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Neomycin: Target animal safety risk assessment report

The reconsideration of registration of the products containing neomycin and approvals of their associated labels

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Director, Public Affairs and Communication
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
KINGSTON ACT 2604 Australia

Telephone: +61 2 6210 4988

Email: communications@apvma.gov.au.

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EXECUTIVE SUMMARY

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is reconsidering the approval and registration of products containing neomycin for use in food-producing animals in Australia. The scope of the reconsideration includes target animal safety and residues and trade.

The current target animal safety assessment for the reconsideration of neomycin was undertaken by the APVMA and considered published and unpublished target animal safety data and information on neomycin. This included a literature review of information available in the public domain, as well as adverse experience reports (AERs) provided to the APVMA and animal safety studies provided by holders.

The most frequently reported AER was for injection site reactions in horses. The frequency of both Australian and global AERs was low and many appeared to be related to reactions to the procaine benzylpenicillin in one of the neomycin-containing parenteral products.

The published and unpublished information indicates that, when administered at high concentrations, for prolonged durations, and/or more than once daily, neomycin causes nephrotoxicity and/or ototoxicity. This is particularly the case for parenteral formulations. However, the risk of developing nephrotoxicity or ototoxicity from either parenteral or oral formulations increases if the individual has compromised renal function, gastrointestinal inflammation or is receiving other potentially nephrotoxic drugs concomitantly.

A close examination of the published and unpublished information for food-producing animals suggests that parenteral neomycin-containing products are generally safe to use in the target species' when administered once daily for short durations. Furthermore, oral neomycin-containing products are generally safe to use in the target species' when administered for short durations and intra-mammary products are safe when administered according to the current approved label directions.

As a prescription animal treatment, products containing neomycin can only be prescribed by a veterinarian and used under veterinary supervision. However, additional label warnings in relation to the application of neomycin products are recommended. This includes warnings about the possibility of nephrotoxicity and ototoxicity, and contraindications for the use of neomycin-containing products in individual animals with compromised renal function, gastrointestinal inflammation or those receiving other potentially nephrotoxic drugs. For parenteral products, the maximum duration of treatment should be clearly indicated and recommended dosage regimens should be based on extended-interval administration that allows for concentration-dependent killing and avoids extended periods of trough concentrations that lead to accumulation of neomycin. For oral products, the potential for adverse effects following prolonged treatment and clear instructions to re-establish diagnosis if no clinical improvement is seen following the recommended duration of treatment should be included on product labels. For intra-mammary products, additional label statements about the potential for local irritation are recommended.

Based on consideration of the available information and that the recommended label changes are adopted, the continued use of neomycin-containing products when applied to food-producing animals is considered safe for target animals.

1 INTRODUCTION

The APVMA Chemical Review team assessed the published and unpublished animal safety data on neomycin. This included a literature review of information available in the public domain including a range of published scientific studies, adverse experience reports (AERs) provided to the APVMA and animal safety studies provided by holders of active constituent approvals, product registrations and label approvals ('holders'). The information assessed included studies conducted on products currently registered in Australia as well as products used overseas but similar to those currently registered in Australia.

1.1 Neomycin

Chemistry

Neomycin [2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-$_{D}$-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-$_{L}$-idopyranosyl)-β-$_{D}$-ribofuranosyl]streptamine] is an aminoglycoside antibiotic produced by *Streptomyces fradiae*. Aminoglycosides are characterised by comprising aminosugars attached to an aminocyclitol group via glycosidic linkages.

There may be several forms of a single aminoglycoside. For example, neomycin is a complex of three separate compounds: neomycin A (neamine; inactive); neomycin B (framycetin); and neomycin C. The commercially available, registered active constituent neomycin consists almost entirely of the sulfate salt of neomycin B (over 90%), with some neomycin C and only traces of neomycin A (otherwise known as fradiomycin; less than 1%) (EMA 2002; Plumb 2002; Renshaw et al. 2003; Boothe 2012).

Mode of action

Aminoglycosides are bactericidal antibiotics, which are active predominantly against aerobic Gram-negative bacteria in a concentration-dependent manner, with a significant post-antibiotic effect. They have little or no action against anaerobic bacteria, as they require oxygen to cross the cell membrane, as described below (Reeves 2011; Boothe 2012). Neomycin is active against strains of Gram-negative bacteria (excluding *Pseudomonas* spp.), such as *E. coli*, *Salmonella* and *Klebsiella* spp. and many strains of *Staphylococcus aureus* (Sweetman 2002), although treatment of staphylococci should be in conjunction with synergistic antibiotics, such as β-lactams (EMA 2002; Plumb 2002; Renshaw et al. 2003; Boothe 2012).

Mechanism of action

Aminoglycosides exert their antibacterial activity by interfering with protein synthesis at the membrane-associated bacterial ribosome (Riviere & Spoo 2001). This is achieved by irreversibly binding to one or more receptor proteins on the 30S subunit of the bacterial ribosome and subsequently interfering with the mRNA translation process, ultimately resulting in the production of a non-functional protein (EMA 2002; Reeves 2011). In order for neomycin to reach the ribosomal binding site of Gram-negative bacteria, it must cross the bacterial cell wall and then the cell membrane. Initially, neomycin diffuses across the cell wall by competitive displacement of bridging divalent cations (such as Mg^{2+} or Ca^{2+}) and subsequent disruption of cross-links between adjacent lipopolysaccharides. This damages the cell wall and increases permeability, which allows the aminoglycoside to enter the periplasmic space in a passive and non-energy-dependent process. From there, it is actively transported across the cytoplasmic membrane via an oxygen- and energy-dependent interaction that is dependent on electron transport. The bacterial cytoplasm is negatively charged with respect to the periplasm and external environment; thus, neomycin is transported across the cytoplasmic membrane by the membrane potential, where it is then able to interact with the ribosome and cause misreading of the mRNA. This further affects cell permeability, which allows more neomycin into the cell and leads to more cell disruption and eventually, cell death (Reeves 2011; Boothe 2012). The efficacy of aminoglycosides is substantially reduced in an anaerobic environment, because the appropriate oxygen-dependent transport mechanisms described above are lacking (Riviere & Spoo 2001; EMA 2002; Huth et al. 2011).

While most antimicrobials that interfere with ribosomal protein synthesis are exclusively bacteriostatic[1], aminoglycosides are bactericidal[2] at higher concentrations.

When aminoglycosides are used in combination with β-lactam compounds (such as benzylpenicillin or cephalosporins) synergism is achieved, as the cell-wall damage produced by the β-lactam compounds allows easier access for the aminoglycosides to the bacterial cell membrane (Boothe 2012). Consequently, neomycin is often administered in conjunction with β-lactam compounds, most commonly procaine benzylpenicillin.

Concentration-dependent killing

Aminoglycosides exhibit concentration-dependent bacterial killing, where the peak aminoglycoside concentration (C_{MAX}) is more important in determining the efficacy of bacterial killing than time above the minimum inhibitory concentration (MIC) (Freeman et al. 1997). Thus, it is more important to achieve optimal peak concentrations than to maintain drug concentrations slightly above the MIC for extended periods of time. While optimum ratios between the peak concentration and MIC have not yet been determined, the literature suggests that peak concentration:MIC ratios of 8:1 to 10:1 are necessary for optimal bactericidal activity while avoiding bacterial regrowth (Freeman et al. 1997; Boothe 2012).

