30 days	kidney, ISR >MRL; no LOQ/LOD	5.11; MRC DAQ.084
Hydropen	Cattle	100-280	4, 3	10.0 [3]	edible	7, 18 days	LOQ>MRL, WHP not addressed	5.7 (no study number)
	Sheep	31-70	4, 3	10.0 [3]	edible	7, 18 days	LOQ>MRL, WHP not addressed	5.7 (no study number)
	Pigs	16-90	4, 3	10.0 [3]	edible	7, 18 days	LOQ>MRL, WHP not addressed	5.7 (no study number)
Penstrep	Cattle	-	84 in total	10.0 [1-7]	kidney	42-93 days	>MRL at points past the WHP	5.12

*2 injections given 12 hours apart; [¥]edible = minimum of liver, kidney, muscle, injection site and fat tissues analysed; ISR = injection site residue.

3.3 Discussion

In the past few years it has been recognised that drugs administered by the intramuscular or subcutaneous route have the potential to cause persistent injection site residues. Since residue levels at injection sites are often at higher concentrations than for other tissues, concern has been expressed that injection site residues should be taken into account when establishing food safety standards and assessing trade implications. In this review, the question of injection site residues will be considered separately from the residue depletion in other tissues.

A review by JECFA of streptomycin and dihydrostreptomycin noted that these two compounds have similar pharmacokinetic and residue profiles and are not distinguishable in measurement of residues by microbial inhibition or enzyme immunological assay methods[40]. For the purposes of the current review streptomycin will be considered synonymous with dihydrostreptomycin.

In answer to a call for residue data to support the continued registration of (dihydro) streptomycin injectable products the NRA received one residue trial for injectable formulations in which (dihydro) streptomycin was the sole active ingredient (target animal was pigs) and 16 reports for (dihydro) streptomycin/ penicillin combination products (cattle, sheep, pigs).

(Dihydro) streptomycin, when formulated alone or in combination with penicillin are administered as daily intramuscular injections at a dose rate of 10-25 mg (dihydro) streptomycin per kg bw. Two exceptions are Vibrostrep, which is administered via the subcutaneous route and preputial infusions, and C.C.D. Formula 4 for which a dose rate of 100 mg/kg bw for poultry is used by either the im or sc route. The duration of treatment depends on the response of the infection but typically should not exceed 3-5 days. The dose rates for some products were not specified in terms of bodyweight but rather according to animal size (small, medium, large). For such products, the maximum label rate cannot be determined precisely but can be estimated.

MRLs are set taking into account good veterinary practice, toxicology, microbiology and information of residue depletion in animals. These are generic for a particular active. On the other hand, WHPs are determined by the time taken for the residue levels to deplete below the MRLs and are formulation-dependent. Excipients in a formulation can affect the pharmacokinetics thereby modifying residue profiles. This is demonstrated in a literature report [41] concerning the pharmacokinetics of three (dihydro) streptomycin /penicillin combination preparations in calves which concluded that the products were not bioequivalent at the 90% confidence intervals because the parameters C_{max}, T_{max} and MRT exceeded the acceptable range of 0.80-1.20. The three products studied differed qualitatively and quantitatively in their composition with respect to excipients. As no bioequivalence data were provided for the current products, no assessment regarding the equivalence of product formulations can be made.

Note was also taken of the recent re-analysis of published (dihydro) streptomycin tissue residue data for dairy cattle [42, 43] which suggested that the current slaughter withholding period of 30 days for (dihydro) streptomycin/ penicillin combination products is inadequate. The authors suggested that a slaughter WHP of greater than 90 days may be

required. The re-analysed data [37, 38, 44, 45] did not include documentation of the formulations used.

3.3.1 Meat Residues (not including injection site tissue)

The information from which the following descriptions were drawn has also been summarised and tabulated in Table 10. The reader is also referred to Table 8 for additional product information. With most of the residue data submissions pertaining to this review, the analytical data were not of a standard suitable for setting MRLs or WHPs.

The residue definition for (dihydro) streptomycin is "inhibitory substance, identified as streptomycin or dihydrostreptomycin". The most appropriate technique for quantitating (dihydro) streptomycin residues according to the residue definition is by microbiological assay. However, the limits of detection for the microbial assay methods are generally too high to give confidence in the analytical results. Ideally, quantitation of (dihydro) streptomycin residues should be confirmed by a validated instrumental technique such as HPLC. The HPLC methods measure the parent compound and so do not address the current residue definition. The one company that did use HPLC to analyse tissue samples was Novartis (Novartis/Heriot/Norbrook) for their Pen & Strep combination product. Unfortunately the LOQs for the HPLC method (LOQ 0.4 mg/kg) were greater than the Australian MRLs for (dihydro) streptomycin (*0.3 mg/kg edible offal and meat mammalian) and no relationship between quantities determined by microbial assays and the HPLC technique was provided. In light of current trends in residue analytical methods it may be advisable in the future to change the residue definition for streptomycin and dihydrostreptomycin to the parent compounds.

3.3.1.1 (Dihydro) streptomycin only products

No data on residues in tissues, milk or injection sites were provided to support the continued registration of (dihydro) streptomycin only products for use in cattle, sheep, pigs or poultry (residue data were submitted for a pig study using a Dopharma product that is not registered in Australia). Despite the lack of supporting data it was considered that the registration of (dihydro) streptomycin injectables should be explored for the following situations.

<u>Leptospirosis</u>

Leptospirosis is a zoonotic disease with serious human health implications. As (dihydro) streptomycin is the only antibiotic that has been proven to remove the carrier state [46, 47] and hence the risk to human health, (dihydro) streptomycin injectable products should remain available to veterinarians. However, there are concerns over the persistence of (dihydro) streptomycin residues in kidneys for at least 90 days after injection with a penicillin (dihydro) streptomycin combination product. Residue levels in excess of 2 mg/kg in kidney were observed at >80 days after administration of an intramuscular injection to cattle. The maximum levels found in cattle kidney were significantly greater than international MRLs and therefore represent a risk to Australian trade. The long interval between administration and slaughter suggests an intrinsic problem with (dihydro) streptomycin residues in kidney rather than a formulation specific effect. This

observation and a recent re-analysis of published (dihydro) streptomycin tissue residue data for dairy cattle [42, 43], which suggested that a slaughter WHP of greater than 90 days may be required, preclude the setting of an appropriate WHP. Continued access by veterinarians to (dihydro) streptomycin can be achieved by off-label use under permit.

Cattle for live export

The use of Leptostrep (Hart Ray Kent) for prophylactic leptospirosis treatment of cattle for live export to Japan also deserves special consideration. Here, steers are injected with (dihydro) streptomycin approximately 1 week prior to shipment in order to satisfy, in part, the Japanese quarantine requirements. While the request to maintain this use pattern was not supported by residue data, it appears worthy of support because the steers spend 270 - 300 days in Japanese feedlots prior to slaughter for human consumption. This period should amply suffice for tissue residues to deplete to low levels prior to slaughter (pers. comm. Allan Parkinson, Toowoomba Veterinary Clinic, Qld). Similar practices apply to the live export of cattle to other markets. In any event, the management of tissue residues from these cattle is the responsibility of the overseas livestock companies and regulatory authorities and any mismanagement should not reflect poorly on Australia.

3.3.1.2 (Dihydro) streptomycin/ penicillin combination products

Cattle Tissues

For cattle treated with Penstrep (Troy), a residue of 1.4 mg/kg (kidney) was detected at the slaughter WHP of 30 days. None of the residue data supplied for Pen & Strep (Novartis/Heriot/Norbrook), Depomycin® (Intervet), Hydropen (Bomac) or Penstrep (Troy) in cattle tissues included time points past the slaughter WHPs of 30 days. The residue data for the Dopharma combination product included residues greater than the edible tissue MRL of *0.3 mg/kg at the last time point of 35 days. Evidence from a residue trial of 84 cattle treated with a (dihydro) streptomycin /penicillin combination product revealed (dihydro) streptomycin residues >MRL at 43-93 days after the last dose for 83% of animals treated and > 2mg/kg for 14% of animals treated. Clearly, the available data do not provide the necessary confidence to set a cattle slaughter WHP for edible tissues. Therefore, based on the residue data submitted for evaluation, the use of (dihydro) streptomycin/ penicillin combination products in cattle cannot be supported.

Cattle Milk

Three milk residue studies were submitted. There is some confusion as to which formulation was used [that employed was either Pen & Strep or Hydropen] where levels of (dihydro) streptomycin and penicillin in milk were below the MRL of *0.2 mg/kg at the milk WHP of 72 hours (for multiple doses). Similar results were submitted for Depomycin®.

From a consideration of the milk residue data alone, residues would not pose an unacceptable risk. However, because dairy cattle are often culled following unsuccessful antimicrobial therapy and as the tissue residue data do not support a slaughter WHP, the continued use of (dihydro) streptomycin/ penicillin combination products in dairy cattle cannot be supported.

