final report

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Study Title: The residues of lignocaine in sheep

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Executive summary

The purpose of this study was to evaluate the decline in lignocaine tissue residues following treatment of sheep at the highest likely dose level of 10 mg/kg with a commercially available injectable formulation of lignocaine (lignocaine hydrochloride 20 mg/mL). This study was conducted under Good Laboratory Practice (GLP) guidelines.

Twenty-five (25) crossbred sheep (15 males and 10 females) weighing 10.8-18.0kg and 3-6 weeks of age were purchased from a commercial sheep farm for enrolment into the study. Sheep were inspected to be in 'normal' health and had not received any treatment containing lignocaine in their lifetime. Sheep were individually identified with uniquely numbered ear tags, weighed and allocated to treatment groups.

On Day 0, male sheep were castrated (elastrator® ring applied at the base of the scrotum) and females tail-docked (elastrator® ring applied at the level of the 3rd-4th joint at the base of the tail). Sheep were concurrently treated sheep with Lignocaine 20® (10mg/kg) to individual bodyweight (applied above the elastrator® ring in each case immediately after ring placement). On Days 2, 3, 7, 14 and 21 post-treatment, sheep were humanely sacrificed and samples of liver, kidney, peri-renal fat, loin muscle and injection site surrounds were collected. Tissues (except injection site surround tissue) were weighed, diced and stored frozen in duplicate. For injection site tissues, skin was removed and remaining tissue was cryoprocessed and stored frozen in duplicate. On the completion of the animal phase of the study, replicate 1 tissue samples were dispatched to the analytical laboratory (Eurofins Agroscience Testing, Lane Cove NSW) for lignocaine residue analyses.

Residue calculations were conducted to determine indicative Withholding Periods (WHP) according to the ACVM Registration Standard and Guidelines for Determination of a Withholding Period for Veterinary Medicines (39 ACVM 03/03) and using Microsoft EXCEL 2010 Version 14.0 in relation to lignocaine. As there was not a current (Australian) Maximum Residue Limit (MRL) for lignocaine in sheep tissue, the previous MRL of 20 µg/kg (0.02 mg/kg) (withdrawn in 2012) and the limit of quantitation (LOQ) (0.02 µg/kg) were used to provide indicative "probable" and "worst case" estimates of possible WHPs, with the latter potentially being more relevant to any future ESI. The levels of 2,6-xylidine (DMA) were quantified such that a metabolism profile for the metabolite in each tissue could be defined.

Quantifiable levels of lignocaine and 2,6-xylidine were observed in all tissues. Residue calculations were performed for lignocaine in muscle, liver, kidney and fat and not for injection site, given the location (in the scrotal sack and tail stump, tissues subject to trimming during processing) and 'worst case' aspect as not being representative of standard edible tissue. Group mean lignocaine residues in muscle, liver and kidney declined to <LOQ by 14 days post-treatment and <LOQ by 21 days post-treatment for fat. Levels of 2,6-xylidine levels were < LOQ in all edible tissues by 14 days post-treatment.

Based on the previously withdrawn MRL for lignocaine in ovine tissues, the limiting tissue appeared to be fat, with an indicative WHP of 3 days when lignocaine was administered via subcutaneous injection to the scrotal neck and tail base of lambs at marking at a nominal dose level of 10 mg/kg. Similarly, if a future MRL or limiting concentration was based on the same figure as the method LOQ, fat would also be the limiting tissue, with an indicative WHP of 22 days.

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1 Background

1.1 Study Purpose

Castration and tail docking are animal husbandry procedures conducted on farm as part of routine sheep management. There is currently increased interest in Australian livestock industries (and abroad) to provide animals with pain relief whilst undergoing these routine procedures. Lignocaine (as hydrochloride) is a commonly used veterinary local anaesthetic and one which may be used with increased frequency in Australian sheep production industries to relieve pain associated with these routine procedures.

Lignocaine does not have an established Export Slaughter Interval (ESI). It is essential that the residue profile of lignocaine is understood to protect Australia's lamb export markets. The overarching aims of this development work was thus to provide data essential to understanding the lignocaine tissue residue decline profile in treated sheep and to assist in understanding a likely ESI for commercially available lignocaine products.

This study was performed to GLP guidelines and was intended to generate data suitable for review by Meat & Livestock Australia and/or regulatory authority personnel.

1.2 Compliance

The study complied with the following national and international standards:

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring:
  - No. 1 - OECD Principles on Good Laboratory Practice (ENV/MC/CHEM(98)17)
  - No. 6 - The Application of the GLP Principles to Field Studies (ENV/JM/MONO(99)22)
  - No. 8 - The Role and Responsibilities of the Study Director in GLP Studies (ENV/JM/MONO(99)24)
  - No. 13 - The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies (ENV/JM/MONO(2002)9)
- NATA Application Document - OECD Principles of Good Laboratory Practice Compliance Monitoring Program (May 17)
- APVMA Node 746 - Food safety studies for veterinary drugs used in food-producing animals (Jul 14)
- APVMA Node 723 – Analytical methodology (Jul 14).

2 Project objective

2.1 Objective

This study aimed to evaluate the decline in lignocaine tissue residues (lignocaine as the parent compound and 2,6-xylidine as a metabolite) following treatment of sheep at the highest likely dose level of 10 mg/kg with a commercially available injectable lignocaine formulation (lignocaine hydrochloride 20 mg/mL). Furthermore, the study aimed to provide indicative WHPs using a historical lignocaine MRL and the LOQ for lignocaine as endpoints.

Data from this study may be used in the establishment of a Withholding Period (WHP) and an Export Slaughter Interval (ESI) based on the lowest international MRLs for the markets for consideration for lamb products.