[1] Bacteriostatic agents prevent bacteria from growing or reproducing, while not necessarily killing them

[2] Bactericidal agents kill bacteria directly

Post-antibiotic effect

Aminoglycosides also exhibit a post-antibiotic effect (PAE), where bactericidal action persists after serum concentrations of neomycin drop below the MIC (Riviere & Spoo 2001; Reeves 2011). The exact mechanism of PAE has not yet been determined. The PAE of aminoglycosides is dependent on the:

- bacterial strain and its MIC
- duration of exposure of bacteria to the aminoglycoside
- inherent potency of the aminoglycoside
- concentration of the aminoglycoside (the higher the concentration, the longer the duration of the PAE).

Longer intervals between dosing (eg once-daily dosing) that provide a drug-free period in which bacteria are not exposed to the drug appear to preserve bactericidal activity of aminoglycosides and reduce the risk of antimicrobial resistance, as well as toxicity (Freeman et al. 1997). Studies in animal models have shown that the degree of cochlear damage induced by aminoglycosides is more dependent on the total daily dose than the frequency with which it is administered. It has been hypothesized that extended-interval dosing may result in less saturation of cochlear cells and accumulation of aminoglycosides than more frequent administration (Freeman et al. 1997).

Pharmacokinetics

Absorption

As for other aminoglycosides, neomycin is a polycation (positively charged) and is highly polar, with the result that it is poorly absorbed (usually less than 10%) from the healthy gastrointestinal tract. However, substantial disruption of the intestinal mucosa (eg from enteritis) may increase permeability (Boothe 2012). In individual animals with impaired renal function, drug concentrations during the trough period may accumulate and result in nephrotoxicity (Boothe 2012).

Neomycin is absorbed rapidly and nearly completely following intramuscular administration, with peak serum concentrations achieved within 30 to 90 minutes. Intrauterine and intra-mammary administration of aminoglycosides also result in effective therapeutic local concentrations, but significant tissue residues have been observed (Reeves 2011).

Distribution

Aminoglycosides do not bind well to plasma proteins, they are poorly lipid-soluble and do not easily enter cells or penetrate cellular barriers. As they are polar at physiologic pH, distribution of aminoglycosides to extracellular fluids is limited and tissue penetration is generally minimal, with the exceptions of the renal tubules and inner ear endolymph, where accumulation is common (Boothe 2012).

Metabolism and excretion

Orally administered aminoglycosides are eliminated unchanged in the faeces in healthy animals. Following parenteral administration, neomycin is excreted unchanged primarily by renal glomerular filtration, with 80–90% of administered neomycin excreted in the urine (within 24 hours following intramuscular administration) (Riviere & Spoo 2001; Huth et al. 2011; Reeves 2011; Boothe 2012).

Glomerular filtration rates vary between species and are usually less in neonates, which are generally more sensitive to aminoglycosides (Boothe 2012). Furthermore, excretion varies as a result of changes to glomerular filtration rates in association with both cardiovascular and renal function, age, etc, and the half-life varies in response to the volume of extracellular fluid.

Aminoglycosides have relatively short plasma half-lives of approximately 1 hour in carnivores and 2 to 3 hours in herbivores and the elimination kinetics generally follow a three-compartment model (Boothe 2012):

- first ‘deep’ phase: binding of drug in renal tubular cell
- β-phase: approximately 90% of the drug is excreted unchanged from the kidneys
- second ‘deep’ (or γ phase): remaining drug excreted over protracted period (gradual release from renal intracellular binding sites; terminal elimination half-life 20–200 hours)

2 TARGET ANIMAL SAFETY

The purpose of this target animal safety assessment was to summarise the published and unpublished information concerning the safety of neomycin in food-producing animals and to present an assessment of the potential risks associated with its use. Where the literature on food-producing animals was lacking, studies conducted in companion or laboratory animals and humans were also included.

The parenteral use of neomycin in human medicine is no longer recommended because of toxicity concerns. As a result, there is very little recent toxicity information available on to the use of neomycin in humans and the majority of the literature relating to the safety of aminoglycosides has been conducted using gentamycin. However, neomycin has a similar mechanism of action to gentamycin, so the information on aminoglycoside toxicity in general will be included in this assessment and any research that was generated using neomycin will be specifically highlighted as such.

Unless indicated otherwise, the cited information has been sourced from peer-reviewed, scientific publications or from other information available in the public domain and not from examination of the original unpublished reports.

2.1 Review of the scientific literature

As well as being potent antimicrobials, aminoglycosides are also capable of causing toxic side effects in the kidney and inner ear. Thus, their use is usually reserved for more serious infections. In food-producing animals, systemic use of some aminoglycosides (including neomycin) is often restricted because of widespread resistance and persistence of residues in kidney tissues, such that they are usually used therapeutically rather than metaphylactically or prophylactically, or as growth promotants (Reeves 2011).

In the kidney, the damage is often reversible but in contrast, damage to the inner ear may be permanent as the hair cells in the cochlea do not regenerate (Masur et al. 1976; Huth et al. 2011). These side-effects are often dose-limiting factors in the use of aminoglycosides; however, they occur independently of each other. In humans, the probability of co-occurrence of nephrotoxicity and ototoxicity is 3.1% and a statistically significant relationship has not been demonstrated (Guthrie 2008).

Generally, toxicity of parenterally-administered aminoglycosides is greatest following intravenous administration, followed by intramuscular administration, with the least toxic route being intraperitoneal injection (Nord et al. 1967). The European Medicines Authority (EMA) reported that the acute toxicity of neomycin is low following oral administration (LD_{50} values greater than 2000 mg/kg bw) but higher following intravenous administration (LD_{50} values of approximately 100 mg/kg bw/day) in mice. Studies in mice reported LD_{50} values between 33.3 and 44 mg/kg following intravenous administration, 109 mg/kg following intramuscular administration, between 128 and 225 mg/kg following intraperitoneal administration, between 260 and 275 mg/kg following subcutaneous administration and >8000 mg/kg for following oral administration (Owada 1962; Black et al. 1963; Nord et al. 1967).

The EMA also reported that although two older, poorly reported mutagenicity studies produced positive results, a number of more recent, well-conducted Good Laboratory Practice (GLP)-compliant studies conducted according to Organisation for Economic Co-operation and Development (OECD) guidelines were unable to demonstrate any evidence of genotoxicity. Furthermore, there is no evidence for carcinogenicity, teratogenicity or adverse effects on reproductive function following neomycin administration (EMA 2002). Administration of neomycin can induce neuromuscular blockade and respiratory insufficiency; however, this is very rare and appears to occur when neomycin is used during surgery with concomitant anaesthetic administration (Gilbert et al. 1998; Mouton 2010).

While all aminoglycosides have the potential to cause ototoxicity and nephrotoxicity, streptomycin is the most ototoxic and neomycin is the most nephrotoxic. Overall, neomycin has been shown to be the most toxic aminoglycoside to mammalian cells, as determined by LD_{50} values for acute exposure in mice and a study that assessed the damage of cultures to hair cells from the outer cochlea of neonate mice (Nord et al. 1967; Kotecha & Richardson 1994).

The risk factors for toxicity that have been identified include prolonged duration of therapy (more than 7–10 days), age, pre-existing renal disease, acidosis and electrolyte disturbances, as well as the administrartion of multiple doses in one day. Aminoglycoside toxicity is related to the trough concentration of the drug, so once-daily treatment is preferable (particularly for parenteral administration) to allow the trough drug concentration to drop below the toxicity threshold. As the efficacy of aminoglycosides is concentration-dependent and there is a prolonged post-antibiotic effect, once-daily dosing is effective and multiple doses over a 24 hour period are not necessary (Reeves 2011; Wargo & Edwards 2014).

