FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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[28–16]

Supporting document 1

Risk assessment – Application A1133

Maximum Residue Limits for Avilamycin in specific Pig Commodities

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1 Dietary Exposure Assessment

1.1 Background

FSANZ conducts and reviews dietary exposure assessments (DEAs) for maximum residue limits (MRLs) using the best available scientific data and internationally recognised risk assessment methodologies. Variations to MRLs in Australia New Zealand Food Standards Code (the Code) will not be supported where estimated dietary exposures to the residues of a chemical indicate a potential public health and safety risk for the population or population sub group.

The steps undertaken in conducting a DEA are:

- determining the residues of a chemical in foods of interest
- calculating dietary exposure to a chemical from relevant foods, using residue data and food consumption data from Australian national nutrition surveys (NNS)
- completing a risk characterisation where estimated dietary exposures are compared to the relevant health based guidance value (HBGV)

Further information on how FSANZ conducts DEAs is available on the FSANZ website[1].

1.2 Food consumption data

1.2.1 1995 National Nutrition Survey (NNS)

The 1995 NNS provides comprehensive information on dietary patterns of a sample of 13,858 Australians aged from 2 years and above. The survey used a 24-hour recall method for all respondents, with 10% of respondents also completing a second 24-hour recall on a second, non-consecutive day. Food frequency data are available for a subset of the national sample (respondents aged 12 years and above) as are responses to a series of short dietary questions about food habits. These data are used unweighted.

1.2.2 2011–12 National Nutrition and Physical Activity Survey (NNPAS)

Mean food consumption data used to estimate exposure were derived from the 2011–12 National Nutrition and Physical Activity Survey (NNPAS) which surveyed 12,153 respondents aged 2 years and above. 7735 (64%) individuals were surveyed for 2 non-consecutive days making it possible to derive average consumption amounts. The two day average exposure was derived based on consumption data from the respondents with two days of data (applying a different set of sample weights to make this survey sub-sample representative of the population).

Consumption amounts were for all respondents that were surveyed over 2 non-consecutive days. Consumption was averaged over the two days. The two day average exposures better reflects longer term estimates of dietary exposure and therefore are a better estimate of chronic dietary exposure.

Consumption data included commodities reported as consumed on their own (e.g. pork chop, glass of milk, boiled egg) and when used in a mixed food (e.g. pork stir fry, quiche).

[1] http://www.foodstandards.gov.au/science/exposure/Pages/dietaryexposureandin4438.aspx

1.2.3 Data used in the dietary exposure assessment

Previous estimates of dietary exposure to avilamycin have used consumption data from the 1995 NNS[2]. In the 1995 NNS, pig liver and pig kidney were not reported to have been consumed. Offal consumption reported as 'not specified as to type' was assigned to both pig liver and kidney and used to determine consumption in the DEA.

Data from the NNPAS[3] has more recently become available to FSANZ for use in dietary exposure estimates. Pig liver and pig kidney were also not reported to have been consumed in this survey. Consequently, cattle liver and kidney were used to represent pig liver and kidney consumption for the DEA. The 2-day average consumption for these was 0.004 and 0.00005 grams per kilogram body weight per day (g/kg bw/d) respectively.

For both the 1995 NNS and 2011–12 NNPAS surveys, consumption included where a food was reported as consumed (for example, fried liver) or where it was consumed as part of a mixed food or recipe (for example, liverwurst). For both surveys, assumptions about pig offal consumption represent a likely over-estimation of consumption and a 'worse-case' scenario for estimating the dietary exposure to avilamycin.

1.3 Dietary Exposure estimates

Only a chronic estimate of dietary exposure, the National Estimated Daily Intake (NEDI) was conducted for avilamycin. The Australian Office of Chemical Safety (OCS) established an acceptable daily intake (ADI) of 1 milligram per kilogram body weight per day (mg/kg bw/day) (1997) and is the relevant HBGV to be used in the NEDI.

A National Estimated Short Term Intake (NESTI) assessment was not required for avilamycin as no relevant acute HBGV has been established by the OCS or the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

The NEDI was calculated encompassing all current (poultry) permissions for avilamycin in Schedule 20 of the Code and proposed commodity MRLs in this Application and using the mean dietary consumption data derived from the relevant NNS.

The dietary exposure estimate as a percentage of the ADI is provided in Table 1.