3 Methodology

3.1 Animal Selection

3.1.1 Animal Requirements

Animals were included in the study if they met the following requirements:

Species:	Ovine	**Number:**	25 (15 male; 10 female)
Breed:	Crossbred	**Source:**	Purchased from commercial sheep farm; all of one line.
Weight:	10.8-18.0 kg at treatment		
Sex:	15 males (intact); 10 females	**Health & special requirements:**	Normal health; not treated with Lignocaine in lifetime.
Age:	3-6 weeks		
Method of ID:	Uniquely numbered ear tag		

3.2 Study Design

3.2.1 Study Outline

Twenty-five (25) crossbred sheep (15 males and 10 females) weighing 10.8-18 kg and 3-4 weeks of age were purchased from a commercial sheep farm for the study. Sheep were inspected to be in 'normal' health and had not had any treatment containing lignocaine in their lifetime. Sheep were individually identified with a uniquely numbered ear tag, weighed and allocated to treatment groups. Study animals remained with their mothers as one group for the duration of the animal phase of the study.

3.2.2 Allocation

Study sheep were allocated to treatment groups based on sex and bodyweight using a randomised blocked design. Allocation was conducted such that each group contained the same proportion of males and females and a similar group mean bodyweight and range of bodyweights within sex across each group.

3.3 Treatment

Dose volumes were calculated according to the dosing regime outlined in Table 1 using bodyweights assessed prior to treatment on Day 0.

Table 1. Study design and treatment regime

Group	Treatment	Dosage Level	Treatment Day	Route of Administration	Number of Animals	Sacrifice Point
1	LIGNOCAINE 20® Local Anaesthetic Injection (20 mg/mL Lignocaine Hydrochloride)	10 mg/kg	Day 0	Subcutaneous Injection above elastrator® ring	5 (3M, 2F)	Day 2
2					5 (3M, 2F)	Day 3
3					5 (3M, 2F)	Day 7
4					5 (3M, 2F)	Day 14
5					5 (3M, 2F)	Day 21

With sheep carefully restrained, the test item was administered via subcutaneous injection at the designated site (males: proximo-cranial to ring applied to scrotal sac; females: proximal to ring applied at the level of the 3rd-4th tail joint at the base of tail) using separate 10 mL syringes and 18-gauge x 1" needles for each animal.

3.4 Animal management

3.4.1 Animal Welfare

Study animals were managed similarly and with due regard for their welfare. Animals were observed three times weekly for health problems according to Animal Ethics Committee (AEC) requirements. Animals were handled in compliance with University of New England (UNE) AEC no. 17-100 approved 17 October 17, and any applicable local regulations.

3.4.2 Housing

Routine management practices were followed. Study animals were grazed together and remained un-weaned from their mothers.

3.4.3 Feed and Water

Animals were grazed on open paddocks with *ad-lib* access to native and improved pastures and water (via trough and stock dam).

3.4.4 Health management

The study animals were observed three times weekly in their paddock commencing on Day 0. No health problems were observed and no concurrent medications were administered during the study.

3.5 Study Procedures

3.5.1 Bodyweights

Study animals were weighed on Day 0 and individual animal weights were recorded. Animals were weighed on stock scales which were checked pre, during and post-weighing with calibrated test weights, to ensure no variation existed when weighing animals.

3.5.2 Sample Collection & Storage

At designated sacrifice times, animals were humanely euthanised via exsanguination after the application of a captive bolt to the head. Samples of injection site surround tissue, loin muscle, kidney, liver and peri-renal fat were collected from each animal on Days 2, 3, 7, 14 & 21 post-treatment. Tissues (except injection site surround tissue) were weighed, diced and stored in duplicate 70mL vials. For injection site tissues, skin was removed and remaining tissue was weight, diced and then cryoprocessed (homogenised with dry ice) and stored in duplicate 70mL vials. All processed samples were stored frozen (~-18^{o}C) in temperature monitored freezers.

3.6 Assessment of Effects

3.6.1 Tissue Residues

Replicate 1 tissue samples were forwarded frozen to Eurofins Agroscience Testing Laboratories (Lane Cove, NSW) for lignocaine and 2,6-xylidine residue analysis after the completion of the animal phase. Lignocaine and 2,6-xylidine residues in ovine tissues were determined using published methodology (Edwards, 2017). Residues of lignocaine and 2,6-xylidine were extracted from ovine tissues by homogenising of the sample with acetonitrile. After sonicating and centrifuging, the extract was then transferred to an EMR dSPE tube and then to an EMR Polish tube for clean-up. The acetonitrile extract was then evaporated to dryness. The residuum was dissolved in methanol and filtered into an LC-MS/MS vial. This analytical method was validated by fortifying sub-samples of untreated control tissues with known amounts of the mixed standard of lignocaine. The fortified samples were then analysed using the defined method and the recovery of the test compounds was determined.

3.6.2 Statistical Analyses

Group mean residue (lignocaine as parent compound and 2,6-xylidine as a metabolite) were calculated by tissue and timepoint (post-treatment) such that metabolism profiles could be determined. Indicative WHPs were calculated according to the ACVM Registration Standard and Guidelines for Determination of a Withholding Period for Veterinary Medicines (New Zealand Food Safety Authority, 2003) and using Microsoft EXCEL 2010 Version 14.0. This methodology was used to provide both indicative WHPs and transparent methodology for subsequent review and use.

No Maximum Residue Limit (MRL) is currently set for lignocaine in sheep tissue by the APVMA. A MRL of 20 µg/kg (0.02 mg/kg) had previously been set; however, this was withdrawn in 2012. A search was unable to find any other relevant MRLs. For calculations purposes both the previous MRL (20 µg/kg) and the LOQ (0.2 µg/kg) were used to provided indicative “probable” and “worst case” estimates of possible WHPs.

In instances where residues were reported as less than the LOQ, a value of 50% of the LOQ (ie 0.1 µg/kg) was substituted and used in calculations. Similarly, in instances where results were reported as less than the relevant Limit of Detection (LOD) a value of 0.07 µg/kg was substituted and used in calculations.