Ototoxicity

It is well established that aminoglycosides are capable of causing ototoxicity by damaging the cochlear hair cells and/or vestibular sensory hair cells of the organ of Corti or vestibular epithelium of the ear, respectively. The early literature indicates that once damaged, the sensory cells do not generally regenerate and the auditory (eighth cranial) nerve degenerates. However, recent research suggests that while there is no evidence for hair cell regeneration in the organ of Corti, the vestibular epithelium of the inner ear may have the capacity to regenerate new hair cells (Wang & Li 2000). Some aminoglycosides primarily damage the vestibular apparatus of the ear, while others (including neomycin) primarily cause cochlear damage, which moves from the base to the apex and from the outer hair cells to the more central structures (Selimoglu et al. 2003; Huth et al. 2011). Cochlear damage can cause permanent hearing loss while vestibular damage results in nausea, vomiting, dizziness, loss of balance and vertigo, ataxia and/or nystagmus (Segal & Skolnick 1998; Renshaw et al. 2003).

In humans, tinnitus is often the first symptom of cochlear damage and if treatment is not discontinued within a few days, hearing loss may follow. This first manifests as a loss in perception of high-frequency sounds, followed by progressive loss of lower-frequency sounds (Langman 1994). As discussed above, the majority of aminoglycoside toxicity research has been conducted using gentamycin, which is primarily vestibulotoxic (Selimoglu et al. 2003), suggesting that this research may not be applicable to neomycin. However, while neomycin more commonly induces cochlear damage, it can also cause vestibular damage (Matz et al. 2004). In a study that assessed damage to cultures of hair cells from the outer cochlea of neonate mice following aminoglycoside administration, the order of potency (from highest to lowest) was neomycin, gentamycin, dihydrostreptomycin, amikacin, neamine and finally, spectinomycin (Kotecha & Richardson 1994). Interestingly, there is some evidence in humans that controlled therapeutic doses of gentamycin to newborns is less ototoxic and vestibulotoxic than in older children or adults (Selimoglu 2007). In contrast, there is evidence that prolonged, supratherapeutic treatment with neomycin is more otototoxic in newbown than adult guinea pigs (N'Guyen et al. 1980).

After parenteral administration, aminoglycosides persist in the inner ear for up to 11 months after treatment, which is partially attributed to slow diffusion back into the bloodstream (Aran et al. 1999). When plasma aminoglycoside concentrations are high, accumulation in the perilymph and endolymph of the inner ear can occur in a dose-dependent manner initially; however, the process is saturable: the half-life of aminoglycosides is 10 to 15 times longer in perilymph than in serum (Lortholary et al. 1995; Renshaw et al. 2003). There is some evidence that uptake into inner ear fluids (endolymph) occurs via the strial capillaries and marginal cells (Warchol 2010; Huth et al. 2011). From there, aminoglycosides can enter the hair cells via endocytosis at the apical or basolateral membrane and/or directly through mechanotransducer channels, transient receptor potential (TRP) channels or adenosine triphosphate (ATP) receptors (Huth et al. 2011).

The cellular process of aminoglycoside-induced ototoxicity is extremely complex and has not yet been fully elucidated. Once inside the hair cell, aminoglycosides can cause damage either directly or indirectly, by disrupting the stereocilia of the inner ear[3] and ultimately leading to apoptotic cell death (Selimoglu 2007; Huth et al. 2011). The generation of reactive oxygen species (ROS) appears to be important in the initiation of hair cell death via oxidative stress (Selimoglu 2007; Warchol 2010). The oxidation caused by the combination of aminoglycosides and iron leads to the formation of ROS (Guthrie 2008; Huth et al. 2011). Iron supplementation has been reported to exacerbate ototoxicity following gentamycin administration via a dose-dependent manner (Guthrie 2008). Iron chelators can protect against aminoglycoside-induced ototoxicity. By a mechanism involving lipid peroxidation, ROS are able to increase membrane fluidity and permeability as well as inhibit protein synthesis and nucleic acids (Willis & Arya 2006; Denamur et al. 2011; Huth et al. 2011; Kamogashira et al. 2015). The latter in turn disrupts the activity of enzymes, ion channels and receptors (Huth et al. 2011). A link between dysfunctional mitochondria and ototoxicity has been confirmed by the discovery of the A1555G deafness mutation within the human mitochondrial rRNA (Guthrie 2008). The mechanism for this effect is not completely understood. However, aminoglycosides impair RNA translation and inhibit mitochondrial protein synthesis, which may lead to decreased ATP production. This decrease in energy production can result in compromised mitochondrial integrity and ultimately, activation of the apoptotic cascade (Huth et al. 2011).

[3] Stereocilia are the mechanosensing organelles of hair cells, which convert fluid pressure and movement into electrical stimuli

There is also some evidence that heat shock proteins (HSPs) may be capable of protecting hair cells from ototoxic injury, although the exact mechanism by which this occurs is not yet known (Warchol 2010). It is known that HSPs maintain normal protein structure during cellular stress by interacting with cytoplasmic proteins.

Recent research suggests that the role of supporting cells (eg cochlear sensory epithelial cells) in hair cell damage has previously been underestimated (Warchol 2010). Various intracellular processes have been implicated in the apoptotic pathway leading to hair cell death following aminoglycoside treatment, including both extrinsic[4] and intrinsic[5] pathways. Components of this intrinsic pathway are regulated by proteins of the B-Cell Lymphoma-2 (Bcl-2) family. Two pathways that belong to the mitogen-activated protein (MAP) kinase family[6] have also been implicated in the apoptotic process. Specifically, there is evidence that activation of both the c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways may be involved in aminoglycoside-induced ototoxicity, as inhibition of both of these pathways can protect against ototoxic damage (Selimoglu 2007; Lahne & Gale 2008; Warchol 2010; Huth et al. 2011).

Aminoglycoside-induced ototoxicity is more likely to occur following parenteral use than any other administration route. Administration of intramuscular neomycin at 2.2 mg/kg bw twice daily for 13 days caused deafness in one of two calves, while intramuscular administration at 4.5 mg/kg twice daily for 12 days also caused deafness in one of two calves (Crowell et al. 1981). The calf administered the higher dose was comatose and euthanised 12 days after treatment; the calf receiving the lower dose was euthanised 24 days after treatment. No adverse effects were observed in two control calves administrered 3600 U/kg bw intramuscular benzylpenicillin twice daily for 7 days (Crowell et al. 1981). These doses are comparable to the recommended doses for registered intramuscular products containing neomycin, as described above (2–4 mg/kg bw every 8 to 12 hours); however, the duration of treatment was considerably longer than that recommended on one product label (2–3 days). The authors utilised the Brainstem Auditory Evoked Response (BAER) technique as well as subjective tests such as hand claps and clicks to assess auditory impairment and determined that the subjective tests were unreliable and difficult to interpret, compared with the BAER technique. While these results provide evidence for the ototoxicity potential of neomycin, the study utilised very small sample sizes (two animals per group), which limits its value for regulatory purposes.

The EMA reported that repeated parenteral but not oral administration of neomycin resulted in ototoxicity in guinea pigs (EMA 2002) and ototoxicity has been reported following once daily subcutaneous administration of neomycin in both newborn (60, 120 or 240 mg/kg/day, 5 days/week for 21 days) and adult guinea pigs (60 ot 120 mg/kg/day, 5 days/week for 8 weeks) (N'Guyen et al. 1980). In the latter study, the ototoxic effects of neomycin were observed in newborn guinea pigs receiving 60 mg/kg neomycin after 18 days of treatment, after 6 days at 120 mg/kg or 240 mg/kg and complete deafness was evident at the end of the trial. By comparison, lower doses of the aminoglycoside amikacin resulted in only mild deafness and the higher dose (240 mg/kg) resulted in profound deafness. In the adult guinea pigs, subcutaneous treatment with 60 mg/kg neomycin elicited signs of ototoxicity after 3 weeks and 120 mg/kg resulted in total deafness after 5–6 weeks. These results indicate that neomycin may be more ototoxic in newborns than in adults.

