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Metabolism study to determine the quantity and identify the nature of parent compounds and metabolites in ovine tissue and fluids following the topical administration of TRI-SOLFEN (lignocaine, bupivacaine plus cetrimide).

INVETUS

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Executive Summary

TRI-SOLFEN is currently in use in Australian sheep industries for the provision of pain relief to lambs undergoing mulesing and castration. Additional requirements of the livestock industries will likely mean that TRI-SOLFEN will be used more extensively in the future for a range of procedures and conditions (eg. shearing cuts, foot abscess) and it is thus diligent to understand the pharmacokinetics and edible tissue residue profiles of the key actives of TRI-SOLFEN and their metabolites. Despite its increasing use, there is minimal published information available on the pharmacokinetics of TRI-SOLFEN in treated lambs.

In this study, routine surgery was performed in Merino lambs (mulesing, castration, tail-docking), topical treatment with TRI-SOLFEN was applied and plasma, edible tissues, urine and faeces were harvested from treated sheep at various time intervals through until 28 days post-treatment. These harvested samples were used to measure the levels of the active's lignocaine, bupivacaine and cetrimide (and their metabolites) of TRI-SOLFEN and describe their metabolism profiles through until 28 days post-treatment. 46 weaned Merino lambs (4 females, 42 male) were allocated to 1 of 12 groups. Blood samples were collected on Day -1. On Day 0, animals were mulesed and/or tail docked and/or castrated and TRI-SOLFEN was applied to the surgical site according to the treatment regime, with dose amount based on animal bodyweights from Day -1. Mulesing was completed by a certified contract surgical muleser. Other study procedures were performed by and treatment was administered by Invetus Veterinarians. From time of treatment until each group was sacrificed (up until 7 days post-treatment), blood samples were collected from all animals at repeat and frequent intervals and processed for plasma collection. At sacrifice, tissues and fluid collected up until 28 days post-treatment included; urine, faeces, kidney, loin and heart muscle, liver, perirenal fat and subcutaneous tissue at the mulesing site. Tissue, fluid and plasma samples were sent frozen to Eurofins Agroscience Testing (Lane Cove, NSW) for analysis of lignocaine, bupivacaine and cetrimide including their metabolites. Following receipt of the analytical results containing the tissue residue data, group means for each metabolite by tissue, timepoints at which metabolites peaked and fell below the LOQ were tabulated and metabolism profiles of the actives in ovine plasma, urine, faeces and edible tissues were determined.

Typically, lignocaine and its metabolites, bupivacaine and its metabolites and cetrimide reached peak levels in ovine plasma by 30-minutes post-treatment and remained quantifiable beyond the final sampling timepoint at 7 days post-treatment. Typically, lignocaine and its metabolites, bupivacaine and its metabolites, and cetrimide reached peak levels in edible ovine tissue by 10-hours post-treatment and remained quantifiable beyond the final sampling timepoint at 28 days post-treatment.

The study presented in this report has provided essential data pertaining to the pharmacokinetics of the key actives (and their metabolites) of TRI-SOLFEN in topically treated lambs having undergone routine surgery. The results of such may be used to guide further studies and allow better characterisation of edible tissue residues and defining markers for

analytical methods and provide supportive data for the definition of the WHP and ESI for TRI-SOLFEN in lambs when applied topically to surgical wounds.

1 Introduction/Hypothesis

Castration and tail docking are animal husbandry procedures conducted on farm as part of routine management. In Merino sheep in particular, this activity is also often associated with mulesing, a procedure involving the removal of wool-bearing skin from a portion of the tail and breech area of sheep, performed to reduce the incidence of flystrike in that area. In efficacy and animal welfare studies, TRI-SOLFEN has been shown to enhance wound healing and assist in the immediate alleviation of pain at the treatment site (Lomax *et al*., 2008).

This development work will provide data to support the registration of TRI-SOLFEN for use as an aid to pain relief in sheep undergoing routine husbandry procedures and specifically provide an understanding of the metabolism profiles of each of the active ingredients and their metabolites within the sheep which may be used in understanding any likely edible tissue residue concerns. Results from this study may assist in tissue residue and metabolism study design that may be required for the extension of the use pattern for TRI-SOLFEN (eg. shearing cuts, foot abscess).