4 Results

4.1 Lignocaine Residues

Quantifiable levels of lignocaine (the parent lignocaine and metabolite 2,6-xylidine) were observed in all tissues (Figs. 1-5; Table 2). Group mean residue levels peaked at 3 days post-treatment in all edible tissues with the highest residue level being lignocaine in ovine fat (3.45 µg/kg). Group mean lignocaine residues in muscle, liver and kidney declined to <LOQ by 14 days post treatment (Figs. 1-3) and <LOQ by 21 days post treatment for fat (Fig 4). Levels of 2,6-xylidine were < LOQ in all tissues by 14 days post-treatment.

Fig.1: Lignocaine metabolism profile in ovine muscle

Fig.2: Lignocaine metabolism profile in ovine liver

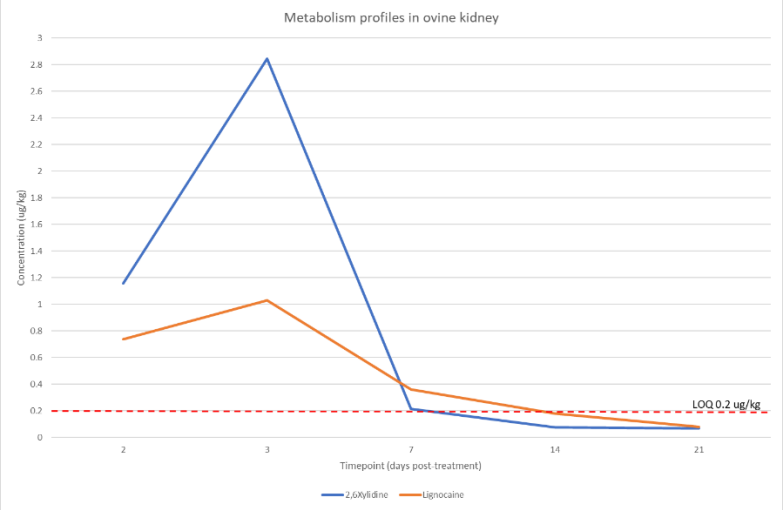

Fig.3: Lignocaine metabolism profile in ovine kidney

Fig.4: Lignocaine metabolism profile in over fat (peri-renal)

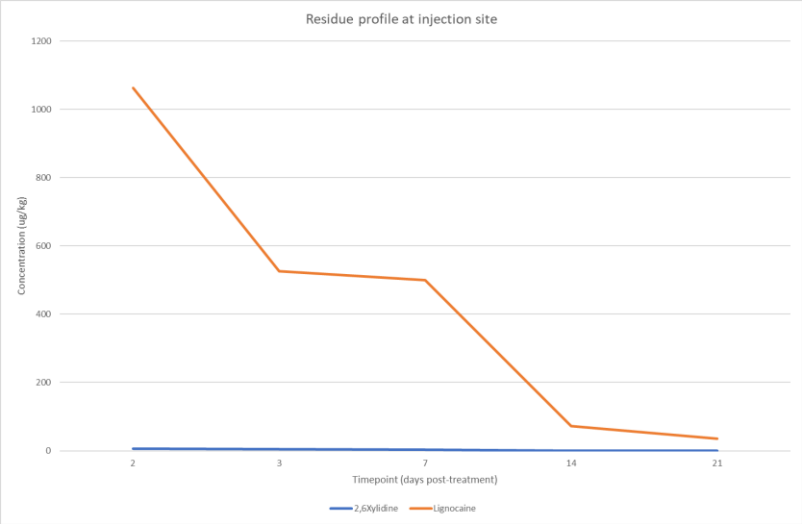

Fig.5: Lignocaine residue profile at the injection site of sheep

For the purposes of indicative WHP/ESI determination, lignocaine was used as the marker residue. Calculations were performed for lignocaine residues in muscle, liver, kidney and fat (Figs 6-9). Injection site data was excluded from calculations given the location (in the scrotal sack and tail stump, tissues subject to trimming during processing) and 'worst case' aspect as not being representative of standard edible tissue. Both the withdrawn lignocaine MRL and the LOQ were used as endpoints in calculations. The analytical method LOQ was substantially lower than the withdrawn MRL (0.2 µg/kg vs 20 µg/kg, 1% of the withdrawn MRL).

Based on the previously withdrawn MRL the limiting tissue appeared to be fat (Table 3), with an indicative WHP (based on withdrawn MRL) of 3 days when lignocaine was applied via subcutaneous injection to the scrotal neck and tail base of lambs at marking at a nominal dose level of 10 mg/kg. Similarly, if a future MRL or limiting concentration was based on the same figure as the method LOQ, fat would also be the limiting tissue (Table 3), with an indicative WHP of 22 days.