Sheep

The comments that immediately follow pertain to what is perceived as being an appropriate slaughter WHP based on the submitted residue data. Subsequent comments relate to the compatibility of the recommended slaughter WHP and good veterinary practice with respect to the three major use patterns applicable to sheep.

In sheep treated with the combination product at 10.0-12.5 mg (dihydro) streptomycin /kg, residues of 0.29-0.46 mg/kg (liver) and 0.31-0.45 mg/kg (kidney) were observed at 30-35 days. The earliest time point at which the residue levels in these tissues were below the MRLs was 42 days for Depomycin® and 46 days for Pen & Strep. However, it is noteworthy that parenteral antibiotics are commonly administered to sheep by intramuscular injection into a hind leg. Residues at injection sites are therefore a necessary consideration. The injection site residues for the Pen & Strep or Depomycin® combination products administered at 10.0-12.5 mg (dihydro) streptomycin /kg bw were above the MRL for all time points. For this reason, a slaughter WHP cannot be set for these products.

Major indications for the use of (dihydro) streptomycin/ penicillin combination products in sheep medicine are the treatment of (i) dermatophilosis (lumpy wool) and actinobacillosis; (ii) the mastitis/metritis complex; and (iii) ovine footrot.

(i) Dermatophilosis is treated 1 month prior to shearing in order to “lift” the scabs and facilitate shearing. Treated sheep are then culled 4 weeks after shearing. The dose rates used for dermatophilosis treatment are high compared to registered label rates. Off-label dose rates of 60-70 mg (dihydro) streptomycin /kg bw and 70,000 U/kg bw procaine penicillin were reported by Scrivener and Vizard[48]. Neither tissue residue data nor injection site residue data are available to support this use pattern.

(ii) Mastitis and metritis of ewes are treated with intramuscular injections. Culling of treated ewes typically occurs at weaning i.e. 10 weeks post-lambing. At face value, the period between treatment of ewes and slaughter (10 weeks) appears adequate. However, the submitted injection site residue data neither supported a slaughter WHP nor defined the period following treatment for injection site residue concentrations to fall below the sheep meat MRL. It can be concluded that continued registration of the claim

for the use of (dihydro) streptomycin/ penicillin combination products on ewes with metritis/mastitis cannot be supported. In this context, it is noteworthy that penicillin is probably the drug of choice for treating these disease entities (pers. comm. Bruce Farquharson).

(iii) Treatment of virulent ovine footrot can involve administering (dihydro) streptomycin/ penicillin every 30 days during summer. Treated sheep are checked 30 days post-treatment at which time "clean" sheep are returned to the mob and any non-responders culled. This use pattern is cause for major concern from a residue perspective. Firstly, a slaughter WHP of 30 days would not cover the high dose rates used (typically 4 times the recommended label rate and as high as 60-70 mg (dihydro) streptomycin /kg bw [49, 50]) or injection site residues. Secondly, large numbers of sheep are treated for footrot and the exposure to residues of sheep-derived food commodities is substantial. For example, in a mob of 4000 sheep, 1500 may require treatment for footrot and of these, approximately 60% (300 sheep) may be culled as soon as 30 days later as non-responders (pers. comm. Bruce Farquharson). It is concluded that major difficulties are associated with the use of (dihydro) streptomycin/ penicillin combination products for treating ovine footrot and that this use pattern cannot be supported as neither tissue residue data nor injection site residue data are available.

Pigs

The following discussion is structured in two parts as was done with sheep. The first addresses an appropriate slaughter WHP based on the submitted residue data while the second assesses the practicality of implementing the recommended slaughter WHP for two major use patterns/indications.

Data in support of a WHP for pigs was submitted for three combination products. Pigs treated with Depomycin® at 12.5 mg (dihydro) streptomycin /kg bw had residues below the MRLs by 35 days but not at 30 days. Hence the slaughter WHP for Depomycin® for use in pigs should be extended from 30 days to 35 days. In the case of the Dopharma product administered at 10 mg (dihydro) streptomycin /kg bw, the residues were >MRL at 36 days following the last dose. As this was the last sacrifice time point, a WHP cannot be set for this product. The residue data for Pen & Strep did not address the current WHP and therefore continued registration of this product in pigs cannot be supported.

The compatibility of a slaughter WHP of 35 days for Depomycin® and good veterinary practice also needs to be considered in respect to the two major use patterns.

(i) Metritis (and mastitis). This is aimed at stopping vaginal discharges and the development of metritis (and possibly mastitis) in sows. Drug administration occurs on the day of farrowing. The residue implications relate to sows, rather than "suckers" which may get small amounts of the drug via the milk and may then be slaughtered at >28 days of age. The problem is sow turnover which typically runs at 60% annually [51]. These sows may be culled at weaning (ie. 14-28 days post-farrowing) for numerous reasons including infertility, lameness, poor condition and age. These animals would need to be retained for the WHP before slaughter, but this does not represent good veterinary practice.

(ii) Leptospirosis. This involves the treatment of carrier sows. While sows do not display clinical symptoms of the disease, they transfer leptospires to growers and finishers. Clinical symptoms are not evident in growers and finishers but leptospirosis may be diagnosed at slaughter. The over-riding concern is that leptospirosis is a zoonotic disease with serious human health implications. Treatment regimens target carrier sows and young pigs. Treatment typically involves dosing sows with in-feed oxytetracycline for 1 month and vaccinating young pigs at weaning and again at 16 weeks of age. However, some farmers prefer to use (dihydro) streptomycin/ penicillin combination products. In this scenario, the culling of non-responders is not an issue but the likelihood of culling recently treated sows for unrelated reasons (e.g. infertility, lameness, poor condition and age) is. These animals would need to be retained for the WHP before slaughter; but again this does not represent good veterinary practice.

In conclusion, from a residues perspective, the continued registration of (dihydro) streptomycin/ penicillin combination products in pigs is not recommended except for Depomycin® for which the slaughter WHP should be 35 days. It should be noted, however, that even at the proposed slaughter WHP of 35 days, injection site residues could be expected to exceed the MRL.

Other uses

The 18 day slaughter WHP assigned to Aquacaine S administered at a rate of 25 mg (dihydro) streptomycin /kg bw for calves, foals, sheep and pigs is not supported by residue data. A residue level of 1.04 mg/kg was observed in kidney of sheep at 36 days. No data were available for sacrifice time points beyond 36 days. The label for this product was amended in 1997 to recommend a dose of 10 mg/kg bw and a slaughter WHP of 30 days. No data were submitted for that label dose rate and therefore a slaughter WHP cannot be set for this product. Since no residue data are available for other food animal species treated with Aquacaine S continued registration cannot be supported.

3.3.2 Injection site residues

The absorption rate of the drug from an injection site depot is commonly a rate-limiting factor for administration of intramuscular injections, and this determines the duration of action for the drug. High concentrations of active ingredients, solvent and suspending agents can cause inflammation of the site, tissue necrosis and scarring. Injection of preparations into connective tissue can lead to additional residue problems. When a problem does occur with intramuscular injections, the site of injection is not always obvious and is likely to remain within the edible tissue. The WHP is usually set on the tissue with the longest tissue depletion profile. This includes the injection site except for cases where this would lead to an impractical WHP, in which case edible tissue away from the injection site is used[52].

In all cases where (dihydro) streptomycin/ penicillin injectable products were administered at the maximum label rates, the maximum observed injection site residues at the recommended WHPs were in violation of the current edible tissue MRLs for

(dihydro) streptomycin of *0.3 mg/kg. The injection site residues were variable, as were the patterns of residue decay.

Although no acute reference dose has been established in Australia for (dihydro) streptomycin, we can assume that the risk to human health posed by consumption of a single dose of injection site muscle is small. This assumption is based on the history of use of these drugs for treatment of humans since the 1950's. In some circumstances the drugs are prescribed for humans as second-line treatments of infections, e.g. tuberculosis where it has been given at 1000-2000 mg daily or twice weekly. The maximum injection site residue detected was 131 mg/kg and for an average meal, the resulting consumption is much less than the human therapeutic dose. The potential health risk posed by injection site residues is mitigated by the low probability of ingestion, poor bioavailability following oral administration, the current low average daily meat consumption rate (*ca* 0.07 kg/day) and any decreases in residues caused by processing.

Sensitive individuals have reported allergic reactions to (dihydro) streptomycin that include redness of skin, rashes, hives, hypotension, headache, nausea and vomiting[53]. Although a sensitive individual could exhibit a severe allergic reaction from eating (dihydro) streptomycin contaminated meat, the probability of consuming residues at an injection site residue is small. Recent estimates put the likelihood of ingesting an injection site as one per 400-4000 portions (assuming an injection site of 300 g which represents 0.1-1.0% of the edible muscular portion of a carcass and that an average portion of meat is 75 g).[54]

(Dihydro) streptomycin has been evaluated in the Forty-third Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Based on an assessment of relevant toxicological and microbiological data, the committee established a temporary acceptable daily intake (ADI) of 0-30 µg/kg bw for the combined residues of both streptomycin and dihydrostreptomycin[61]. Based on data from the JECFA report, an Australian ADI of 30 µg/kg bw has also been established (4/06/97). The ADI relates to chronic dietary exposure. A theoretical maximum daily intake (TMDI) calculation for (dihydro) streptomycin using the Australian MRLs indicates that the ADI will not be exceeded by the current use patterns. The TMDI is estimated to be 6% of the ADI.