[4] Extrinsic pathways involve, for example, tumour necrosis factors and caspases, which are involved in cellular degeneration.

[5] Intrinsic pathways predominate and are characterised by permeabilisation of the outer mitochondrial membrane

[6] The mitogen-activated protein (MAP) kinase family is involved in cellular proliferation, survival and apoptosis

It should be noted that the treatment regimen followed in this study used doses and treatment durations considerably higher and longer, respectively, than the recommended parenteral therapeutic dose regimen of 2–5 mg/kg/day for 2–3 days for both small and large animals. Furthermore, the subcutaneous route of administration was used; however, none of the parenteral products registered for use in Australia are indicated for subcutaneous administration. Similarly, administration of parenteral (the specific route of administration was not reported) neomycin to guinea pigs at 30 mg/kg/day for 60 days, followed by 40 mg/kg/day for an additional 30 days, revealed cochlear degeneration and complete disappearance of the organ of Corti and the outer and inner hair cells, but no evidence of vestibular damage (Chin 1963). While these studies collectively provide support for an ototoxicity mechanism for neomycin in guinea pigs, the doses and duration of treatment in these studies are not applicable to registered dosage regimens, making them of limited value for regulatory purposes.

Intramuscular administration of neomycin at a dose of 50 or 60 mg/kg bw to kittens for 15–17 days was used in an animal model of congenital or very early acquired profound deafness. This dose is 5 times higher and of longer duration than the recommended dose for dogs and cats (total daily dose of 10 mg/kg bw/day administered in divided doses every 6 to 8 hours for up to 3 days days) (Leake-Jones et al. 1980; Leake et al. 1997). Similarly, the intramuscular administration of neomycin (50 to 75 mg/kg daily for 10 consecutive days) to adult cats resulted in ototoxicity which was observed two days after cessation of treatment (Shepherd & Clark 1985). Finally, evidence of ototoxicity and cochlear damage were demonstrated in adult cats following 6, 7, 10 and 12 days of high doses of neomycin (100 mg/kg/day) administered subcutaneously (Brown & Daigneault 1973).

Although the majority of orally administered neomycin is excreted unchanged in faeces, there is evidence for systemic absorption leading to ototoxicity. For example a number of trials and case studies published in the 1960s and 1970s describe signs of ototoxicity,including deafness and cochlear damage in humans following oral administration of neomycin (Greenberg & Momary 1965; Ward & Rounthwaite 1978). However, these cases were usually associated with the administration of higher than recommended doses of neomycin for prolonged durations (in some cases, months or years), as well as concomittant therapy with various other treatments (including gentamycin) and usually concommitant gastrointestinal inflammation or renal failure (Ward & Rounthwaite 1978; Kavanagh & McCabe 1983; Langman 1994). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reported a French study that described the loss of hearing in the high-frequency range in 9 of 17 (53%) children aged 2 to 7 years and suffering gastroenteritis who had been administered 50–100 mg/kg bw/day orally, for 6 to 9 days (Renshaw et al. 2003).

Collectively, the evidence available in the literature indicates that prolonged treatment with high parenteral doses of neomycin administered more than once per day to animals is associated with the development of ototoxicity. Neomycin is generally poorly absorbed from the gastrointestinal tract; however, prolonged high doses of orally-administered neomycin appear to be associated with ototoxicity in some individual animals, usually with concommitant gastrointestinal inflammation or renal failure.

Nephrotoxicity

While most parenterally-administered aminoglycoside is excreted unchanged in the urine, the remainder (approximately 10%) accumulates in the renal proximal tubules, following glomerular filtration. This accumulation can result in concentrations of aminoglycosides in the renal cortex several times higher than in blood plasma. As a result, aminoglycosides have a long half-life in renal proximal tubule cells. When neomycin accumulates in high concentrations in the proximal tubule cells, it induces both structural changes and functional impairment of the cells (Nagai & Takano 2004; 2014).

The three recognised mechanisms by which aminoglycosides exert their nephrotoxic effects are renal tubular toxicity, reduced glomerular filtration rate (GFR) and reduced renal blood flow (Lopez-Novoa et al. 2011; Wargo & Edwards 2014). The primary mechanism is renal tubular toxicity, which occurs as a result of cell death via both apoptosis and necrosis. Apoptosis is more commonly observed than necrosis; however, apoptosis is an ATP-dependent process and once the ATP reserve of the cell is depleted, necrosis occurs (Lopez-Novoa et al. 2011). Once inside the proximal tubule of the nephron, aminoglycosides undergo endocytosis and transport through the endosomal compartment to accumulate in the Golgi body, lysosomes and the endoplasmic reticulum presumably until a threshold is reached. At that time, the endosomal membrane is compromised and its contents enter the cytosol and activate the intrinsic apoptotic pathway in the mitochondria, which interrupts the respiratory chain, impairs ATP production and produces oxidative stress. This leads to cell death via necrosis and inhibition of transporters in the proximal tubule, subsequently affecting tubular resorption and compromising cell viability (Lopez-Novoa et al. 2011).

A second nephrotoxic mechanism results in decreased GFR. Aminoglycosides increase intracellular calcium concentrations via a number of mechanisms (including increasing ROS and oxidative stress), which causes the smooth muscle mesangial cells to contract, leading to decreased GFR.

A third nephrotoxic mechanism is triggered by damage to the proximal tubule. The associated increased vascular resistance causes reduced renal blood flow in an effort to prevent fluid and electrolyte loss, which ultimately reduces GFR and results in tubular cell death by reducing oxygen and ATP availability (Lopez-Novoa et al. 2011; Wargo & Edwards 2014).

Aminoglycoside-induced nephrotoxicity occurs after a few days of treatment and is characterised initially by increased urinary excretion of proteins, enzymes, glucose, potassium, calcium, magnesium and phospholipids despite unaltered urinary volume. When the damaged kidneys are no longer capable of compensating, plasma creatinine and blood urea nitrogen (BUN) concentrations increase as does the excretion of potassium and sodium (Rougier et al. 2003; Nagai & Takano 2014; Wargo & Edwards 2014).

A number of risk factors for the development of aminoglycoside-induced nephrotoxicity have been identified. These include older age, compromised kidney and liver function, pregnancy, dehydration, metabolic acidosis and sodium depletion, as well as longer treatment duration, higher doses and split doses. In addition, concomitant therapy with nonsteroidal anti-inflammatory drugs (NSAIDs), diuretics, amphotericin B, cisplatin, cyclosporine, iodide-containing contrast media, vancomycin and/or cephalosporins have also been demonstrated to increase the risk of developing aminoglycoside-induced nephrotoxicity (Wargo & Edwards 2014). There is some evidence in horses that younger individuals may be less susceptible to neomycin-induced nephrotoxicity than adults, which is in contrast to the neomycin-induced ototoxic effects. Following experimental exposure to *Rhodococcus equi*, two foals were administered 4 g neomycin/day for 23 days while a third foal was administered 3 g neomycin on Day 1 of treatment, followed by 2 g neomycin/day for 18 days. The foals did not show any signs of nephrotoxicity; serum creatinine and BUN remained within normal limits and post-mortem examination of kidney tissue revealed no abnormalities (Barton 1986).