Table 1: Dietary exposure estimate for proposed MRLs for Avilamycin

Commodity	Pre-A1133 MRL	Proposed Post-A1133 MRL (mg/kg)	MRL change in the Code	Origin of requested MRL	Consumption amount (g/kg bw/d)	NEDI* 1995 NNS†	NEDI* 2011-12 NNPAS††
Pig meat	n/a	0.2	New	Codex	0.422	<0.01% of the ADI	0.01% of the ADI
Pig fat/skin	n/a	0.2	New	Codex	0.046		
Pig kidney	n/a	0.2	New	Codex	0.004 (cattle)		
Pig liver	n/a	0.3	New	Codex	0.00005 (cattle)		

* The NEDI represents an estimate of chronic dietary exposure from the whole diet for the general population aged 2 years and over, expressed in this table as a proportion of the ADI.
† Food consumption data were derived from the 1995 NNS, for all survey respondents using food consumption data from Day 1 only. The design of this survey and key attributes of each are set out in Section 1.2.
†† Food consumption data were derived from the 2011-12 NNPAS for all survey respondents using food consumption data from 2 non-consecutive days. The design of this survey and key attributes are set out in section

[2] 1995 Australian National Nutrition Survey (1995 NNS): http://www.foodstandards.gov.au/science/exposure/pages/foodconsumptiondatau4440.aspx (accessed 3/2/2016)
[3] 2011-12 National Nutrition and Physical Activity Survey (NNPAS): http://www.foodstandards.gov.au/science/exposure/pages/foodconsumptiondatau4440.aspx (accessed 3/2/2016)

1.4 Conclusion

The NEDI for avilamycin, taking into account all currently permitted and newly requested MRLs, is <1% of the ADI.

The inclusion of MRLs for avilamycin (with the proposed residue definition: measured as Dichloroisoeverninic acid) at 0.2 mg/kg for pig fat/skin, pig kidney and pig meat and 0.3 mg/kg for pig liver did not substantially increase dietary exposure.

2 Microbiological evaluation

2.1 Activity against human gastrointestinal microflora

Avilamycin is an orthosomycin antibiotic that inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit (Treede et al 2003). Avilamycin is highly active against numerous gram-positive bacteria that are a normal component of the gastrointestinal microflora, including *Enterococcus*, *Peptostreptococcus*, *Eubacterium* and *Clostridium* spp., and less active against *Lactobacillus*, *Bifidobacterium* and *Bacteroides* (JECFA 2009). Avilamycin is highly active *in vitro* against clinical isolates of *C. difficile* (Aarestrup and Tvede 2011). Avilamycin is inactive against the gram-negative *Escherichia coli* (Delsol et al 2005; JECFA 2009).

2.2 Microbiological activity of residues in edible pork

Avilamycin is poorly absorbed and is extensively metabolised in the gastrointestinal (GI) tract of pigs and poultry. Studies in pigs and poultry indicate that the majority of avilamycin consumed in feed is excreted in faeces and a small proportion (<10% in pigs) is in the parent form (JECFA 2009). Several residue studies indicate that meat, skin, kidney and fat tissue of pigs and poultry, contain little or no avilamycin residues and low levels of residue may be detected in liver. The residues detected in edible tissues of poultry and pigs are not microbiologically active as determined by bioautographic methods. Furthermore, avilamycin residues are rapidly and irreversibly bound to faecal material (JECFA 2009). Taken together, it is highly unlikely for avilamycin residues in edible pork products to have a disruptive effect on the colonisation barrier of consumers or select for antimicrobial resistance.

2.3 Antimicrobial resistance

Avilamycin is structurally closely related to evernimicin, and has a similar binding site in the 50S ribosomal subunit (Adrian et al 2000a; Adrian et al 2000b; Aarestrup and Jensen 2000; McNicholas et al 2000; Mann et al., 2001; Treede et al 2003). Cross-resistance between avilamycin and evernimicin has been demonstrated for poultry, pig and human isolates of *Enterococcus* (Aarestrup 1998; Aarestrup and McNicholas 2002), although high level resistance to evernimicin has been demonstrated for only a small proportion of avilamycin resistant strains (Aarestrup and McNicholas 2002). Evernimicin was developed for, but not introduced into human medicine due to toxicity issues (JECFA 2009).

Resistance to both avilamycin and evernimicin is mediated by either point mutations in the L16 50S subunit ribosomal protein or methyltransferases acting on the 50S subunit ribosomal RNA (Aarestrup and Jensen 2000; Mann et al., 2001; Treede et al. 2003), but only one has been associated with horizontal gene transfer. A methyltransferase (EmtA) was identified in a strain of *E. faecium* and specifically modifies helix 89 of 23S rRNA (Mann et al., 2001) and confers high level cross-resistance to both avilamycin and evernimicin (Aarestrup and McNicholas 2002).