If registration of selected products were to continue, it is recommended that in order to minimise the risk to human health from injection site residues, a risk management approach be adopted and treated animals be clearly identified during the WHP. A similar approach has already been successfully adopted with long-acting oxytetracycline injectable products in Australia.

3.3.3 Trade

Farm animals treated with (dihydro) streptomycin or (dihydro) streptomycin/ penicillin injections have the potential to impact adversely on Australian trade in cattle, sheep, pig and poultry commodities if the residue standards applied by importing countries differ from Australian standards. Of the three major commodity importers only the EU and USA have assigned MRLs for (dihydro) streptomycin. The US FDA has deregistered all combination (dihydro) streptomycin/ penicillin injectable products. In Canada, as well as Japan, South Korea and other Asian countries, the default tolerance is nil.

(Dihydro) streptomycin MRLs (mg/kg)

Tissue	Australia [mammalian] (November 1997)	USA (cattle, swine)	EU[1](cattle, sheep, pigs, poultry)	Codex
Edible offal	*0.3	2.0	T1.0	1.0
Meat	*0.3	0.5	T0.5	0.5
Milks	*0.2	0.125	T0.2	0.2 (cattle)
Eggs	*0.2			

[1]to expire 1 January 2000
*at or about the limit of analytical quantitation

Considering that MRLs for (dihydro) streptomycin have not been established in major export markets other than the EU and the USA, there exists a potential significant threat to Australian trade in animal commodities. The residue tolerances for edible tissues in the EU and the USA are higher than their Australian counterparts and, apart from injection site residues and sheep/poultry commodities, there would be little risk of violations occurring providing an adequate WHP could be set. In other countries, any dispute concerning residue levels would reference Codex MRLs for arbitration.

Temporary MRLs for (dihydro) streptomycin were adopted by Codex at the 43rd session of the Codex Committee on Residues in Veterinary Drugs in Foods[(40)]. The committee recommended in its report that further information from experiments or expert opinion be sought on farm animal metabolism of (dihydro) streptomycin as well as residue data for eggs. Information on the relationship between antimicrobial activity of the residues and the measurement of residues by specific chemical methods was also requested. Injection site residues are likely to be exaggerated when high dose volumes are involved. The same lack of information is noted in this report.

The NRS's *Randomised Monitoring Program* indicated greater than 99.8% compliance with the current Australian MRLs. The reasons for low level non-compliance are not clear due to the problems with trace-back but are thought to be due to non-observance of the relevant WHP. It would also appear that approved WHPs may not be adequate. This conclusion is reinforced by a residue trial of 84 cattle which were treated with a (dihydro) streptomycin /penicillin combination product. (Dihydro) streptomycin residues in cattle kidney were >MRL at 43-93 days after the last dose for 83% of animals treated and > 2mg/kg for 14% of animals treated. These kidney residue data do not allow a viable slaughter WHP for cattle to be determined. The detection of residue levels in edible offal at levels greater than the USA, EU or CODEX tolerances indicates a significant trade risk exists.

Injection site residues also constitute a risk to Australian trade. One incident often cited is the 1991 violation in Japan by Australian beef. The injection site lesion was identified by a retail butcher during fine slicing and not by a routine surveillance program. While the evidence to date is that any disruption to trade from such detections is likely to be rare, the consequences of any detection of non-compliant residues can be severe.

Should the use of (dihydro)streptomycin injectable products continue for food-producing animals by off-label use or under permit, the most appropriate way to deal with injection site residues is to manage the risk[55]. An approach has been taken similar to that taken by the NRA to minimise injection site residues from long-acting oxytetracyclines[52].

3.4 Conclusions

(a) (Dihydro) streptomycin only products

No data on residues in tissues, milk or injection sites were provided to support the continued registration for the use of (dihydro) streptomycin only products in cattle, sheep, pigs or poultry.

Leptospirosis (cattle and pigs)

Due to the importance of (dihydro) streptomycin in the treatment of the carrier state for leptospirosis, and the consequential risk to human health, access by veterinarians to (dihydro) streptomycin injectable products should continue.

Cattle for live export

The continued use of these products to meet the requirements of the live cattle export trade, with respect to leptospirosis, can be accommodated despite a lack of supporting residue data. This is justified on the basis that the time between treatment and slaughter is likely to exceed 12 months, thereby ensuring that any residues at slaughter will be have depleted to low levels (pers. comm., Allan Parkinson, Toowoomba Veterinary Clinic, Qld).

Chronic respiratory disease and coryza in poultry

No data on residues in tissues, injection sites or eggs were provided to support the continued registration of (dihydro) streptomycin products in poultry. Therefore the continued use of (dihydro) streptomycin in poultry intended for slaughter for human consumption or egg production cannot be supported as no supporting residue data were provided. Existing MRL for (dihydro) streptomycin poultry meat, offal and eggs should be deleted from the *MRL Standard* and Standard A14 of the Food Code.

(b) (Dihydro) streptomycin/ penicillin combination products

No residue data were submitted for the following (dihydro) streptomycin/ penicillin combination products: Biodexamine (Intervet); Streptopen (Jurox Pty Ltd); Vetstrep Injection (Jurox Pty Ltd). Their continued registration for use in food animals cannot be supported. Residue depletion profiles in animals are dependent upon a number of factors including the formulation and the route of administration. Extrapolations from data generated for other formulations are not acceptable for determining withholding periods.

Beef and Dairy Cattle

Residue data were submitted for the following products: Aquacaine S Suspension (Boehringer Ingelheim); Depomycin® (Intervet); Dopharma penicillin/streptomycin (Dopharma Australia P/L); Hydropen (Bomac); Penstrep (Troy Lab/Ilium) and Pen & Strep (Novartis/Heriot/Norbrook). The data did not support continued registration for use on beef or dairy cattle due to concerns over residues. While acceptable milk residue data were submitted, these data *per se* did not support continued use on lactating dairy cattle because non-responders to antibiotic therapy are likely to be culled for slaughter for human consumption before the expiry of an acceptable WHP.

Sheep

Residue data were submitted for the following products: Aquacaine S Suspension (Boehringer Ingelheim); Depomycin® (Intervet); Hydropen (Bomac); and Pen & Strep (Novartis/Heriot/Norbrook). The residue data for Aquacaine S Suspension and Hydropen do not allow a WHP to be set for these products and so do not support continued registration. Residue data for Pen & Strep and Depomycin® did not support continued registration for use on sheep with a slaughter WHP of 30 days but could support a slaughter WHP of 49 days. However, compliance with this WHP gives no assurance that injection site residues will not violate the (dihydro) streptomycin MRLs. This is problematic from a residue perspective because it is customary to administer antibiotics to sheep by the intramuscular (hind leg) route. The situation is further compounded by off-label uses which involve much higher dose rates (6-7 times) than those dose rates on which a 49 day slaughter WHP could be based. There is the potential for serious residue implications if these antimicrobial agents continue to be used following current use patterns.

On balance, the submitted residue data do not support the continued registration of (dihydro) streptomycin/ penicillin combination products in sheep. Moreover, effective alternative therapies are available for those disease entities.

Pigs

Residue data were submitted for the following products: Depomycin® (Intervet); Dopharma penicillin/streptomycin (Dopharma Australia P/L); Hydropen (Bomac); and Pen & Strep (Novartis/Heriot/Norbrook). The residue data for Dopharma, Hydropen and Pen & Strep do not allow a WHP to be set and do not support continued registration. While residues data for Depomycin® did support use in pigs with a slaughter WHP of 36 days the injection site residues still exceeded the MRL at this time point.

Two critical use patterns needed to be considered to determine whether a slaughter WHP of 35 days is compatible with good veterinary practice. The first of these was metritis/mastitis and the second indication, *albeit* of dwindling importance, was the treatment of sows that are carriers of leptospirosis. From a residue perspective, the issue here is the practicality of observing the WHP. It is good farm practice to keep good records enabling farmers to identify, isolate and hold animals over for the WHP. Despite this, holding animals for extended periods to comply with lengthy WHPs is not compatible with good farm practice.

Injection site residues

History shows that injection site residues of aminoglycosides have already impacted adversely on Australia's trade and against this background, injection site residues were considered separately in this review. Should registration of injectable aminoglycosides continue, risk management would appear to be the preferred approach for dealing with injection site residues. This approach has already been implemented successfully with long-acting oxytetracycline injections.

Other aminoglycoside products

During the course of the current review, industry concerns were expressed in relation to neomycin tissue residues in particular and aminoglycoside residues in general. The former probably arises as a result of improved analytical methodologies becoming available that allow residues to be positively identified. Previously, these residues were identified only as "inhibitory substances". The latter pertains to residues of a range of aminoglycosides that are administered enterally and/or parenterally to major food-producing species. For example, it is generally assumed from studies in healthy cows that aminoglycosides do not cross the udder to become systemically available. However, recent evidence with gentamicin intramammary treatments has shown that in cows with clinical mastitis up to 80% of the drug was systemically absorbed[(56)]. Tissue residues of (dihydro) streptomycin resulting from the use of intramammary aminoglycoside products were outside the scope of this review.