A study by Paterson et al (1998) in elderly patients in an aged care facility assessed the incidence of toxicity following once-daily administration of gentamicin (83% of patients) or tobramycin (17% of patients) at an initial dose of 4 mg/kg/day y. The authors observed that 15% of the patients (13/88) developed nephrotoxicity. When this data was further analysed, it was observed that no patient who received less than 3 days of therapy developed nephrotoxicity, and 3.9% of patients who received aminoglycoside therapy for a week or less developed nephrotoxicity. Although none of the patients received neomycin, the results highlight the importance of administering aminoglycosides at low, once-daily doses for only 2 to 3 days.

Neomycin administered intramuscularly to four adult horses with no evidence of renal dysfunction at 10 mg/kg bw every 12 hours for 10 days did not result in nephrotoxicity (Edwards et al. 1989). Within four days, some evidence of renal tubular injury was evident; however, this subsided following cessation of treatment. Similarly, when neomycin was administered at 10 mg/kg bw every 12 hours for 15 days to 8 adult horses, four of which were euthanased after cessation of treatment, there was no histological evidence of nephrotoxicity or alterations in serum or urinary creatinine. However, urinary gamma-glutamyl transferase (GGT), which is an indicator of renal toxicity was significantly increased in treated horses. Together, these studies demonstrate some effect of neomycin on renal function in horses; however, these studies provided no definitive evidence of nephrotoxicity.

Intramuscular administration of neomycin to four calves at 2.25 mg/kg or 4.5 mg/kg twice daily for 13 or 12 days, respectively, induced signs of nephrotoxicity by five days of treatment (Crowell et al. 1981). Nephrotoxicity was diagnosed based on abnormal urinalysis results (granular casts, proteinuria and low specific gravity), increased urinary enzymes (alanine aminopeptidase and gamma-glutamyltranspeptidase), azotaemia, increased creatinine and BUN, decreased creatinine clearance and transient polyuria and polydipsia. The two calves administered the higher dose were euthanased on day 12 and 14 due to anorexia and depression, while the two calves administered the lower dose survived until day 19 and 24. By comparison, two calves treated with penicillin did not display any clinical abnormalities. These doses and durations of treatment are higher than those recommended for parenteral administration of neomycin in calves.

The EMA reported nephrotoxic effects following repeated subcutaneous administration of neomycin to mice (300 mg/kg/day) and guinea pigs (10 to 60 mg/kg/day) as well as repeated intramuscular administration to dogs (24 to 96 mg/kg/day) (EMA 2002). Again, these doses are considerably higher than the recommended dose rate for parenteral neomycin administration.

In rats, nephrotoxicity was demonstrated following oral administration of neomycin calculated from recommended human doses (4 g/day) using a previously described formula (Freireich et al. 1966). In this study, 47 mg/100 g neomycin/day was administered by gavage to six rats for 14 days, which resulted in increased blood urea nitrogen and creatinine concentrations compared with untreated controls and rats administered with oral ampicillin (70 mg/100 g/day). In 2/6 rats, histological evidence of interstitial nephritis was also observed (Narendranathan et al. 1982). It was not clear from the paper whether treatments were administered once daily or more frequently.

In 1965, a published case report described renal failure in a woman who received oral neomycin prior to (total of 9 g over two days) and following (1 g four times daily for 6 days, followed by 0.5 g thrice daily for 5 days) surgery (Greenberg & Momary 1965). This patient had no clinical signs of renal disease prior to treatment.

A review of the scientific literature by Wargo & Edwards (2014) suggests that the risk of nephrotoxicity can be reduced by avoiding the use of aminoglycosides in patients with elevated baseline serum creatinine concentrations, limiting treatment duration to less than 7 days, monitoring pharmacokinetic parameters and trough concentrations, avoiding concomitant nephrotoxic medications and the use of extended-interval dosing strategies. In particular, the effectiveness of extended-interval dosing (i.e. once-daily) aminoglycoside regimens at reducing the incidence of treatment-associated nephrotoxicity has been extensively evaluated. Numerous studies conducted in humans or animals have demonstrated equal or less toxicity following once-daily administration (Nicolau et al. 1995; Ali & Goetz 1997; Bailey et al. 1997; Freeman et al. 1997; Rybak et al. 1999; Contopoulos-Ioannidis et al. 2004); however, these results generally do not include data collected following neomycin administration. Nevertheless, Rybak et al. (1999) concluded that a single daily dose regimen results in a lower daily intracellular accumulation rate, providing a longer time of administration until the threshold for toxicity is met. Thus, a once-daily dosing regimen may not reduce the overall incidence of aminoglycoside-induced nephrotoxicity but it may lengthen the time taken to cause nephrotoxicity.

Collectively, the literature indicates the potential for neomycin, along with other aminoglycosides, to induce nephrotoxicity in various species, particularly following parenteral administration. However, as described for ototoxicity, nephrotoxicity was reported to occur when dose rates and the duration of treatment consistently did not adhere to an extended-interval dosing regimen that allows for effective concentration-dependent killing and a PAE, without promoting accumulation of aminoglycosides.

The risk of developing nephrotoxicity is greater in humans with compromised renal function or those receiving concomitant medications that may also predispose to renal damage, as well as when treatment is administered more than once daily. There is no evidence that neomycin causes nephrotoxicity when used according to recommended dose regimens in individuals without impaired renal function. It has been hypothesised that the high dose be fixed but the interval between treatments lengthened in patients with decreased renal function (based on an assessment of creatinine clearance) (Freeman et al. 1997). This would ensure effective bactericidal activity while allowing an adequate drug-free period to prevent aminoglycoside accumulation and subsequent toxicity.

2.2 Adverse drug experience reports for neomycin in food-producing animals

Adverse experience reports may not represent a complete picture of the adverse effects of a drug and cannot be used to calculate an overall incidence because the number of unreported AERs is always unknown. In addition, various confounding factors, such as concurrent drug administration, underlying disease processes (diagnosed or subclinical) and the variable ability of owners, managers and veterinarians to recognise an adverse events may also reduce the reliability of the data. Nevertheless, AERs are a valuable source of data, as they may highlight previously unknown problems with a newly registered pharmaceutical as part of an ongoing pharmacovigilance process or amplify clinical observations that were not significant in smaller clinical trials.

AERs in Australia

The information for AERs in Australia was obtained from the APVMA's Adverse Experience Reporting Program (AERP) database (1995-2014). Table 1 and Table 2 include AERs that were classified as 'probable'[7] or 'possible'[8]. No AERs relating to neomycin use in pigs, poultry or sheep were reported.

Between 1995 and 2014, one AER relating to safety in cattle following the use of an intra-mammary product containing neomycin was reported. The report described lethargy followed by death in one of two treated cows following treatment.

The majority of AERs relating to target animal safety were reported for use of neomycin-containing products in horses, with a total of 25 AERs reported in 15 horses (Table 1). The most frequently reported AER was for injection site reactions, involving swelling and occasionally, pain on palpation at the injection site. Other reported AERs included pyrexia, muscle twitching, collapse or recumbency and in one case, death. The signs classed as 'behavioural' included agitation and pawing at the ground.