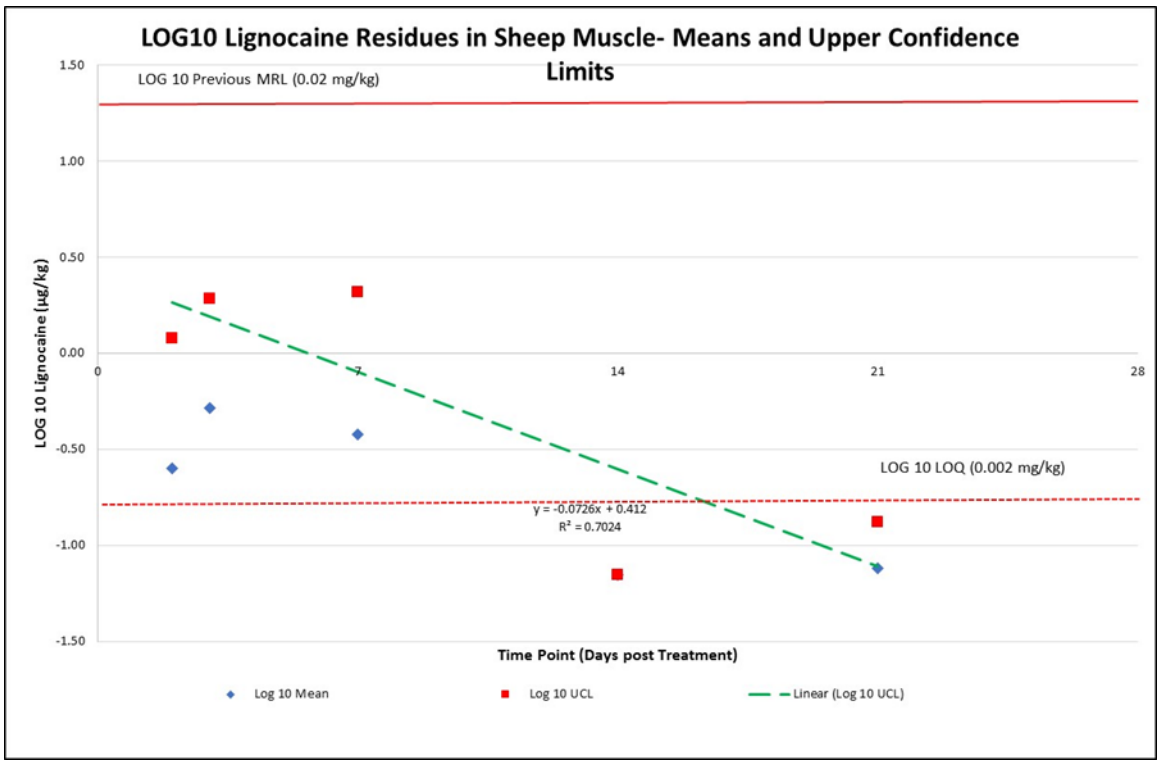

Fig.6: Log10 lignocaine residue in ovine muscle

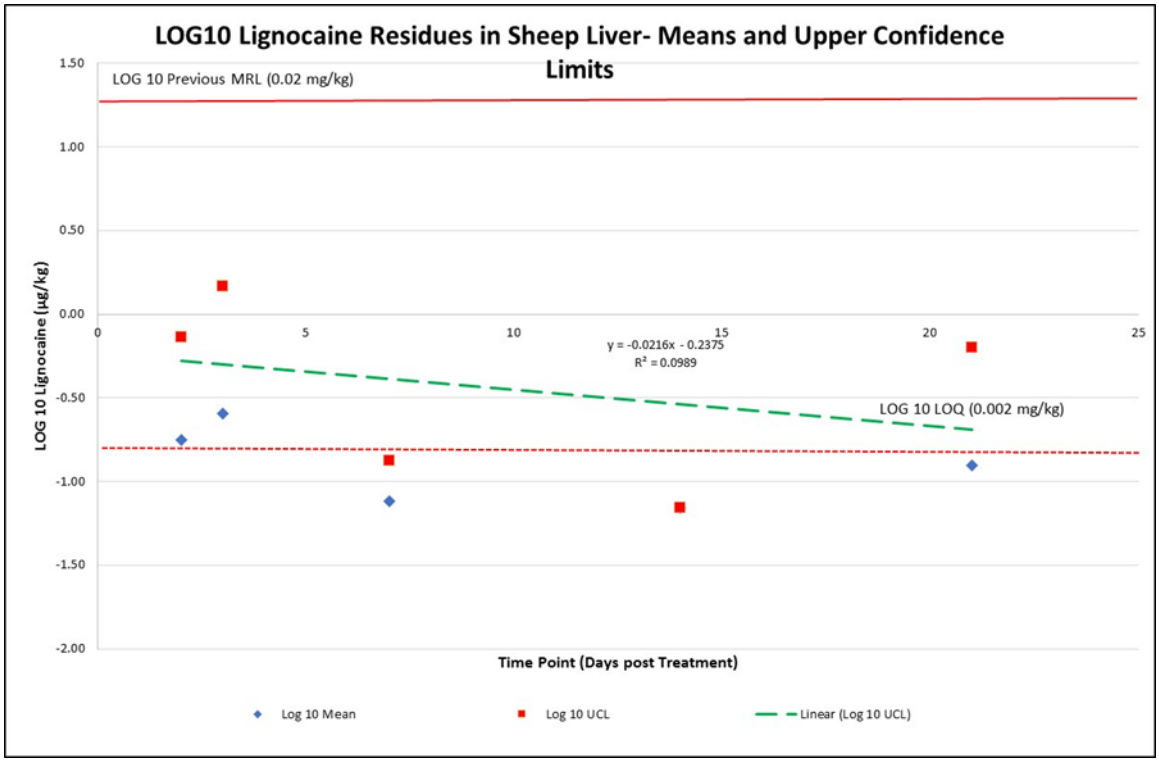

Fig.7: Log10 lignocaine residue in ovine liver

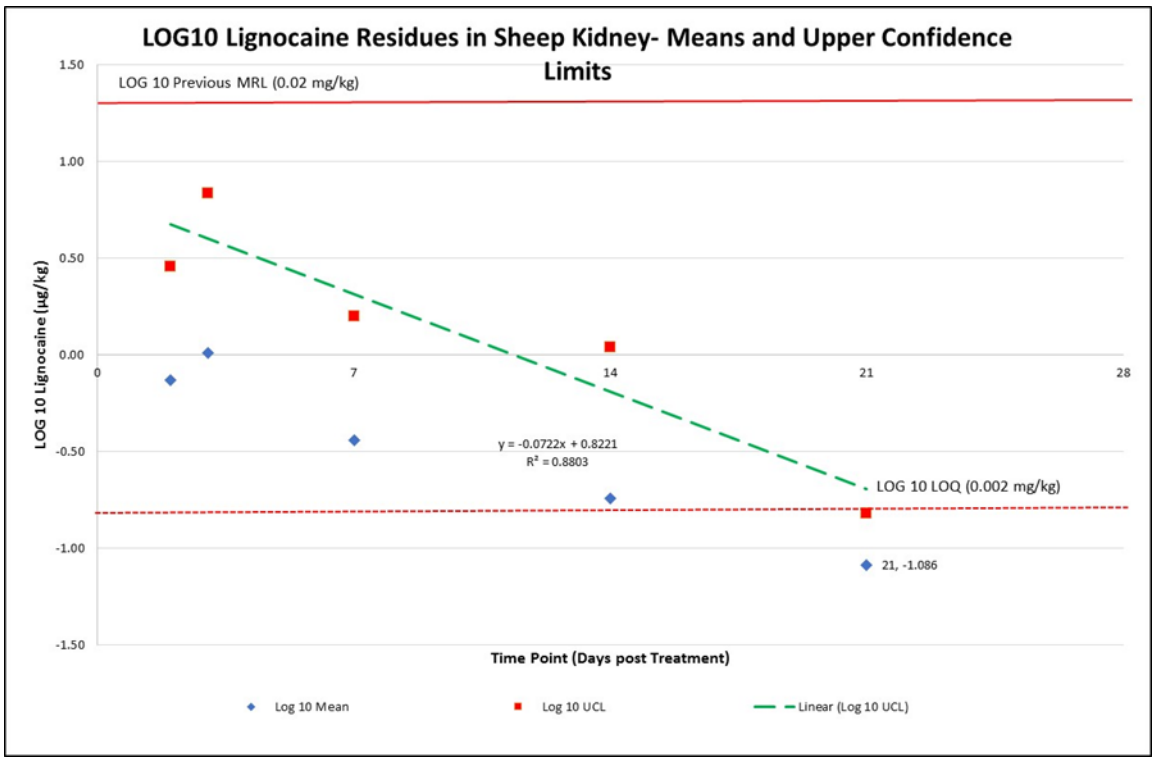

Fig.8: Log10 lignocaine residues in ovine kidney

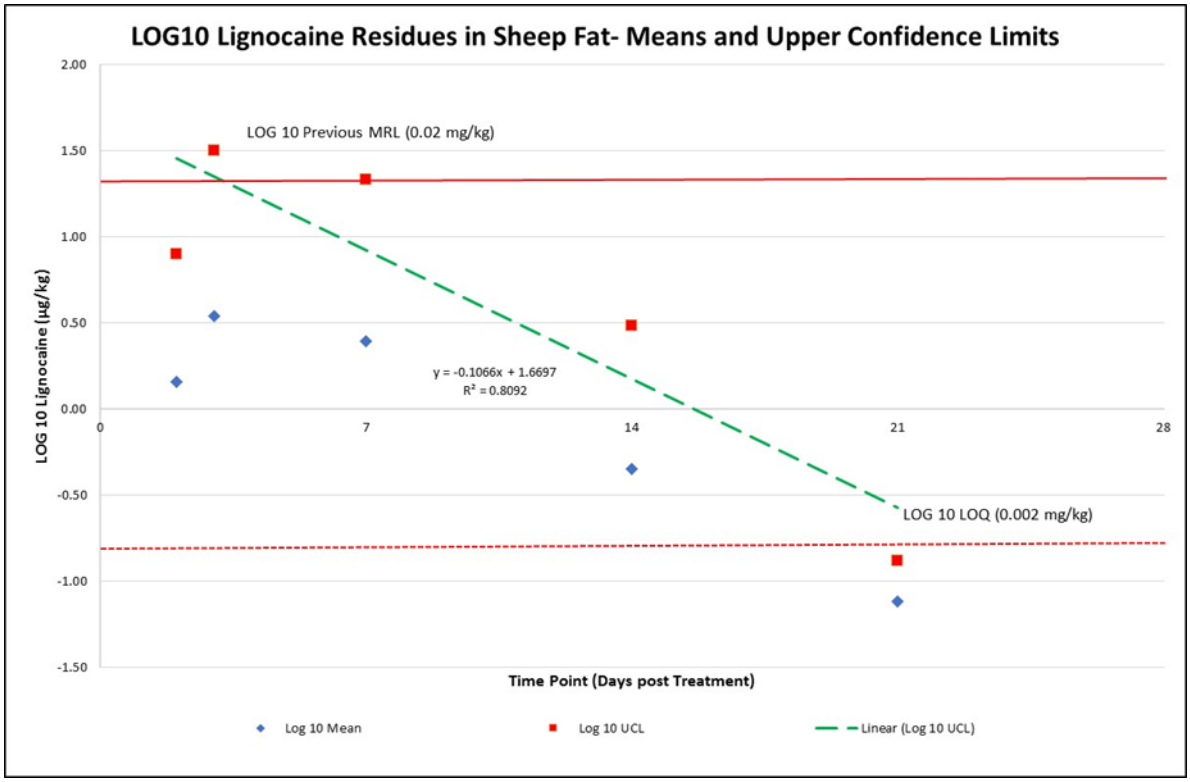

Fig.9: Log10 lignocaine residues in ovine fat

Table 2. Lignocaine and 2,6-xylidine residues by tissue and timepoint in edible ovine tissue

Time Point	Mean Lignocaine Residues (µg/kg)	Lignocaine Residue StDev (µg/kg)	Log 10 Mean	Log 10 UCL	2,6-xylidine (µg/kg)
Muscle					
2	0.252	0.224	-0.599	0.077	0.07
3	0.517	0.337	-0.287	0.286	0.176
7	0.376	0.406	-0.425	0.318	0.07
14	0.070	0.000	-1.155	-1.155	0.07
21	0.076	0.013	-1.119	-0.878	0.07
Liver					
2	0.176	0.132	-0.754	-0.136	0.928
3	0.256	0.286	-0.592	0.164	1.504
7	0.076	0.013	-1.119	-0.878	0.216
14	0.070	0.000	-1.155	-1.155	0.082
21	0.124	0.121	-0.907	-0.200	0.082

Time Point	Mean Lignocaine Residues (µg/kg)	Lignocaine Residue StDev (µg/kg)	Log 10 Mean	Log 10 UCL	2,6-xylidine (µg/kg)
Kidney					
2	0.738	0.510	-0.132	0.460	1.156
3	1.030	1.387	0.013	0.836	2.846
7	0.362	0.292	-0.441	0.201	0.216
14	0.182	0.217	-0.740	0.040	0.076
21	0.082	0.016	-1.086	-0.821	0.07
Fat					
2	1.450	1.539	0.161	0.899	0.532
3	3.454	6.740	0.538	1.502	1.156
7	2.474	4.519	0.393	1.332	0.306
14	0.450	0.615	-0.347	0.482	0.07
21	0.076	0.013	-1.119	-0.878	0.07