It is therefore recommended that a separate review of all aminoglycoside products used on the major food animal species be undertaken. Until such time as this review has been completed, aminoglycoside residue definitions cannot be amended to be in line with modern instrumental analytical techniques. In addition, the development of Control of Use legislation (which is currently being drafted by the States) could well benefit from knowledge of the findings of such a review.

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ATTACHMENTS

Attachment I Residue Studies

I.1 Reference: Study No. 043/88 "A study to determine the concentration of penicillin and (dihydro) streptomycin in milk after treatment with Pen Strep", Bradley, M., Wilson, W. and Kennedy, L, Norbrook Laboratories Limited.

Experiment: Six lactating cows (450-525 kg) were treated with PEN & STREP (Novartis/Heriot/Norbrook) at 1 mL/25 kg once daily for 3 days (procaine penicillin G **8 mg/kg bw**; (dihydro) streptomycin **10 mg/kg bw**). Milk samples were collected a.m. and p.m. for 96 hours after the last treatment. Samples were analysed using a microbiological assay.

Recoveries: Procaine penicillin G, 95-110% at 0.01-0.5 mg/L.

Results: Residues of penicillin in milk were below the MRL of *0.0015 mg/kg by 48 hours after the last injection.

Maximum (dihydro) streptomycin residues (μg/mL) in milk after 3 successive daily intramuscular injections with PEN & STREP (Novartis/Heriot/Norbrook); n = 6.

Time after last treatment					
	8 hours	**24 hours**	**32 hours**	**48 hours**	**56 hours**
mean±sd	0.57±0.47	0.17±0.13	<0.10	<0.10	<0.10
maximum	2.10	0.71	0.14	<0.10	<0.10

LOD < 0.1 μg/mL

Comment: Residues in milk were below the MRL for penicillin by 48 hours and (dihydro) streptomycin by 32 hours post-dose.

I.2 Reference: Exp No. 89/3712 "A milk residue study of dihydrostreptomycin and penicillin in cows treated with Depomycin®", Intervet.

Experiment: Four lactating cows (crossbred Holstein Friesian and Dutch Friesian, 450-600 kg) were treated with Depomycin® at 4 mL/100 kg (procaine penicillin G **8 mg/kg bw**; (dihydro) streptomycin **10 mg/kg bw**) intramuscularly once daily for 3 days. Milk samples were collected a.m. and p.m. for 6 days after the last treatment. Samples were analysed using a microbiological assay; method nr. PEN-L (Mycofarm) for penicillin and method nr. DHS (Mycofarm) for (dihydro) streptomycin.

Results: Maximum penicillin and (dihydro) streptomycin residues were detected at 12 hours post- dosing and were 0.16 IU/mL and 0.97 mg/L, respectively. Residues were

below the LOD (<0.007 IU/mL) by 5 milkings for penicillin and below LOD (0.2 mg/L) by 3 milkings for (dihydro) streptomycin.

Comment: Residues of both penicillin and (dihydro) streptomycin were below the MRL for milk by 5 milkings (60 h) and 3 milkings (36 h), respectively.

I.3 Reference: Report No. 042/95/1093, Nouws, J.F.M., Milk residue study of penicillin and dihydrostreptomycin following intramuscular injections of pen-strep 20-20 inj. to dairy cows, Farma Research B.V., Nijmegen, 14 July 1995.

Experiment: Eight dairy cows (610-800 kg bw; milk yield 9.6-23.0 L) were treated with **intramuscular injections** of Pen-Strep 20-20 (10 mg (dihydro) streptomycin /kg bw) in alternated neck regions for 5 consecutive days. Quarter milk samples were collected just before the first and last injections and 8, 21, 32, 44, 56, 68, 80 and 92 hours after the last injection. (dihydro) streptomycin contents were determined by microbiological assay (LOQ 0.1 µg/mL; LOD 0.074 µg/mL).

Results: Penicillin residues in milk were below the MRL of 0.0016 mg/kg by 44 hours after the last injection (LOD 0.003 µg/mL).

(Dihydro) streptomycin residues (µg/mL) in milk after 5 successive daily intramuscular injections with Pen-Strep (Dopharma); n = 8.

Time after last treatment								
	8 hours	**21 hours**	**32 hours**	**44 hours**	**56 hours**	**68 hours**	**80 hours**	**92 hours**
range	0.20-5.30	<LOQ-3.86	<LOQ-2.45	<LOQ-0.68	<LOQ-0.56	<LOQ-**0.32**	<LOQ-**0.25**	<LOQ-**0.19**
mean	1.19±1.09	0.69±0.87	0.34±0.53	0.28±0.19	0.29±0.17	**0.25±0.08**	**0.20±0.04**	**0.17±0.03**

Comment: Residues in milk were below the MRL by 92 h post-dose.

I.4 Reference: Cattle tissue residue studies, Norbrook Laboratories Limited.

Protocol No.: 079/95, A tissue residue study of penicillin G and dihydrostreptomycin in cattle, 14 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 3 October 1996.

Protocol No.: 072/95, A tissue residue study of penicillin G and dihydrostreptomycin in cattle, 18 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 30 September 1996.

Protocol No.: 014/96, A tissue residue study of penicillin G and dihydrostreptomycin in cattle, 21 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 13 September 1996.

Experiment: Twelve beef cattle (4-6 month males; 135-205 kg bw) were treated with an intramuscular injection of PEN-STREP at 1 mL/25 kg (procaine penicillin G **8 mg/kg bw**; (dihydro) streptomycin **10 mg/kg bw**) once daily for 3 days. Groups of 4 animals were slaughtered at 14, 18 and 21 days post-dose and samples (liver, kidney, fat, muscle and injection site) taken for analysis. Tissue samples were homogenised and stored at <-20 °C before analysis. Analytical method was HPLC SOM No: CRD/PPT/010 and CRD/DST/010.

Results: Penicillin residues were below the MRL for all time points.

Maximum (dihydro) streptomycin residues (mg/kg) in cattle tissues after administration of PEN STREP 10 mg/kg bw intramuscularly daily for 3 days (4 animals per time point)

Post-dose (days)	Muscle	Liver	Kidney	Fat	Injection site muscle
14	<0.4	<0.4	<0.4	<0.4	0.982
18	<0.4	<0.4	<0.4	<0.4	1.14
21	<0.4	<0.4	<0.4	<0.4	<0.4

LOQ = 0.4 mg/kg

Comment: Residues of penicillin were below the MRLs for all time points. (dihydro) streptomycin LOQ > MRLs and the WHP was not addressed. The residue definitions for (dihydro) streptomycin and penicillin were not addressed.

I.5 Reference: Sheep tissue residue studies, Norbrook Laboratories Limited.

Protocol No.: 085/95, A tissue residue study of penicillin G and dihydrostreptomycin in sheep, 14 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 12 September 1996.

Protocol No.: 074/95, A tissue residue study of penicillin G and dihydrostreptomycin in sheep, 18 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 12 October 1996.

Protocol No.: 015/96, A tissue residue study of penicillin G and dihydrostreptomycin in sheep, 28 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 13 September 1996.

Experiment: Twelve crossbred sheep (Suffolk×, Texel× and Cheviot×, ca. 5.5 yrs old, female, 58.5-85.5 kg bw) were treated with an intramuscular injection of PEN-STREP at 1 mL/25 kg (**8 mg/kg bw** procaine penicillin G; **10 mg/kg bw** (dihydro) streptomycin) once daily for 3 days. Groups of 4 animals were slaughtered at 14, 18 and 28 days post-dose and samples (liver, kidney, fat, muscle and injection site) taken for analysis. Tissue samples were homogenised and stored at <-20 °C before analysis. Analytical method was HPLC SOM Nos: CRD/PPT/010 and CRD/DST/010.

Results: Penicillin residues were below the respective MRLs at all time points.

Maximum (dihydro) streptomycin residues (mg/kg) in sheep tissue after intramuscular administration of PEN & STREP at 10 mg/kg bw daily for 3 days (4 animals per time point)

Post-dose (days)	Muscle	Liver	Kidney	Fat	Injection site muscle
14	<0.4	<0.4	<0.4	<0.4	0.982
18	<0.4	<0.4	<0.4	<0.4	0.691
28	<0.4	<0.4	<0.4	<0.4	<0.4

LOQ = 0.4 mg/kg

Comment: Residues of penicillin were below the MRLs for all time points. (dihydro) streptomycin LOQ > MRLs and the WHP was not addressed. The residue definitions for (dihydro) streptomycin and penicillin were not addressed.

I.6 Reference: Pig tissue residue studies, Norbrook Laboratories Limited.

Protocol No.: 087/95, A tissue residue study of penicillin G and dihydrostreptomycin in pigs, 14 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 6 September 1996.