Table 1: AERs reported for use of neomycin-containing products in horses in Australia (1995–2014)

AER	Number of reports
Site reaction ± swelling and/or pain	12
Pyrexia	2
Behavioural	3
Muscle twitching/stiffness	3
Collapse/recumbency	4
Death	1

[7] ie there is a reasonable association between exposure to the product and the adverse experience, the description of presenting signs is consistent with the known pharmacology and toxicology of the product and there are no other equally plausible explanations for the adverse experience

[8] ie there is a reasonable association between exposure to the product and the adverse experience but the association does not meet the criteria for a probable classification

As described in Section 1.1, neomycin is often administered in conjunction with procaine penicillin, either in a combination product or as two separate injections. Procaine benzylpenicillin is widely used, has a wide safety margin and is usually well tolerated; however, adverse reactions can occur (Woodward 2005). The common clinical signs associated with an allergic response to benzylpenicillin may be mild and transient (e.g. oedema) or more severe and potentially fatal (anaphylaxis; eg respiratory distress, collapse). However, while there are some reports in the literature of allergy or anaphylaxis in horses attributable to benzylpenicillin, it is likely that most of the reactions to procaine benzylpenicillin are due to other causes.

Procaine is used to stabilise benzylpenicillin for intramuscular use. If procaine is inadvertently injected into the venous circulation, it can stimulate the central nervous system (CNS) and induce frantic and uncontrollable locomotor and behavioural changes, along with other CNS-related clinical signs (Olsen et al. 2007).
A report of 11 case studies in which adverse reactions to procaine benzylpenicillin were observed following intramuscular injection concluded that the clinical findings of most cases were indicative of central nervous involvement (Nielsen et al. 1988). Initial clinical signs in horses that did not become recumbent included behavioural signs (startled behaviour, evidence of fright and terror, sudden backing, rearing, aimless galloping), loss of coordination and muscle tremors. Additional clinical signs in the six horses that became recumbent included collapse, gasping followed by apnoea, cardiac arrest and, in five horses, death. Post mortem findings were consistent with acute procaine toxicity in the majority of cases (10/11) and anaphylaxis in one horse. Intravenous administration of 2, 5 and 10 mg/kg procaine hydrochloride induced behavioural (agitation, restlessness, vocalisation, sniffing the ground, staring into the distance, lip curling and changes in respiratory pattern), locomotor (muscle tremors, incessant running, ataxia and falling over) and vascular reactions (hyperaemia of the conjunctival blood vessels), similar to the adverse reactions reported following administration of procaine benzylpenicillin although no fatalities occurred (Chapman et al. 1992).

The adverse experiences described in Table 1 are consistent with either benzylpenicillin anaphylaxis or procaine toxicity. Thus, it is possible that some of the AERs described above in horses may be attributable to procaine benzylpenicillin used in conjunction with neomycin.

Global AERs

One of the holders presented a summary of suspected AERs to a product containing neomycin in animals reported globally for a 5½ year period between 2001 and 2006. The AERs associated with registered use originated from Australia, Ireland, Belgium, the Netherlands and the United Kingdom, while AERs relating to off-label use were reported in Belgium, Germany and the United Kingdom (Table 2).

As discussed above, many of the global AERs listed in Table 2 are consistent with either benzylpenicillin allergy or anaphylaxis, or a procaine-induced reaction. In particular, clinical signs associated with benzylpenicillin allergy or anaphylaxis that were reported include oedema, site/skin reactions, respiratory distress/failure, tachycardia, collapse/recumbency, convulsions, shock and death. Clinical signs commonly seen following inadvertent intravenous procaine administration that were reported include ataxia, behavioural changes, site reactions and recumbency (Chapman et al. 1992; Olsen et al. 2007; Omidi 2009). The signs classed as ‘behavioural’ included hyperaesthetic behaviour, lethargy, signs of distress and crashing into stable walls.

Table 2: Global AERs reported for use of neomycin-containing products (2001–2006)

AER	PIG	COW	SHEEP	HORSE			
	Belgium (n=60)	Netherlands (n=2)	Netherlands (n=3)	Ireland (n=1)	Australia (n=1)	Netherlands (n=1)	United Kingdom (n=10)
Ataxia				1			2
Behavioural							2
Tachycardia							1
Vomiting ± diarrhoea	1						
Weak	1						
Site reaction							1
Skin reaction	1						
Sweating		1				1	1
Oedema		1					3
Respiratory distress/ failure		2					
Collapse/ recumbency		1					
Convulsions				1			
Shock							1
Death	4	1	3	1	1		2

One of the AERs reported above in which a horse died (United Kingdom) was classified by the registrant as unlikely to be caused by the product. This causality assessment was based on a post mortem examination that indicated that the most likely cause of death of the horse was disseminated intravascular coagulation related to cellulitis; the causality assessment appears justified. In addition, the causality of death of three sheep in the Netherlands following treatment is not clear, although nephrotoxicity may explain the death of one lamb (increased risk in young animals), thus the AER was considered ‘unclassifiable’ by the registrant.

The United States Food and Drug Administration (US FDA) publishes Cumulative Adverse Drug Event (ADE) Summary Reports compiled by the Centre for Veterinary Medicine, which contain reported ADEs from 1987 until 2013. It is important to note that only paper reports are currently incorporated into these summaries and that currently, electronically submitted data is not contained. Nevertheless, two ADEs from horses (parenteral) and two ADEs from cattle (oral) were included in the summary. The ADEs observed in horses involved site reactions and skin irritation, while one ADE reported in cattle related to oedema and the other reported the death of an animal.

Off-label use of neomycin

Two reports of AERs following ‘off-label’ use that relate to target animal safety in food-producing animals in Australia were noted. There was one report of an injection site reaction and one report of pyrexia and pain associated with off-label use of neomycin in horses.

Four reports of AERs following ‘off-label’ use in food-producing animals overseas that relate to target animal safety were provided by a holder. Two cows died 11 and 21 days after receiving the product during abomasum displacement surgery; however, the long time period between treatment and death suggest that it is unlikely that the product was the cause of death and that post-operative complications were the likely cause. One cow died following an anaphylactic reaction to the product after it was used off-label. One horse collapsed and died immediately following administration of the product; however, it is likely that the product was administered intravascularly, rather than intramuscularly, suggesting death was the result of procaine toxicity.

2.3 Animal safety studies

The target animal safety assessment considered animal safety data provided by the holders for the reconsideration. A total of 15 safety studies in food-producing animals were provided for assessment. These included:

- studies conducted with the Neomycin Penicillin 100/200 aqueous solution for intramuscular injection product applied at twice the recommended dose (5 mL/50 kg bw) for three consecutive days to calves, sheep, pigs and ponies
- a study conducted with a soluble powder formulation (no longer registered as of 1997) applied at 0, 10, 30 or 50 mg/kg bw in drinking water of broiler chickens for 7 consecutive days
- studies conducted using the intra-mammary formulations applied at the recommended dose to lactating cows.

Discussion of safety studies

Generally, the studies analysed a consistent and wide range of endpoints including haematology and clinical chemistry parameters, as well as clinical observations. However, several data gaps were identified. For example, not all treatment scenarios were covered by the submitted data, with one study each conducted in calves, sheep, pigs and ponies to assess the tolerance of intramuscular administration of a product containing neomycin, one study conducted in broiler chickens to assess an oral powder formulation administered in drinking water and ten studies assessing an intra-mammary formulation. In addition, the studies that were submitted for the parenteral product utilised very small sample sizes (n=4) in predominantly young, female animals. International guidelines for conducting target animal safety studies for pharmaceuticals recommend that eight animals per treatment group at an age that represent the intended treatment population are assessed, and that both males and females are included (VICH 2008). The studies conducted using the intra-mammary products were again conducted in small numbers of animals (n=5 or n=6) and were not designed to comprehensively assess product safety but were instead designed to determine potential milk residues and local irritation only. However, while all recommended parameters were not assessed, the key variables for safety assessment are signs of local irritation, elevated SCC and changed milk production, which were assessed in a number of the submitted studies. The APVMA data guidelines stipulate that eight target animals should be used in each treatment group to determine the margin of safety (unless a strong scientific justification is provided for the use of a smaller sample size). The one study submitted on an oral formulation relates to a product that is no longer registered; however the study was included in this assessment as the dose was similar to that of currently registered products and it follows a similar administration pattern. No data were submitted for any of the currently registered oral formulations. Finally, the studies were generally conducted using only one dose (usually either the recommended dose or twice the recommended dose). In line with internationally recognised guidelines (VICH 2008), the APVMA's data guidelines stipulate that target animal safety studies are conducted using 0, 1, 3 and 5 times the recommended dose of the test product. The APVMA's data guidelines also require that animals receive the recommended dose for a period greater than that recommended on the label (e.g. twice the recommended duration), while international guidelines recommend that each group be treated for at least three times the proposed duration up to a maximum of 90 days (VICH 2008).