Table 3. WHP estimates based on varying end-points

	Withdrawn MRL	LOQ
ug/kg	20	0.2
Muscle WHP (Days)	0	15
Liver WHP (Days)	0	21
Kidney WHP (Days)	0	21
Fat WHP (Days)	3	22
Limiting WHP (Days)	3	22

5 Discussion

Injectable lignocaine formulations have been used in Veterinary medicine over a long period of time. For use in production animals, these products typically have had *nil* withholding periods and have not had established ESI's. Given the potential increased use of lignocaine in the Australian sheep industries to alleviate perceived pain associated with routine husbandry practices (lamb marking) and its potential future administration by non-Veterinary professional personnel, it is timely to assess likely residue implications of the use of lignocaine in lambs as part of routine husbandry. This is important from domestic food safety perspectives and for preservation of our export markets.

Interpretation of the results of this study need to account for the fact that in Australia there is not currently an established MRL for lignocaine in ovine tissue. However, the recently withdrawn MRL and the LOQ make for good starting points. Based on the previously withdrawn MRL an indicative withholding period for lignocaine is small (3 days); however, still quantifiable and above the current WHP described for the product (nil). An indicative WHP/ESI based on the LOQ of 0.2 µg/kg is potentially of more concern with 22 days. These indicative WHP/ESIs are based on targeted delivery of the test item and certainly would not apply should the product be administered at other locations within the animal.

With the likely increased use of injectable lignocaine formulations in Australian sheep industries it is apparently timely for an MRL for lignocaine to be re-established in Australia. Subsequently, any label claims for the use of lignocaine hydrochloride injectable formulations for the alleviation of pain associated with lamb marking would need to take the findings of this study into account and thus a suitable domestic WHP and potential ESI adopted.

6 Conclusions/recommendations

6.1 Concluding Remarks

Based on the previously withdrawn Australian MRL for lignocaine in ovine tissue, the limiting tissue was found to be fat, with an indicative WHP of 3 days when lignocaine was applied via subcutaneous injection to the scrotal neck and tail base of lambs at marking at a nominal dose level of 10 mg/kg. Similarly, if a future MRL or limiting concentration was based on the same figure as the method LOQ, fat would also be the limiting tissue, with an indicative WHP/ESI of 22 days.

7 Key messages

- The administration of lignocaine hydrochloride at 10 mg/kg at lamb marking results in the presence of quantifiable lignocaine residues in edible ovine tissue.
- Based on previously withdrawn Australian lignocaine MRL an indicative domestic WHP for lignocaine hydrochloride would be 3 days.
- A more conservative calculation based on the LOQ is 22 days which may be more indicative of a likely ESI for lignocaine hydrochloride when used in sheep at lamb marking.

8 Bibliography

Edwards, Scott (2017). Pharmacokinetics, residue depletion studies for lignocaine and bupivacaine in sheep and cattle", Meat and Livestock Australia Report B.AWW.0247.

New Zealand Food Safety Authority (2003). ACVM Registration Standard and Guideline for Determination of a Residue Withholding Period for Veterinary Medicines 39 ACVM 03/03. Wellington, New Zealand.

9 Appendix

9.1 Abbreviations

AEC	Animal Ethics Committee
APVMA	Australian Pesticides and Veterinary Medicines Authority
ESI	Export Slaughter Interval
g	Grams
GLP	Good Laboratory Practice
ID	Identification
kg	Kilograms
mg	Milligrams
mL	Millilitres
NATA	National Association of Testing Authorities, Australia
µg	Micrograms
UNE	University of New England
WHP	Withholding Period

9.2 Tabulated Data

Table 5. Residue data for lignocaine as reported and as used in calculations- ½ LOQ used for data reported as "<LOQ(value)" and LOD used for values reported as <LOD.

ID	Group	Substrate	Days After Treatment	Lignocaine as Reported (µg/kg)	Lignocaine-Substituted (µg/kg)	LOG10 Lignocaine Substituted
805	1	Muscle	2	0.54	0.54	-0.268
806	1	Muscle	2	0.45	0.45	-0.347
811	1	Muscle	2	<LOQ (0.16)	0.10	-1.000
816	1	Muscle	2	<LOQ (0.11)	0.10	-1.000
821	1	Muscle	2	<LOD	0.07	-1.155
802	2	Muscle	3	0.47	0.47	-0.328
804	2	Muscle	3	0.3	0.30	-0.523
807	2	Muscle	3	<LOQ (0.08)	0.10	-1.000
817	2	Muscle	3	0.86	0.86	-0.066
824	2	Muscle	3	0.85	0.85	-0.069
808	3	Muscle	7	0.83	0.83	-0.081
812	3	Muscle	7	0.81	0.81	-0.092
819	3	Muscle	7	<LOQ (0.09)	0.10	-1.000
820	3	Muscle	7	<LOD	0.07	-1.155
825	3	Muscle	7	<LOD	0.07	-1.155
803	4	Muscle	14	<LOD	0.07	-1.155
813	4	Muscle	14	<LOD	0.07	-1.155
815	4	Muscle	14	<LOD	0.07	-1.155
826	4	Muscle	14	<LOD	0.07	-1.155
829	4	Muscle	14	<LOD	0.07	-1.155
801	5	Muscle	21	<LOQ (0.14)	0.10	-1.000
809	5	Muscle	21	<LOD	0.07	-1.155
810	5	Muscle	21	<LOD	0.07	-1.155
814	5	Muscle	21	<LOD	0.07	-1.155
818	5	Muscle	21	<LOD	0.07	-1.155
805	1	Liver	2	0.33	0.33	-0.481
806	1	Liver	2	0.31	0.31	-0.509
811	1	Liver	2	<LOQ (0.15)	0.10	-1.000
816	1	Liver	2	<LOD	0.07	-1.155
821	1	Liver	2	<LOD	0.07	-1.155
802	2	Liver	3	<LOQ (0.10)	0.10	-1.000
804	2	Liver	3	<LOQ (0.19)	0.10	-1.000
807	2	Liver	3	<LOD	0.07	-1.155
817	2	Liver	3	0.26	0.26	-0.585
824	2	Liver	3	0.75	0.75	-0.125
808	3	Liver	7	<LOQ (0.18)	0.10	-1.000