The study was performed to GLP guidelines and VICH GL48, VICH GL 49 and VICH GL46. Invetus, a NATA recognised GLP facility for the conduct of animal residue studies, is a privately owned and operated independent contract research organisation based in Armidale in northern New South Wales, Australia. Invetus has extensive experience in the design and conduct of animal research studies.

2 Literature Review

The active ingredients of TRI-SOLFEN are the local anaesthetics lignocaine (LIG) and bupivacaine (BUP), together with adrenaline as a vasoconstrictor and the antiseptic cetrimide. These are formulated together as TRI-SOLFEN and the product is used as to provide post-procedural analgesia to lambs having undergone mulesing, tail-docking and/or castration. The recommended age for mulesing, castrating and tail-docking lambs is 2–12 weeks, with the maximum age allowed under the present Commonwealth Government Model Code of Practice for Welfare of Animals being 12 months.

LIG and BUP do not persist in mammals because they are rapidly metabolised, particularly in the liver. The plasma half-lives of LIG and BUP in all species are very short (range 1–190 minutes), depending on the species, age, dose and route of administration. The metabolic pathways are common to humans and other mammalian species, including sheep, cattle and pigs. Excretion of parent compounds and their metabolites is rapid and predominantly in the urine. Metabolism results in degradation of the compounds, which is responsible for the loss of local anaesthetic action (McLean, 2014).

Metabolism of the amide local anaesthetics is extensive in all species and is the primary factor limiting the length and intensity of anaesthesia and governing elimination from the body. General pathways in metabolism include aromatic ring and side-chain hydroxylation, N-dealkylation, and hydrolysis of the amide bond. In primates (including man) and dogs, N-dealkylation appears to be the predominant pathway to metabolites detected in urine samples, with subsequent hydroxylation primarily in the 4-position of the xylidine moiety. In the rat and guinea pig, hydroxylation products, primarily in the 3-position of the xylidine moiety, are the predominant species excreted, with minor amounts of dealkylated products detected. Hydroxylation at the 4-position of 2,6-xylidine may predominate in all species, probably due to the lack of steric interference by the alkyl chain or pipecolyl ring. Hydroxylation may occur primarily prior to amide hydrolysis, with minor amounts of metabolites being hydroxylated after hydrolysis. The local anaesthetics along with their metabolites are predominantly excreted in the urine, with a small fraction excreted in the faeces. Almost all of the hydroxylated metabolites are recovered in the urine as glucuronide or sulfate conjugates.

3 Project Objectives

This study aimed to provide data for each of the three active components of TRI-SOLFEN Topical Anaesthetic & Antiseptic Solution for Pain Relief in Lambs and Calves (lignocaine, bupivacaine and cetrimide) with the purposes of:

- Identify and quantify the individual component parent compound and metabolites that potentially comprise the residues of concern in edible tissues;
- Determine the residue(s) for each compound that can serve as a marker for analytical methods intended for compliance purposes; and
- Identify target tissue or tissues, as applicable to residue monitoring programmes as well as provide some indication of the depletion of the identified residues of concern (target residues) in edible tissues.

4 Success in Achieving Objectives

All animals enrolled in the study completed the study, and all samples were collected, and analyses completed. Overall, this study successfully provided information on the metabolism pharmacokinetics of TRI-SOLFEN. The analytical residue data and metabolism profiles identified and quantified the individual compounds and metabolites that comprise the residues of concern in edible tissues and determined the residues for each compound that will provide markers for future analytical methods for compliance purposes. The study allowed for identification of target tissues and depletion of residues of concern in edible tissues.

5 Methodology

Fifty-two animals (46 entire males and 6 females) considered suitable for enrolment were individually identified and weighed on Day -1, ranked in order of sex, then heaviest to lightest on bodyweight (within sex). Four of the 6 females were allocated to Group 6 (selected by the closest weight distribution), the remaining two females were discarded. Of the 46 males, the 3 heaviest animals and the lightest animal were discarded. With the remaining 42 ranked heaviest to lightest on bodyweight, the median bodyweight was calculated and 2 animals closest to that median weight were allocated to Group 12. The remaining 40 animals were blocked into 4 groups of 10 and randomly allocated to treatment groups 1, 2, 3, 4, 5, 7, 8, 9, 10 and 11 from within each block via the "draw from the hat" methodology such that each treatment group consisted of four animals. Statistical analysis was conducted to ensure no significant difference existed between treatment group based on bodyweight at $P < 0.05$. Allocation summary data is shown in Table 1.