Protocol No.: 076/95, A tissue residue study of penicillin G and dihydrostreptomycin in pigs, 18 days following intramuscular administration of PEN & STREP (Norbrook Laboratories Limited; PL 2000/4100), Henning, R., Rostrevor, Co. Down, Northern Ireland, 2 October 1996.

Experiment: Eight Landrace× pigs (3F, 5M; 35.5-43.0 kg bw) were treated with an intramuscular injection of PEN & STREP at 1 mL/25 kg (**8 mg/kg bw** procaine penicillin G; **10 mg/kg bw** (dihydro) streptomycin) once daily for 3 days. Groups of 4 animals were slaughtered at 14 and 18 days post-dose and samples (liver, kidney, fat, muscle and injection site) taken for analysis. Tissue samples were homogenised and stored at <-20 °C before analysis. Analytical method was HPLC SOM Nos: CRD/PPT/010 and CRD/DST/010.

Results: Penicillin residues were below the MRLs of *0.06 mg/kg for all time points (LOQ 0.01 mg/kg).

Maximum (dihydro) streptomycin residues (mg/kg) in pig tissues after intramuscular administration of PEN & STREP at 10 mg/kg bw daily for 3 days (4 animals per time point)

Post-dose (days)	Muscle	Liver	Kidney	Fat	Injection site muscle
14	<0.4	<0.4	<0.4	<0.4	<0.4
18	<0.4	<0.4	<0.4	<0.4	<0.4

LOQ = 0.4 mg/kg

Comment: Residues of penicillin were below the MRLs for all time points. (dihydro) streptomycin LOQ > MRLs and the WHP was not addressed. The residue definitions for (dihydro) streptomycin and penicillin were not addressed.

I.7 Reference: Tissue residue study; no title or reference.

Experiment: Seven cattle (100-280 kg bw), sheep (31-70 kg bw) and pigs (16-90 kg bw) were treated with Hydropen at the recommended dose rate (procaine penicillin G **8 mg/kg bw** and (dihydro) streptomycin **10 mg/kg bw**) by intramuscular injection for 3 days. Seven days after the last injection 4 cattle, 4 sheep and 4 pigs were slaughtered. The remaining animals were sacrificed at 18 days post-dose. Tissue samples were collected from both groups. Analysis of penicillin (LOD 0.008 mg/kg) and (dihydro) streptomycin (LOD 1 mg/kg) was by quantitative microbiological assay.

Results: For cattle and sheep, penicillin residues were below the MRL of *0.06 mg/kg for all time points. For pigs the penicillin MRL was exceeded in muscle and at the injection site at day 7 but was less than the MRL by 18 days post-dose.

Maximum (dihydro) streptomycin residues (mg/kg) in animal tissues after 3 successive daily treatments with Hydropen at the recommended dose rate

Species	WHP (days)	Muscle	Injection site	Heart	Liver	Kidney	Kidney fat	Subcutaneous fat
Cattle	7	<1.0	6.70	<1.0	3.98	11.70	<1.0	<1.0
	18	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Sheep	7	1.5	12.8	3.0	20.5	26.0	2.4	14.4
	18	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Pigs	7	1.31	<1.0	2.02	7.93	24.50	5.27	3.38
	18	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

LOD = 1.0 mg/kg

Comment: Residues of penicillin were below the MRLs by 18 days. (dihydro) streptomycin LOQ > MRLs and the WHP was not addressed.

I.8 Reference: Ref. No. 870316, Tissue residue study of an injectable procaine penicillin G/dihydrostreptomycin formulation (Depomycin®) in cattle, sheep and pig (Food-producing animals), Nouws, J.F.M. and van Raay, A.A.M.G., 16 March 1987, Intervet.

Experiment: Nine cattle (Friesian-Holstein× females, 82-140 kg bw), 9 sheep (Texel, 50-60 kg bw) and 14 pigs (Dutch Landrace/Yorkshire×, 50-120 kg bw) were treated with an intramuscular injection of Depomycin® at 1 mL/25 kg (**8 mg/kg bw** procaine penicillin G; **10 mg/kg bw** (dihydro) streptomycin) once daily for 3 days. Groups of cattle and sheep were slaughtered at 14 (n=5) and 21 (n=4) days after the last dose and samples (liver, kidney, fat, muscle and injection site) were taken for analysis. For pigs, the slaughter points were 3 (n=4), 14 (n=5) and 21 (n=5) days following the last dose. Tissue samples were homogenised and stored at <-20 °C before analysis. Analytical method: microbiological assay within 4 days of sample collection. Confirmations were quantitated using high voltage electrophoresis; penicillin LOD 0.02 IU/g; (dihydro) streptomycin LOD 0.1-0.2 mg/L (tissue).

Results: No penicillin residues were detected for any of the animals, LOD > 0.01 IU/g muscle; >0.02 IU/g kidney or liver; >0.006 IU/mL plasma. Injection site residues in

cattle were <1.7 IU/mL at 14 days and <1.2 IU/mL at 21 days. For sheep, the maximum injection site residues were <0.8 IU/mL at 14 days and <1.3 IU/mL at 21 days. In the case of pigs, 1139 IU/mL at 3 days, <1.2 IU/mL at 14 days and <0.81 IU/mL at 21 days.

Maximum (dihydro) streptomycin residues (mg/kg) in animal tissues after intramuscular administration of Depomycin® at 10 mg/kg bw daily for 3 days

Animal	Post-dose (days)	Muscle	Liver	Kidney	Plasma	Urine	Injection site muscle (µg/kg)
calves	14	<0.1	0.64	2.85	<0.16	<0.16	185
	21	<0.1	**0.63**	**0.76**	<0.16	<0.16	9.2
sheep	14	<0.1	2.6	5.6	<0.16	<0.16	263
	21	<0.1	**1.81**	**1.84**	<0.16	<0.16	<4.2
pigs	3	<0.1	4.9	18.8	<0.16	4.8	1275
	14	<0.1	0.98	2.09	<0.16	<0.16	346
	21	<0.1	**0.64**	**0.72**	<0.16	<0.16	314

Comment: Penicillin residues were below the MRLs for all time points. This study did not address the slaughter WHP of 30 days. (Dihydro) streptomycin residues still exceeded the MRL at 21 days post-dose.

I.9 Reference: Ref. No. 90363-3, Residue study of Depomycin® administered intramuscularly to sheep, Nouws, J.F.M., July 1989, Intervet.

Experiment: Twelve sheep (Texel, 36-43 kg bw) were treated with an intramuscular injection of Depomycin® at 1 mL/20 kg (**10 mg/kg bw** procaine penicillin G; **12.5 mg/kg bw** (dihydro) streptomycin) once daily for 3 days. Groups of 4 sheep were slaughtered at 4, 5 and 6 weeks after the last dose and samples (liver, kidney, fat, muscle and injection site) taken for analysis. Tissue samples were homogenised and stored at <-20 °C before analysis. Analytical method: microbiological assay within 4 days of sample collection. Confirmations were quantitated using high voltage electrophoresis; penicillin LOD 0.02 IU/g; (dihydro) streptomycin LOD 0.1-0.2 mg/L (tissue).

Results: At 28 days after the last injection, no penicillin residues were detected in edible tissues, plasma or urine.

Maximum (dihydro) streptomycin residues (mg/kg) in sheep tissue after intramuscular administration of Depomycin® at 12.5 mg/kg bw daily for 3 days (4 animals per time point)

Post-dose (days)	Fat	Muscle	Liver	Kidney	Plasma	Urine	Injection site muscle
28	<LOD	<LOD	1.17	0.47	<LOD	<LOD	0.81
35	<LOD	<LOD	**0.46**	**0.31**	<LOD	<LOD	**1.69**
42	<LOD	<LOD	<LOD	0.20	<LOD	<LOD	**2.36**

LOD = 0.1-0.2 mg/kg

Comment: Penicillin residues were below the MRLs for all time points. (dihydro) streptomycin residues still exceeded the MRL at 35 days post-dose.

I.10 Reference: Ref. No. 90353-3, Residue study of Depomycin® in pigs after intramuscular injections, Nouws, J.F.M., July 1989, Intervet.

Experiment: Sixteen pigs (Dutch Landrace, 38-64 kg bw) were treated with an intramuscular injection of Depomycin® at 1 mL/20 kg (**10 mg/kg bw** procaine penicillin G; **12.5 mg/kg bw** (dihydro) streptomycin) once daily for 3 days. Groups of 4 pigs were slaughtered at 4, 5, 6 and 7 weeks following the last dose and samples (liver, kidney, fat, muscle and injection site) taken for analysis. Tissue samples were homogenised and stored at <-20 °C before analysis. Analytical method was a microbiological assay; samples were assayed within 4 days of collection. Confirmations were quantitated using high voltage electrophoresis, penicillin LOD 0.02 IU/g, (dihydro) streptomycin LOD 0.1-0.2 mg/L (tissue).

Results: At 28 days after the last injection, no penicillin residues were detected in edible tissues, plasma or urine.