ID	Group	Substrate	Days After Treatment	Lignocaine as Reported (µg/kg)	Lignocaine-Substituted (µg/kg)	LOG10 Lignocaine Substituted
812	3	Liver	7	<LOD	0.07	-1.155
819	3	Liver	7	<LOD	0.07	-1.155
820	3	Liver	7	<LOD	0.07	-1.155
825	3	Liver	7	<LOD	0.07	-1.155
803	4	Liver	14	<LOD	0.07	-1.155
813	4	Liver	14	<LOD	0.07	-1.155
815	4	Liver	14	<LOD	0.07	-1.155
826	4	Liver	14	<LOD	0.07	-1.155
829	4	Liver	14	<LOD	0.07	-1.155
801	5	Liver	21	<LOD	0.07	-1.155
809	5	Liver	21	<LOD	0.07	-1.155
810	5	Liver	21	<LOD	0.07	-1.155
814	5	Liver	21	<LOD	0.07	-1.155
818	5	Liver	21	0.34	0.34	-0.469
805	1	Kidney	2	1.28	1.28	0.107
806	1	Kidney	2	1.27	1.27	0.104
811	1	Kidney	2	0.6	0.60	-0.222
816	1	Kidney	2	0.21	0.21	-0.678
821	1	Kidney	2	0.33	0.33	-0.481
802	2	Kidney	3	0.51	0.51	-0.292
804	2	Kidney	3	0.24	0.24	-0.620
807	2	Kidney	3	<LOQ (0.10)	0.10	-1.000
817	2	Kidney	3	0.84	0.84	-0.076
824	2	Kidney	3	3.46	3.46	0.539
808	3	Kidney	7	0.75	0.75	-0.125
812	3	Kidney	7	0.47	0.47	-0.328
819	3	Kidney	7	0.45	0.45	-0.347
820	3	Kidney	7	<LOD	0.07	-1.155
825	3	Kidney	7	<LOD	0.07	-1.155
803	4	Kidney	14	<LOD	0.07	-1.155
813	4	Kidney	14	<LOQ (0.14)	0.10	-1.000
815	4	Kidney	14	<LOD	0.07	-1.155
826	4	Kidney	14	<LOQ (0.072)	0.10	-1.000
829	4	Kidney	14	0.57	0.57	-0.244
801	5	Kidney	21	<LOQ (0.085)	0.10	-1.000
809	5	Kidney	21	<LOD	0.07	-1.155
810	5	Kidney	21	<LOD	0.07	-1.155
814	5	Kidney	21	<LOD	0.07	-1.155
818	5	Kidney	21	<LOQ (0.079)	0.10	-1.000
805	1	Fat	2	1.95	1.95	0.290
806	1	Fat	2	3.94	3.94	0.595
811	1	Fat	2	0.61	0.61	-0.215

ID	Group	Substrate	Days After Treatment	Lignocaine as Reported (µg/kg)	Lignocaine-Substituted (µg/kg)	LOG10 Lignocaine Substituted
816	1	Fat	2	0.4	0.40	-0.398
821	1	Fat	2	0.35	0.35	-0.456
802	2	Fat	3	0.34	0.34	-0.469
804	2	Fat	3	0.42	0.42	-0.377
807	2	Fat	3	<LOQ (0.089)	0.10	-1.000
817	2	Fat	3	0.91	0.91	-0.041
824	2	Fat	3	15.5	15.50	1.190
808	3	Fat	7	10.5	10.50	1.021
812	3	Fat	7	0.32	0.32	-0.495
819	3	Fat	7	1.38	1.38	0.140
820	3	Fat	7	<LOD	0.07	-1.155
825	3	Fat	7	<LOQ (0.075)	0.10	-1.000
803	4	Fat	14	<LOD	0.07	-1.155
813	4	Fat	14	<LOQ (0.11)	0.10	-1.000
815	4	Fat	14	<LOQ (0.11)	0.10	-1.000
826	4	Fat	14	1.51	1.51	0.179
829	4	Fat	14	0.47	0.47	-0.328
801	5	Fat	21	<LOQ (0.11)	0.10	-1.000
809	5	Fat	21	<LOD	0.07	-1.155
810	5	Fat	21	<LOD	0.07	-1.155
814	5	Fat	21	<LOD	0.07	-1.155
818	5	Fat	21	<LOD	0.07	-1.155
805	1	Injection Site	2	1881	1881.0	3.274
806	1	Injection Site	2	1999	1999.0	3.301
811	1	Injection Site	2	877	877.0	2.943
816	1	Injection Site	2	505	505.0	2.703
821	1	Injection Site	2	48.2	48.2	1.683
802	2	Injection Site	3	182	182.0	2.260
804	2	Injection Site	3	288	288.0	2.459
807	2	Injection Site	3	12.5	12.5	1.097
817	2	Injection Site	3	265	265.0	2.423
824	2	Injection Site	3	1884	1884.0	3.275
808	3	Injection Site	7	557	557.0	2.746