The rRNA methyltransferase encoded *emtA* gene is located on a plasmid-borne transposonable element (Mann et al., 2001) and has been detected in *Enterococcus* isolates from broiler chickens, pigs and humans (Aarestrup and McNicholas 2002; Delsol et al 2005). Horizontal gene transfer of the *emtA* gene has been documented in laboratory studies of poultry and human isolates, whereby resistance was transferred *in vitro* to previously susceptible *E. faecium* isolates (Aarestrup and McNicholas 2002). The study of Aarestrup and McNicholas (2002) found no co-transfer of resistance determinants to bacitracin, chloramphenicol, erythromycin, gentamicin, kanamycin, penicillin, streptomycin, quinupristindalfopristin, tetracycline, and vancomycin with plasmids containing *emtA*. No data could be identified that shows avilamycin resistance determinants are co-located on mobile genetic elements (MGE) associated with resistance to antibiotics important for human use.

Inclusion of avilamycin in feed selects for avilamycin resistance in the native enterococci population of pigs (Delsol et al 2005). In the Delsol et al. (2005) study, treatment and control pigs were inoculated with *Salmonella* Typhimurium DT104 and the treatment group fed *ad libitum* with feed supplemented with avilamycin at 100 mg/kg; the control group was fed the same feed mix without avilamycin. Avilamycin resistant *Enterococci* isolates were first detected in the treatment group 33 days after treatment commenced and all isolates were resistant after 82 days of treatment. No resistant *Enterococci* were detected in the control pigs. In all isolates with high level resistance to avilamycin (MIC≥32 mg/L), the *emtA* gene was detected. No avilamycin resistant *Enterococci* isolates were detected in the treatment pigs 2 weeks after ceasing the treatment, indicating transient resistance and an inability of the resistant isolates to outcompete susceptible isolates in the absence of selection pressure (Delsol et al 2005). No changes in the *Salmonella* and *Campylobacter* populations of the treatment and control groups were detected during or post-treatment.

All *E. coli* isolates in the Delsol et al. (2005) study were intrinsically resistant to avilamycin. However, avilamycin indirectly effected the enteric *E. coli* population whereby the *tetB* gene, which encodes for an efflux pump with high affinity for tetracycline, was dominant in tetracycline resistant *E. coli* isolates both during and post-treatment (Delsol et al 2005). This was not seen in the untreated control pigs, where the *tetA* resistance gene dominated the resistant population (Delsol et al 2005). Furthermore the proportion of highly resistant tetracycline resistant *E. coli* in the avilamycin treatment group was 76% of isolates after 62 days. At no time point during the 4 month trial did the proportion of tetracycline resistant *E. coli* isolates in the control group of pigs exceed 20%. This increase in resistance to tetracycline was determined to be due to a dominant *E. coli* clone in the treatment group containing the *tetB* gene (Delsol et al 2005). The mechanism of clonal selection for *tetB* in the enteric *E. coli* population was not determined. There were no changes in the *E. coli* population for susceptibility to avilamycin, nalidixic acid, chloramphenicol, erythromycin, trimethoprim, ampicillin, and cyclohexane before, during and post-treatment (Delsol et al 2005).

Tetracycline is used in human medicine in Australia and is designated as being of low importance due to the number of alternative agents in different classes available to treat most infections even if antibacterial resistance develops (ASTAG 2015).

The use of avilamycin in pig and poultry feed has not been associated with clonal selection of *Enterococci isolates* resistant to antibiotics used in human medicine. In a study of antibiotic resistant *Enterococci* in New Zealand broilers, where avilamycin was used by producers as a growth promoter, the proportion of avilamycin resistant isolates was similar in vancomycin resistant and susceptible *Enterococci* populations, indicating that avilamycin was not selecting for vancomycin resistance (Manson et al 2004). These data together with data that shows *emtA* is not co-transferred with other resistance determinants (Aarestrup and McNicholas 2002), indicates it is unlikely that pigs exposed to avilamycin will be more likely to harbour *Enterococci* containing resistance determinants for clinically important antibiotics.

2.4 Conclusion

The available data indicates that it is highly unlikely for avilamycin residues in edible pork products to have a disruptive effect on the colonisation barrier of consumers or select for antimicrobial resistance. Neither avilamycin nor evernimicin are used in human medicine and no cross-resistance or co-resistance to other antibiotics used in veterinary or human medicine have been identified. FSANZ concludes that an avilamycin MRL of 0.2 mg/kg on selected pork products and 0.3 mg/kg for pig liver does not present a risk to consumers for the development of resistance to antimicrobials commonly used in human medicine.

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