Maximum (dihydro) streptomycin residues (mg/kg) in pig tissue after intramuscular administration of Depomycin® at 12.5 mg/kg bw daily for 3 days (4 animals per time point)

Post-dose (days)	Fat	Muscle	Liver	Kidney	Plasma	Urine	Injection site muscle
28	<LOD	<LOD	0.75	0.62	<LOD	<LOD	1.08
35	<LOD	<LOD	<LOD	0.18	<LOD	<LOD	**1.56**
42	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.34**

LOD = 0.1-0.2 mg/kg

Comment: Penicillin residues were below the MRLs for all time points. (dihydro) streptomycin residues exceeded the MRL at 28 days after the final dose.

I.11 Reference: MRC DAQ.084 Development and validation of improved test procedures for antimicrobial residues in meat, section 4, tissue distribution study, pp 330-333.

Experiment: Three cattle (18-24 months old, Braford, 279-348 kg bw) were injected intramuscularly with PENSTREP at 2 mL/50 kg bw once in the early morning and once in the late afternoon (**10 mg/kg bw** procaine penicillin G; **10 mg/kg bw** (dihydro) streptomycin). One animal was slaughtered at 5, 15 and 30 days after the last dose and samples (kidney, liver, muscle and injection site) collected. Urine samples were collected each day and analysed using microbiological assay.

Results: No penicillin residues were detected in edible tissues. The maximum injection site residues were 0.03 mg/kg and occurred at 5 days after the last dose. Penicillin injection site residues were <LOD by 15 days. Urine samples tested positive for penicillin G for up to day 3.

(Dihydro) streptomycin residues (mg/kg) in cattle tissue after intramuscular administration of PENSTREP at 10 mg/kg bw twice daily for one day (1 animal per time point)

Post-dose (days)	Muscle	Kidney	Liver	Injection site muscle
5	0.07	27	3.3	5.3
15	<0.02	3.3	1.0	7.7

30	<0.02	1.4	0.16	1.8

Urine samples tested positive with a microbiological assay up to and including day 10 for (dihydro) streptomycin.

Comment: No analytical method or LOQ/LOD were supplied.

I.12 Reference: MRC DAV.085 Development and validation of improved test procedures for antimicrobial residues in meat, antibiotic residues after parenteral administration to sheep of penicillin-dihydrostreptomycin, pp 1-7.

Experiment: Six merino wethers (17-25 kg bw) were treated with an intramuscular injection of PEN & STREP (Heriot Agvet Pty Ltd) at 2 mL/50 kg bw (**8 mg/kg bw** procaine penicillin G; **10 mg/kg bw** (dihydro) streptomycin) once daily for three days. Groups of 2 sheep were slaughtered at 30, 45 and 60 days post-dose and tissue samples taken. Samples were stored at -70 °C prior to analysis. Tissue samples were analysed by HPLC.

Results:

(Dihydro) streptomycin residues (mg/kg) in sheep tissue after intramuscular administration of PEN & STREP 10 mg/kg daily for 3 days (2 animals per time point)

Post-dose (days)	Muscle	Kidney	Liver	Injection site muscle
30	nd	0.45	0.29	0.70
46	nd	nd	nd	**1.30**
64	nd	nd	nd	**2.11**

In urine, the microbial assay detected penicillin G and (dihydro) streptomycin up to 3 days post-dose and 48 days respectively after the final dose.

Comment: No LOD/LOQ were given for the HPLC method. The residue definitions for (dihydro) streptomycin and penicillin were not addressed.

I.13 Reference: No study number, Dihydrostreptomycin residues after parenteral administration to sheep of AQUACAINE S, Webber, J.J., Walsh, J.R. and Plozza, T.E., Centaur International, March 1995

Experiment: Six merino wethers (17-25 kg bw) were treated with intramuscular injections of Aquacaine S (Boehringer Ingelheim) at 1 mL/10 kg bw (**20 mg/kg bw** procaine penicillin G; **25 mg/kg bw** (dihydro) streptomycin). Two sheep were slaughtered 18 days after a single injection. The other four animals were injected once daily for three days and slaughtered in 2 groups of 2 at 18 and 36 days after the last dose. Samples were stored at -70 °C prior to analysis. Tissue samples were analysed by HPLC. Urine samples were collected at days 1, 2, 3, 18 and 36 for analysis using a microbiological assay.

Results:

(Dihydro) streptomycin residues (mg/kg) in sheep tissue after a single intramuscular administration of Aquacaine S at 25 mg/kg bw (2 animals per time point)

Post-dose (days)	No. of injections	Muscle	Kidney	Liver	Injection site muscle
18	1	0.13	1.04	0.16	0.21
18	3	nd	3.65	0.61	1.08
36	3	nd	**1.04**	0.19	**25.39**

Urinary penicillin and (dihydro) streptomycin were detected by microbial assay up to and including 3 days and 18 days, respectively, following the last dose.

Comment: No LOD/LOQ were given for the HPLC method. The residue definitions for (dihydro) streptomycin and penicillin were not addressed.

I.14 Reference: Study No: 029/87, "To determine the concentrations of penicillin and dihydrostreptomycin in the blood plasma of cattle, pigs and sheep during and following a course of treatment with Pen & Strep", Norbrook Laboratories Limited.

Experiment: Ten cattle, 6 pigs and 6 sheep were injected intramuscularly with Hydropen at 1 mL/25 kg bw (**8 mg/kg bw** procaine penicillin G; **10 mg/kg bw** (dihydro) streptomycin) once daily for three days. Blood samples were taken at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48, 49, 50, 51, 52, 54, 56, 60 and 72 hours after the last dose A microbiological assay was used to quantify penicillin and (dihydro) streptomycin.

Results:

Pharmacokinetic parameters for procaine penicillin in plasma of farm animals after intramuscular injection of Hydropen once daily for 3 days

Animal	Injection	C_{max} (mg/L)	T_{max} (hours)	AUC_{0-24} (mg.h/L)	$t_{½}$ (hours)	Vd_{area} (1/kg)
Cattle	1st	0.54±0.18	2.5±1.4	4.72±0.93	5.40±1.6	14.17±6.5
	3rd	0.59±0.18	2.2±1.1	4.70±1.17	5.03±1.2	12.98±4.2
Sheep	1st	0.78±0.08	1.0±0	3.17±0.46	2.28±0.3	8.36±1.1
	3rd	1.05±0.19	1.2±0.4	3.82±0.79	1.98±0.7	6.09±2.4
Pigs	1st	1.79±0.42	1.0±0	5.71±0.82	2.24±0.5	4.68±1.5
	3rd	1.25±0.17	1.2±0.4	5.66±0.66	2.29±0.8	4.69±1.6

Pharmacokinetic parameters for (dihydro) streptomycin in plasma of farm animals after intramuscular injection of Hydropen once daily for 3 days

Animal	Injection	C_{max} (mg/L)	T_{max} (hours)	AUC_{0-24} (mg.h/L)	t½ (hours)	Vd_{area} (1/kg)
Cattle	1st	23.0±5.7	1.4±0.8	104.2±20.8	2.71±0.6	0.39±0.1
	3rd	23.8±3.2	1.5±0.5	110.1±27.6	2.62±0.4	0.35±0.05
Sheep	1st	23.0±6.4	1.7±0.5	79.5±18.2	1.63±0.3	0.31±0.1
	3rd	23.2±5.0	1.0±0	64.3±15.7	1.76±0.1	0.41±0.1
Pigs	1st	27.1±9.3	1.2±0.4	86.1±13.2	2.33±0.2	0.40±0.1
	3rd	23.2±6.1	1.3±0.5	86.5±18.3	2.34±0.3	0.41±0.1

I.15 Reference: Report No. 042/95/1265, Nouws, J.F.M., Kinetic and residue depletion study of pen-strep 20-20 inj. in pigs after multiple intramuscular injections, Farma Research B.V., Nijmegen, 12 September 1995.

Experiment: Seventeen Dutch Landrace pigs (9F, 8M; 50-65 kg bw) were given intramuscular injections of Pen-Strep 20-20 inj. at 10,000 IU penicillin and **10 mg (dihydro) streptomycin /kg bw** daily for 5 consecutive days. Heparinized blood samples were collected from eight of the pigs before the start of treatment and at 1, 2, 4, 8, 12 and 24 hours after the first injection, at 2, 8 and 24 hours after the second, at 2.5, 8 and 24 hours after the third and fourth injections and 1, 2, 4.5, 8.5, 12 and 24 hours after the fifth injection. Four animals were slaughtered at 7, 14, 28 and 36 days after the last injection. Samples of kidney, liver, muscle, fat, skin, injection site, plasma and urine were taken for analysis. (dihydro) streptomycin contents were determined by microbiological assay (LOQ 0.10-0.15 µg/g).

Results: Based on mean residue values, the depletion half-lives for (dihydro) streptomycin in kidney and liver were 7.9 and 10.7 days, respectively.