The primary issue regarding the target animal safety of neomycin-containing products are the effects on hearing and kidney function, particularly following parenteral administration. None of the studies assessed the possibility of ototoxicity following treatment, other than assessing general clinical signs in the animals. However, there was no description of clinical signs that would be used to specifically indicate deafness in any study. While some behavioural signs may indicate deafness in animals, such as an inability to localise sounds outside the field of view or orientating a good ear towards the ground, they may be unreliable and subjective.

Increased serum creatinine and BUN following treatment may be indicative of kidney failure or nephrotoxicity and can be monitored to determine whether to either commence or continue treatment with neomycin. Four studies were conducted to determine the tolerance of administration of a neomycin-containing product by intramuscular injection at twice the recommended dose (5 mL/50 kg bw) for 3 consecutive days in calves (Coert 1989a), sheep (Coert 1989c), pigs (Coert 1989b) and horses (Coert 1989d). In general, the product appeared to be well tolerated, with some evidence of local reactions in horses (increased segmented neutrophil/lymphocyte ratio, creatine phosphate kinase and glutamic oxalacetic transaminase, local swelling, increased temperature and some lethargy, stiffness and inappetence) (Coert 1989d) and sheep (increased creatine phosphate kinase) (Coert 1989c). These clinical signs had subsided within 14 days of the cessation of treatment. There was no evidence of increased serum creatinine or BUN in calves or sheep immediately following treatment (Coert 1989a; c). However, in pigs, serum creatinine ($p<0.05$) and BUN ($p<0.01$) were significantly increased 14 days after treatment (Coert 1989b) and serum BUN was significantly increased ($p<0.01$) 24 hours after treatment in sheep (Coert 1989c). Additionally, although the effect was not significant when results were pooled, three of the four treated pigs had elevated serum creatinine concentrations immediately after treatment (Coert 1989b). While the small (n=4) sample size limits the study (VICH 2008), it does suggest that there is some evidence for impaired renal function following treatment with a neomycin-containing product in pigs. In contrast, serum creatinine was significantly reduced 24 hours after treatment in horses ($p<0.01$) (Coert 1989d).

One study investigated the effect of oral administration of a powder formulation (no longer registered for use in Australia) in drinking water at 0, 10, 30, 50 mg/kg/day to 25–day-old broiler chickens for 7 consecutive days (Ibayashi et al. 1994). The US FDA approved label for this product recommends a dose of 10 mg/lb bw/day for up to 5 days. A similar product currently registered for use in broiler chickens in Australia recommends a dose of between 11 and 100 mg/kg/day, administered dissolved in drinking water for 3 to 5 days. Three other oral powders registered for use in broiler chickens recommend a minimum daily dose of 8 mg/kg when added to feed for 3 to 5 days. Thus, the data submitted by the holder relating to the archived product was included in this review. No dose-related changes were observed in any of the parameters assessed that appeared to be related to treatment (Ibayashi et al. 1994), Post mortem examination revealed yellowish livers in some birds; however, this finding was not dose-dependent and histological analysis revealed mild fatty liver, which is a spontaneous change often seen in broiler chickens. Thus, these changes were not considered to be associated with neomycin administration. Clinical pathology and post mortem examination did not reveal evidence of nephrotoxicity; however, the post mortem examination only involved weighing the organs. Histopathological analysis was performed on liver if required, but no histopathological analysis of kidney was performed. As renal function is a primary consideration for neomycin-induced toxicity, the lack of histologic analysis of kidney tissue reduces the value of the study for regulatory purposes.

There was some evidence for local irritation for up to 24 hours following intra-mammary infusion, with one study observing slight pain at milking and increased somatic cell counts (Raynaud et al. 1976). Two studies also concluded that intra-mammary infusion of neomycin-containing products was slightly irritating (increased somatic cell counts, abnormal visual appearance) when only one or two quarters were infused daily for 3 days (Raynaud & Brunault 1977; Raynaud & Brunault 1978b) and one of these studies also concluded that the product was more irritating (swelling, pain, clots) when all four quarters were infused (Raynaud & Brunault 1977). A fourth study found that after treatment for three days, increased somatic cell counts were observed in the treated quarters, but these had returned to normal by 7, 9 or 11 milkings after the last infusion (Raynaud & Brunault 1978c). One study reported increased somatic cell counts in milk following intra-mammary infusion of neomycin for three infusions at 24 hour intervals, which had returned to pre-treatment values by 8 milkings (96 hours) post-treatment or 7 milkings (84 hours) for mastitic or normal quarters, respectively (Raynaud & Brunault 1978a). In contrast, another study saw no visual evidence of local inflammation or systemic disease (swelling, heat, redness or pain) attributable to neomycin, nor a rise in somatic cell count following intra-mammary infusion (Deluyker et al. 1996). A further 7 studies assessed signs of local inflammation and irritation following intra-mammary infusion of neomycin-containing products. Collectively, these studies demonstrated that some local irritation (assessed via leucocyte counts, strip cup and/or California mastitis test) may be present by 12 hours post-treatment (Symonds 1967c; b; e; d; a; f; Hawbaker & Coon 1968).

2.4 Conclusion

A close examination of the published and unpublished information concerning neomycin use in food-producing animals suggests that neomycin-containing products are generally safe to use in individuals without renal impairment at recommended doses and routes of administration. Information provided by the holders and global and Australian AERs show a particularly low incidence of problems encountered in food-producing animals, when used according to the label directions. Many of the AERs that have been reported were either site reactions or may be due to benzylpenicillin anaphylaxis or procaine toxicity (especially in horses). However, there were some data gaps that should be addressed by changes to product labelling or in advice to veterinarians when dispensing the products:

- While it is appreciated that the global and Australian incidence of adverse experiences related to the use of neomycin-containing products is low, it is clear that administration of neomycin at high doses and/or for prolonged duration is associated with nephrotoxicity and ototoxicity. These should be noted as possible adverse reactions on product labels and the recommended maximum duration of treatment should be clearly indicated.
- The risk of developing nephrotoxicity and/or ototoxicity is increased in individuals with compromised renal function, gastrointestinal inflammation and those being treated with other potentially nephrotoxic drugs. These situations should be listed as contraindications on product labels.