ID	Group	Substrate	Days After Treatment	Lignocaine as Reported (µg/kg)	Lignocaine-Substituted (µg/kg)	LOG10 Lignocaine Substituted
812	3	Injection Site	7	1260	1260.0	3.100
819	3	Injection Site	7	379	379.0	2.579
820	3	Injection Site	7	53.8	53.8	1.731
825	3	Injection Site	7	247	247.0	2.393
803	4	Injection Site	14	0.36	0.4	-0.444
813	4	Injection Site	14	106	106.0	2.025
815	4	Injection Site	14	9.86	9.9	0.994
826	4	Injection Site	14	1.83	1.8	0.262
829	4	Injection Site	14	245	245.0	2.389
801	5	Injection Site	21	60	60.0	1.778
809	5	Injection Site	21	9.13	9.1	0.960
810	5	Injection Site	21	3.5	3.5	0.544
814	5	Injection Site	21	64.5	64.5	1.810
818	5	Injection Site	21	42.3	42.3	1.626

LOD = 0.07 µg/kg; LOQ = 0.2 µg/kg

Table 6. Residue data for 2,6-Xylidine as reported

Animal ID	Group	Substrate	Days After Treatment	2,6-DMA (µg/kg)
805	1	Muscle	2	<LOD
806	1	Muscle	2	<LOD
811	1	Muscle	2	<LOD
816	1	Muscle	2	<LOD
821	1	Muscle	2	<LOD
802	2	Muscle	3	<LOD
804	2	Muscle	3	<LOD
807	2	Muscle	3	<LOD
817	2	Muscle	3	<LOD
824	2	Muscle	3	0.6
808	3	Muscle	7	<LOD
812	3	Muscle	7	<LOD
819	3	Muscle	7	<LOD
820	3	Muscle	7	<LOD
825	3	Muscle	7	<LOD
803	4	Muscle	14	<LOD
813	4	Muscle	14	<LOD
815	4	Muscle	14	<LOD
826	4	Muscle	14	<LOD
829	4	Muscle	14	<LOD
801	5	Muscle	21	<LOD
809	5	Muscle	21	<LOD
810	5	Muscle	21	<LOD
814	5	Muscle	21	<LOD
818	5	Muscle	21	<LOD
805	1	Liver	2	1.12
806	1	Liver	2	1.32
811	1	Liver	2	0.86
816	1	Liver	2	0.56
821	1	Liver	2	0.78
802	2	Liver	3	0.69
804	2	Liver	3	0.23
807	2	Liver	3	<LOQ (0.097)
817	2	Liver	3	1.16
824	2	Liver	3	5.34
808	3	Liver	7	0.37
812	3	Liver	7	0.20
819	3	Liver	7	0.20
820	3	Liver	7	0.21
825	3	Liver	7	<LOQ (0.12)
803	4	Liver	14	<LOD

Animal ID	Group	Substrate	Days After Treatment	2,6-DMA (µg/kg)
813	4	Liver	14	<LOQ (0.098)
815	4	Liver	14	<LOD
826	4	Liver	14	<LOD
829	4	Liver	14	<LOQ (0.14)
801	5	Liver	21	<LOQ (0.10)
809	5	Liver	21	<LOD
810	5	Liver	21	<LOD
814	5	Liver	21	<LOQ (0.099)
818	5	Liver	21	<LOD
805	1	Kidney	2	1.94
806	1	Kidney	2	1.19
811	1	Kidney	2	1.34
816	1	Kidney	2	0.62
821	1	Kidney	2	0.69
802	2	Kidney	3	0.65
804	2	Kidney	3	0.53
807	2	Kidney	3	0.64
817	2	Kidney	3	0.91
824	2	Kidney	3	11.5
808	3	Kidney	7	0.46
812	3	Kidney	7	0.35
819	3	Kidney	7	<LOQ (0.12)
820	3	Kidney	7	<LOQ (0.096)
825	3	Kidney	7	<LOD
803	4	Kidney	14	<LOD
813	4	Kidney	14	<LOD
815	4	Kidney	14	<LOD
826	4	Kidney	14	<LOD
829	4	Kidney	14	<LOQ (0.078)
801	5	Kidney	21	<LOD
809	5	Kidney	21	<LOD
810	5	Kidney	21	<LOD
814	5	Kidney	21	<LOD
818	5	Kidney	21	<LOD
805	1	Fat	2	0.62
806	1	Fat	2	0.62
811	1	Fat	2	0.7
816	1	Fat	2	0.34
821	1	Fat	2	0.38
802	2	Fat	3	0.51
804	2	Fat	3	0.28
807	2	Fat	3	<LOQ (0.094)
817	2	Fat	3	0.69

Animal ID	Group	Substrate	Days After Treatment	2,6-DMA (µg/kg)
824	2	Fat	3	4.2
808	3	Fat	7	0.86
812	3	Fat	7	0.31
819	3	Fat	7	0.22
820	3	Fat	7	<LOD
825	3	Fat	7	<LOD
803	4	Fat	14	<LOD
813	4	Fat	14	<LOD
815	4	Fat	14	<LOD
826	4	Fat	14	<LOD
829	4	Fat	14	<LOD
801	5	Fat	21	<LOD
809	5	Fat	21	<LOD
810	5	Fat	21	<LOD
814	5	Fat	21	<LOD
818	5	Fat	21	<LOD
805	1	Injection Site	2	1.85
806	1	Injection Site	2	5.61
811	1	Injection Site	2	15
816	1	Injection Site	2	4.87
821	1	Injection Site	2	0.68
802	2	Injection Site	3	0.83
804	2	Injection Site	3	5.95
807	2	Injection Site	3	0.31
817	2	Injection Site	3	2.3
824	2	Injection Site	3	15.4
808	3	Injection Site	7	10.1
812	3	Injection Site	7	3.11
819	3	Injection Site	7	1.89
820	3	Injection Site	7	<LOQ (0.12)
825	3	Injection Site	7	1.31
803	4	Injection Site	14	<LOD
813	4	Injection Site	14	<LOQ (0.077)
815	4	Injection Site	14	<LOD
826	4	Injection Site	14	<LOD
829	4	Injection Site	14	0.45
801	5	Injection Site	21	0.5
809	5	Injection Site	21	<LOQ (0.088)
810	5	Injection Site	21	<LOD
814	5	Injection Site	21	<LOQ (0.19)
818	5	Injection Site	21	0.25

LOD = 0.07 µg/kg; LOQ = 0.2 µg/kg