Pharmacokinetic parameters for (dihydro) streptomycin in plasma of pigs after intramuscular injections with Pen-Strep 20-20 inj. (Dopharma) once daily for 5 days

		C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)	t½ (h)
1st injection	range	14.0-20.8	1	45-83.8	1.99-5.79
	mean	18.0±2.1		60±14	3.36±143
5th injection	range	20.1-26.4	1	55.8-80.0	1.73-2.47
	mean	23.6±2.5		67.5±8.8	2.05±0.27

(Dihydro) streptomycin residues (mg/kg) in pig tissue after intramuscular administration of Pen-Strep 20-20 inj. (Dopharma) once daily for 5 days

Days after last injection		Kidney	Liver	Muscle	Skin	Fat	Plasma	Injection site
7	range mean	3.68-5.33 4.14±0.79	2.09-2.84 2.38±0.34	<LOQ	<LOQ	<LOQ	<LOQ	1.00-3.37
14	range mean	2.21-2.33 2.28±0.06	1.32-2.27 1.74±0.41	<LOQ	<LOQ	<LOQ	<LOQ	0.36-2.53
28	mean range	0.51-0.73 0.60±0.11	0.51-0.62 0.54±0.06	<LOQ	<LOQ	<LOQ	<LOQ	0.36-4.23
36	range mean	0.30-**0.35** 0.34±0.01	0.44-**0.46** 0.45±0.01	<LOQ	<LOQ	<LOQ	<LOQ	<0.15-**2.42**

LOQ 0.15 mg/kg (kidney, liver, muscle), 0.10 mg/kg (fat, skin, plasma).

Comment: Liver, kidney and injection site residues > MRLs at 36 days after the final treatment.

I.16 Reference: Report No. 042/95/1186, Nouws, J.F.M., Kinetic and residue depletion study of pen-strep 20-20 inj. in calves after multiple intramuscular injections, Farma Research B.V., Nijmegen, 12 September 1995.

Experiment: Seventeen calves (11M, 6F, 7-24 months, 143-270 kg bw) were administered Pen-Strep 20-20 inj. as an intramuscular injection at a dose rate of 10,000 IU penicillin and **10 mg (dihydro) streptomycin /kg bw** daily for 5 consecutive days. After the first injection, blood samples were collected at 1, 2, 4, 8, 12, and 23.5 hours post-dose. For the second and fourth injections blood samples were taken at 2, 8 and 23.5 hours post-dose and at 1.5, 8 and 23.5 hours post-dose for the third injection. After the last injection, samples were obtained at 1, 2, 4, 8, 12 and 24 hours. Four calves were slaughtered at times 7, 14, 28 and 35 days after the last injection. Blood, urine, kidney, liver, muscle, fat and injection site samples were collected. Samples were homogenised and frozen at ≤-20°C before analysis. A microbiological assay was used to determine (dihydro) streptomycin concentrations (LOQ 0.08-0.32 mg/kg).

Results: The depletion half-lives for (dihydro) streptomycin in kidney and liver were 11.4 and 9.3 days, respectively (based on mean residue values).

Pharmacokinetic parameters for (dihydro) streptomycin in plasma of calves after intramuscular injections with Pen-Strep 20-20 inj. (Dopharma) daily for 5 days

		C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)	t½ (h)
1st injection	range mean	14.8-24.0 18.2±3.4	<1	62.5-90.5 75.8±10.5	4.15-10.7 5.6±2.1
5th injection	range mean	13.8-22.4 18.9±2.8	<1	69.5-112 84±13	4.40-10.1 6.3±1.8

(Dihydro) streptomycin residues (mg/kg) in calf tissue after intramuscular administration of Pen-Strep 20-20 inj. (Dopharma) daily for 5 days

Days after last injection		Kidney	Liver	Muscle	Skin	Fat	Plasma	Injection site
7	range mean	9.54-14.3 11.4±2.1	1.97-3.46 2.51±0.67	<LOQ	<LOQ	<LOQ	<LOQ	<0.16-4.97
14	range mean	3.29-4.71 3.93±0.69	1.23-1.66 1.44±0.24	<LOQ	<LOQ	<LOQ	<LOQ	<0.16-4.63
28	range mean	1.60-2.54 2.02±0.42	0.31-0.75 0.44±0.21	<LOQ	<LOQ	<LOQ	<LOQ	0.20-5.95
35	range mean	0.78-**3.80** 1.88±1.34	0.20-**0.59** 0.33±0.18	<LOQ	<LOQ	<LOQ	<LOQ	<0.16-**2.19**

LOQ 0.16 mg/kg (kidney, liver, muscle, skin, plasma), 0.08 mg/kg (fat).

Comment: (dihydro) streptomycin residues in kidney, liver and injection site muscle were >MRLs at 35 days after the final treatment.

I.17 Reference: Report No. 042/95/1287, Nouws, J.F.M., Kinetic and residue depletion study of (dihydro) streptomycin -250 inj. in pigs after multiple intramuscular injections, Farma Research B.V., Nijmegen, 21 August 1995.

Experiment: Thirteen Dutch Landrace pigs (ca 16-18 wks old; 7F, 6M; 46-60 kg bw) were given an **intramuscular injection** of (dihydro) streptomycin -250 inj. at **25 mg (dihydro) streptomycin /kg bw** daily for 5 consecutive days. Blood samples were collected from 9 pigs immediately before the first injection and 1, 2, 4, 8, 12 and 23.5 hours after the first injection and at 2, 8 and 24 hours after the second, third and fourth injections. Blood samples were also collected at 1, 2, 4, 8, 12 and 24 hours after the fifth injection. Groups of 4 pigs were slaughtered at 14, 35 and 56 days after the last injection and samples of kidney, liver, muscle, fat, skin, injection site, plasma and urine collected. Kidney, liver and muscle samples were homogenised and stored at ≤-20°C before analysis. Samples of fat and skin were stored intact. (dihydro) streptomycin contents were determined by microbiological assay (LOQ 0.08-0.16 mg/kg).

Results:

Pharmacokinetic parameters for (dihydro) streptomycin in plasma of pigs after intramuscular injection with (dihydro) streptomycin 250 inj. (Dopharma) daily for 5 days

		C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)	t½ (h)
1st injection	range mean	38.8-37.9 43.5±3.3	1	117-175 142±20	2.76-3.27 3.01±0.17
5th injection	range mean	35.0-50.4 43.9±4.8	1	93.4-177 132±22	3.17-4.21 3.61±0.33

(Dihydro) streptomycin residues (mg/kg) in pig tissues after intramuscular administration of (dihydro) streptomycin 250 inj. (Dopharma) daily for 5 days

Days after last injection		Kidney	Liver	Muscle	Skin	Fat	Plasma	Injection site
14	range mean	3.70-6.75 5.59±1.32	3.31-4.38 3.74±0.46	<LOQ	<LOQ	<LOQ	<LOQ	<0.16-2.76
35	range mean	0.39-**0.63** 0.53±0.11	0.14-**0.40** 0.24±0.12	<LOQ	<LOQ	<LOQ	<LOQ	0.76-**2.45**
56	range mean	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<0.16-**1.61**

LOQ 0.16 mg/kg (kidney, liver, muscle, skin, plasma), 0.08 mg/kg (fat).

Comment: (dihydro) streptomycin residues in kidney and liver >MRL at day 35. Injection site muscle residues of (dihydro) streptomycin > meat MRL at day 56.

I.18 Reference: Bennett, G.K., Britt, A.G., Determination of dihydrostreptomycin residues in feedlot cattle following parenteral administration with a dihydrostreptomycin/penicillin combination, Dept of Natural Resources & Environment, Victoria, Australia

Experiment: Eighty-four cattle in a Victorian feedlot were administered PENSTREP® intramuscularly in the neck at 10 mL/250 kg bw once daily for up to seven consecutive days. Fifty-nine animals received a single injection, 5 two injections, 14 three injections, 4 four injections and 1 seven injections. Dose rates were based on an estimation of the animal's liveweight by feedlot staff. Animals were slaughtered at intervals of 43 - 93 days after the last injection and samples of urine and kidney collected for analysis. Urine samples were analysed using a microbiological assay while kidney samples were analysed by HPLC (LOD 0.1 mg/kg).

Results: Of the 82 urine samples tested using a multi-plate inhibition test (MIT), only 4 had detectable residues. The average concentration of (dihydro)streptomycin in kidney was 1.0 mg/kg and the range was <0.01 mg/kg to 8.2 mg/kg. Eight-five percent of animals had residues levels in excess of the Australian MRL of *0.3 mg/kg.

(Dihydro) streptomycin residues (mg/kg) in cattle kidneys after intramuscular administration of PENSTREP® at ca. 10 mg/kg bw daily for one to seven days

Post-dose (days)	Number tested	(Dihydro)streptomycin residues			
		<0.3 mg/kg	0.3-1.0 mg/kg	1.0-2.0 mg/kg	>2.0 mg/kg
43-49	11	1	3	5	2
50-59	28	5	15	5	3
60-69	20	3	15	1	1
70-79	14	1	9	0	4
80-89	9	2	3	2	2
90-93	2	1	1	0	0
Total	84	13	46	13	12

Comment: (Dihydro)streptomycin kidney residues did not decline with time. The data indicate a WHP of 30 days is not adequate.