- The risk of nephrotoxicity following parenteral administration of neomycin-containing products decreases when extended-interval (once daily) administration is employed. This information is critical from a toxicity perspective and should be clearly outlined on product labels. The package inserts for parenteral products should elaborate on the importance of once-daily dosing that achieves concentration-dependent killing and sub-toxic trough concentrations, as well as the post-antibiotic effects association with treatment with neomycin. The directions for use should indicate a recommended dosage regimen that includes once-daily dosage for parenteral products.
    - The following precaution statement is recommended for all parenteral products: ‘Neomycin exhibits concentration-dependent killing and a post-antibiotic effect. Repeated daily administration or overdosage with neomycin can cause renal damage and deafness. The risk of toxicity can be reduced by extending the dosage interval to 24 hours. Care should be taken in individuals with known or suspected impaired renal function. Care should be taken in individuals being treated with other potentially nephrotoxic substances, such as NSAIDs. Where practical, it is recommended to weigh young stock to ensure accurate dosage calculation based on bodyweight’.
- The risk of nephrotoxicity following oral administration of neomycin-containing products increases when the duration of treatment is prolonged. This information is critical from a toxicity perspective and should be clearly outlined on product labels. The package inserts for parenteral products should elaborate on the importance of re-establishing the diagnosis if no improvement is observed following the recommended duration of treatment.
    - The following precaution statement is recommended for all oral products: ‘Neomycin exhibits concentration-dependent killing and a post-antibiotic effect. Overdosage with neomycin can cause renal damage and deafness. Care should be taken in individuals with known or suspected impaired renal function. While this is unlikely at therapeutic doses, care should be taken in individuals being treated with other potentially nephrotoxic substances, such as NSAIDs. Where practical, it is recommended to weigh young stock to ensure accurate dosage calculation based on bodyweight’.
    - For all products that recommend a minimum duration of treatment of 5 days for salmonellosis, the following direction should be included: ‘If no improvement is seen after 5 days, the diagnosis should be re-established’.
- The following precaution statement is recommended for all intra-mammary products: ‘Neomycin exhibits concentration-dependent killing and a post-antibiotic effect. Overdosage with neomycin can cause renal damage and deafness. While this is unlikely at therapeutic doses, care should be taken in individuals with known or suspected impaired renal function. Care should also be taken in individuals being treated with other potentially nephrotoxic substances, such as NSAIDs’.
- There is some evidence for local irritation following intra-mammary infusion of neomycin for the treatment of mastitis in dairy cattle. This should be noted as a possible adverse reaction on product labelling.
    - The following precaution statement is recommended for all intra-mammary products: ‘If abnormal milk, redness, irritation or swelling persists or increases, discontinue use and redetermine diagnosis’

APPENDICES

APPENDIX A—LIST OF PRODUCT REGISTRATIONS AND LABEL APPROVALS

Table 3: Product registrations and associated label approvals included in the review

Product number	Product name	Registrant	Product type	Species	Label approval number
36026	SCOURBAN ORAL ANTI-DIARRHOEAL SUSPENSION	BAYER AUSTRALIA LTD (ANIMAL HEALTH)	Oral solution/ suspension	Calf, horse, dog, cat	36026/100547 36026/56642 36026/1201 36026/01
36237	JUROX NEOMYCIN SULFATE INJECTION	JUROX PTY LTD	Parenteral liquid/solution/ suspension	Cattle, horse, pig, sheep, dog, cat	36237/50976 36237/100ML/0305 36327/02
36693	NEOJECT 200 ANTIBIOTIC INJECTION	CEVA ANIMAL HEALTH PTY LTD	Parenteral liquid/solution/ suspension	Cattle, horse, pig, sheep, dog, cat	Ψ
37241	NEOMYCIN PENICILLIN 100/200 AQUEOUS SUSPENSION FOR INTRAMUSCYLAR INJECTION	INTERVET AUSTRALIA PTY LIMITED	Parenteral liquid/solution/ suspension	Cattle, horse, pig, sheep, dog, cat	37241/100M/0410 37241/250M/0410 327241/100M/1006 37241/100M/0405 37241/250M/0405 37241/100M/0504 37241/250M/0504 37241/0301
38696	SPECIAL FORMULA 17900 FORTE-V LACTATING INTRAMAMMARY ANTIBIOTIC SOLUTION	ZOETIS AUSTRALIA PTY LTD	Intramammary	Cattle	38696/60502 38696/20X10/0809 38696/0402 38696/0899
38698	LINCOCIN FORTE LACTATING INTRAMAMMARY ANTIBIOTIC SOLUTION	ZOETIS AUSTRALIA PTY LTD	Intramammary	Cattle	38698/0402 38698/0699
46414	NEO-SULCIN SCOUR TABLETS	JUROX PTY LTD	Oral tablet	Calf, horse	46414/40/0410 46414/0101

Product number	Product name	Registrant	Product type	Species	Label approval number
49788	SCOUR-X ORAL ANTI-DIARRHOEAL SUSPENSION	AUSRICHTER PTY LTD	Oral solution/ suspension	Calf, horse, dog, cat	49788/0101 49788/01
49851	MASTALONE INTRAMAMMARY SUSPENSION FOR LACTATING COWS	ZOETIS AUSTRALIA PTY LTD	Intramammary	Cattle	49851/20X10/0709 49851/10/0709 49851/01
52621	NEOMYCIN SULPHATE UPJOHN FEED ADDITIVE POWDER	ZOETIS AUSTRALIA PTY LTD	Oral powder, pre-mix	Cattle, pig, poultry	52621/25KG/0510 52621/25KG/0508 52621/0802 52621/0100
52782	CCD NEOMYCIN (NEOMYCIN SULPHATE WATER SOLUBLE POWDER)	CCD ANIMAL HEALTH PTY LTD	Oral powder, pre-mix	Poultry	52782/2/0705 52782/1003 52782/1100
58671	NEOPHARM ANTIBIOTIC FEED ADDITIVE	BAYER AUSTRALIA LTD (ANIMAL HEALTH)	Oral powder, pre-mix	Cattle, pig, poultry	58671/101686 58671/500G/0205 58671/1KG/0205 58671/10KG/0205
67805[a]	ABBEYNEO ANTIBIOTIC FEED ADDITIVE	ABBEY LABORATORIES PTY LTD	Oral powder, pre-mix	Cattle, pig, poultry	67805/56898

Ψ *Labels transitioned from the states and so not having an approval number*

a *Products registered after the review started*

ABBREVIATIONS

ADE	Adverse drug event
AER	Adverse experience report
AERP	Adverse Experience Reporting Program (through APVMA)
Agvet Codes	Agricultural and Veterinary Chemicals Codes Act 1994
APVMA	Australian Pesticides and Veterinary Medicines Authority
ATP	Adenosine triphosphate
BAER	Brainstem Auditory Evoked Response
Bcl-2	Beta-cell lymphoma-2
bw	Bodyweight
bw/day	Bodyweight per day
BUN	Blood urea nitrogen
Ca^{2+}	Calcium
C_{MAX}	Maximal or peak concentration
CNS	Central nervous system
EMA	European Medicines Agency
ERK1/2	Extracellular signal-regulated kinase ½
g	Gram
GFR	Glomerular filtration rate
GLP	Good Laboratory Practice
GGT	Gamma-glutamyl transferase
HSP	Heat Shock Protein
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JNK	c-Jun N-terminal kinase
LD_{50}	Median lethal dose
MAP	Mitogen-activated protein
MIC	Minimum inhibitory concentration

mg/kg	Milligrams per kilogram
mg/kg bw	Milligrams per kilogram of bodyweight
mg/kg bw/day	Milligrams per kilogram of bodyweight per day
Mg^{2+}	Magnesium
mL	Millilitre
mRNA	Messenger RNA
NSAID	Non-Steroidal Anti-Inflammatory Drug
OECD	Organisation for Economic Co-operation and Development
PAE	Post-antibiotic effect
RNA	Ribonucleic Acid
ROS	Reactive oxygen species
TRP	Transient receptor potential
US FDA	United States Food and Drug Administration

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