Attachment II Analytical Methodology

Several analytical methods have been developed for (dihydro) streptomycin and penicillin G in animal tissues and body fluids. Microbiological assays are well suited for rapid screening, working best with milk though applied to other tissues. (dihydro) streptomycin limits of detection are < 1 mg/kg for tissues and 0.1-0.2 mg/L for milk. Although chromatographic methods are not as well developed, HPLC and GC methods are utilised for confirmatory purposes in Australia. The reported limits of quantitation for HPLC and TLC methods for (dihydro) streptomycin are 0.04 mg/kg for muscle and kidney [57] and 0.01-0.02 mg/L for milk[58, 59].

The microbiological assays typically involve homogenisation of the tissues in a phosphate buffer. The sample is centrifuged to remove tissue debris and denatured proteins and the supernatant collected for microbiological assay. The supernatant is placed into a well cut from inoculated agar and diffuses into the medium at a specific rate. If antibiotic is present growth of the test organism is inhibited thereby creating a zone of inhibition. The antibiotic concentration is determined using interpolation of a standard curve of inhibition zones diameters versus concentration. The LOD is dependent on a variety of factors including the microorganism used, pH, tissue and incubation temperature.

The residue definition for (dihydro) streptomycin is inhibitory substance identified as (dihydro) streptomycin. The limit of detection for the current microbiological assay methods for (dihydro) streptomycin in tissues and milk are too high to give confidence in analytical results that approximate the LOQ. For this reason, quantitation of (dihydro) streptomycin residues should be confirmed using validated instrumental techniques such as HPLC. However, we are unaware of any studies that have demonstrated a

relationship between the antimicrobial activity of residues and their measurement by instrumental methods such as HPLC.

Organisms used in microbiological assay plates for penicillin G and (dihydro) streptomycin analysis

Organism/Tissue	Compound	Recovery	Concentration (mg/kg)	LOD (mg/kg)
Milk/Blood				
Micrococcus luteus NCIB 9660 [1, 2, 3, 4]	penicillin G	98-105%	0.01-0.1	<0.1
Bacillus stearothermophilus ATCC 10419 [5]	low levels penicillin G	?	0.0025-0.08	0.0025
Bacillus subtilis NCIB 8054 [6, 7, 8, 9]	(dihydro) streptomycin	54-65%	1.0 - 5.0	1.0
Bacillus subtilis ATCC 10649 [10]	(dihydro) streptomycin	93%	0.1-3.2	0.074
Micrococcus luteus ATCC 9341 [10]	penicillin G	86%	0.003-0.096	0.003
Animal Tissues				
Micrococcus luteus ATCC 9341 [11]	penicillin G	74-93%	0.01-0.32	0.005-0.008 0.01 muscle, liver, kidney
Bacillus stearothermophilus var calidolactis [12]	penicillin G		0.01-0.065	0.02
Bacillus subtilis NCIB 8054 [13, 14]	(dihydro) streptomycin	44-61%	1.0-5.0	1.0
Bacillus subtilis NCIB 8054 [12]	(dihydro) streptomycin		0.156-20.0	0.1
Bacillus subtilis 10649 pH 8 [15, 16, 17, 18]	(dihydro) streptomycin	99-107%	0.08-5.12	0.11-0.15 kidney, liver, muscle 0.02-0.045 fat

(1) Microbiological assay for penicillin G in the presence of dihydrostreptomycin, SOP NO: MRD/AM/065, Norbrook Laboratories Limited, 12 January 1987

(2) Validation for the recovery of (i) procaine penicillin G and (ii) procaine penicillin G in the presence of dihydrostreptomycin sulphate from bovine blood, Norbrook Laboratories Limited.

(3) Microbiological assay for penicillin G in the presence of dihydrostreptomycin in milk, SOP NO: MRD/APDS/020, Norbrook Laboratories Limited, 5 January 1988

(4) Validation for the recovery of (i) procaine penicillin G and (ii) procaine penicillin G in the presence of dihydrostreptomycin sulphate from milk, Norbrook Laboratories Limited.
(5) Microbiological assay for low levels of penicillin G in the presence of dihydrostreptomycin in milk using *Bacillus stearothermophilus* ATCC 10419, SOP No: MRD/APDS/030, Norbrook Laboratories Limited, 5 January 1988
(6) Microbiological assay for dihydrostreptomycin in the presence of penicillin G
(7) Validation for the recovery of (i) dihydrostreptomycin sulphate and (ii) dihydrostreptomycin in the presence of penicillin from bovine blood, Norbrook Laboratories Limited.
(8) Microbiological assay for dihydrostreptomycin in the presence of penicillin G in milk, SOP NO: MRD/APDS/020, Norbrook Laboratories Limited, 5 January 1988
(9) Validation for the recovery of (i) dihydrostreptomycin sulphate and (ii) dihydrostreptomycin in the presence of penicillin from milk, Norbrook Laboratories.
(10) Validation of the quantitative analysis of dihydrostreptomycin and penicillin in milk, 14 July 1995, van Duuren, E., Farma Research BV, Nijmegen, Report No. 042/95/1093
(11) Validation of the recovery of penicillin G from animal tissue, Norbrook Laboratories Limited.
(12) Tissue residue studies of an injectable procaine penicillin G/dihydrostreptomycin formulation (Depomycin®) in cattle, sheep and pigs (food-producing animals), 16 March 1987, Nouws, J.F.M. and van Raay, A.A.M.G., Ref No.: 870316, Intervet.
(13) Extraction and determination of dihydrostreptomycin residues in the presence of procaine penicillin G and vice versa from animal tissue, SOP No: M/RD/AM/046, Norbrook Laboratories Limited, 12 January 1987
(14) Validation of the recovery of dihydrostreptomycin from animal tissue, Norbrook Laboratories Limited.
(15) Validation of the quantitative analysis of dihydrostreptomycin, 6 September 1995, van Duuren, E., Farma Research BV, Nijmegen, Report No. 042/95/1186
(16) Validation of the quantitative analysis of dihydrostreptomycin, 12 September 1995, van Duuren, E., Farma Research BV, Nijmegen, Report No. 042/95/1265
(17) Validation of the quantitative analysis of dihydrostreptomycin, 21 August 1995, van Duuren, E., Farma Research BV, Nijmegen, Report No. 042/95/1287
(18) Validation of the quantitative analysis of dihydrostreptomycin and penicillin in milk, 14 July 1995, van Duuren, E., Farma Research BV, Nijmegen, Report No. 042/95/1093

HPLC Methods
Method of analysis of penicillin G in tissue, Norbrook Laboratories Limited, 23 February 1994
Validation of the method of analysis for penicillin G in bovine muscle, liver, kidney and fat (Som No.: CRD/PPT/010), Norbrook Laboratories Limited
Validation of the method of analysis for penicillin G in ovine muscle, liver, kidney and fat (Som No.: CRD/PPT/010), Norbrook Laboratories Limited
Validation of the method of analysis for penicillin G in porcine muscle, liver, kidney, fat and skin (Som No.: CRD/PPT/010), Norbrook Laboratories Limited

Penicillin G in tissue samples (HPLC).

Penicillin G is extracted from homogenised tissues using aqueous extraction followed by deproteinisation and isolation/clean-up on a C-18 Sep-Pak column. The eluant is

derivatised to form a mercuric mercaptide complex which is analysed by HPLC. Quantitation is by comparison with a standard curve. The response was linear over the concentration range 0.01-0.5 mg/kg, CV < 12%, the accuracy at 0.01 and 0.5 mg/kg were 45-89%, **LOQ = 0.01 mg/kg**. (dihydro) streptomycin did not interfere with the assay although small peaks from blank samples were a problem. These were accounted for by averaging the integrations from blanks and subtraction from the assay samples.

Method of analysis of dihydrostreptomycin in tissue, Norbrook Laboratories Limited, 6 October 1995
Validation of the method of analysis for dihydrostreptomycin in bovine muscle, liver, kidney and fat (Som No.: CRD/DST/010), Norbrook Laboratories Limited
Validation of the method of analysis for dihydrostreptomycin in ovine muscle, liver, kidney and fat (Som No.: CRD/DST/010), Norbrook Laboratories Limited
Validation of the method of analysis for dihydrostreptomycin in porcine muscle, liver, kidney, fat and skin (Som No.: CRD/DST/010), Norbrook Laboratories Limited

Method of analysis of (dihydro) streptomycin in tissue (HPLC).

(Dihydro) streptomycin is recovered from homogenised tissue samples via a protein precipitation step followed by isolation of the drug using cation exchange. The sample is derivatised with 1,2 napthoquinone-4-sulphonic acid before analysis using HPLC with fluorescence detection. Validation data were supplied. The response was linear over the concentration range 0.4-5 mg/kg, CV < 12%, the accuracy at 0.4 and 5.0 mg/kg were 86-109%, **LOQ = 0.4 mg/kg**. Penicillin did not interfere with the assay.