Table 1: Allocation Summary Data

Group	Number per Group	Sex	Mean BWt (kg)	StDev BWt	Minimum BWt (kg)	Maximum BWt (kg)
1	4	M	20.8	2.3	17.5	23.5
2	4	M	21.3	2.5	18.5	25.0
3	4	M	21.0	2.7	17.0	24.0
4	4	M	21.0	2.2	18.0	23.5
5	4	M	21.5	2.7	18.0	25.0
6	4	F	22.6	0.4	22.0	23.0
7	4	M	20.0	2.4	17.0	23.5
8	4	M	20.8	2.5	18.5	24.5
9	4	M	21.0	2.9	17.5	25.5
10	4	M	21.4	2.2	18.5	23.5
11	4	M	20.5	2.6	17.5	24.5
12	2	M	23.5	3.0	20.5	26.5

BWt: Bodyweight; StDev: Standard Deviation; M: Male; F: Female.

Based upon a treatment regime, groups were mulesed, tail-docked and/or castrated, and treatment applied (based on Day -1 bodyweights), with dosage and administration methodology as follows:

1. **Mulesing & Tail Docking** — **Total Dose**

   Lambs (5-10 kg) — 6 mL

   Lambs (11-15 kg) — 8 mL

   Lambs (16-20 kg) — 10 mL

   Lambs (over 20kg) — 12 mL

Dosage and Administration

TRI-SOLFEN spray was applied to the wound immediately (within 1 minute) following mulesing and tail docking, ensuring that the entire wound area was evenly covered with the spray, particularly the wound edges. Did not spray to run-off. Note, that the label has a tail-docking (if not concurrently mulesed) dosage which was used in Group 9 animals only as all other treatment group lambs were mulesed.

2. Castration	**Total Dose**
Lambs up to 10 kg	3 mL
Lambs over 10 kg	4.5 mL

1. Incised skin of scrotum and gently extruded the testis to expose the spermatic cord (without cutting or tearing it).
2. Inserted the nozzle through skin incision (prior to removal of testicle) and carefully advanced the applicator along the length of the exposed spermatic cord as far as possible within the scrotum.
3. Ejected one third (1/3) of total TRI-SOLFEN dose as the applicator was withdrawn from the scrotum to coat the spermatic cord.
4. Inserted the nozzle through the other scrotal incision and applied one third (1/3) of total TRI-SOLFEN dose to the spermatic cord (as described above).
5. After application of TRI-SOLFEN to the spermatic cords, the testicles were extracted from the scrotum and removed as per normal procedure.
6. Lastly, the final one third (1/3) of total TRI-SOLFEN dose was applied to the edges of the scrotal skin wound(s).

Furthermore, based upon group number, all animals were bled at frequent time-points for plasma sample collection and sacrificed for collection of tissues (liver, kidney, perirenal fat, loin muscle, heart muscle, subcutaneous tissue at the site of mulesing), urine and faecal samples for residue analysis (timepoints for sample collection is shown in Table 2, study schedule of events).

Replicate 1, 2 and 3 samples were stored frozen (~20°C) at Invetus facilities, prior to shipment of replicate 1 samples via same day dispatch to the analytical laboratory. Details of sample storage, dispatch and disposal of samples were recorded.

All study animals were managed with due regard for their welfare and observed daily for the duration of the study, housed in four group pens with 1 animal from each group housed in each pen to remove any pen effect. Animals had ad lib access to water and were maintained

on a lucerne chaff/sheep pellet mix. Animals were humanely euthanised at set intervals by captive bolt and exsanguination, and group 12 was re-homed at the conclusion of the study.

All study activities were performed as per Invetus standard operating procedures and completed under the University of New England Animal Ethics number AEC17-053.

Table 2: Schedule of Study Events

Study Day	Date	Event
Induction	22Jun17	Weighed all sheep with verified scales, ear-tagged, recorded sex and drenched with 6 mL STARTECT.
-14	04 Jul 17	Weighed 52 of the lightest animals available.
-13	05 Jul 17	Relocated these 52 animals to groups pens in the UNE Animal House Facility and commenced daily observations.
-6	12 Jul 17	Installed dataloggers to record temperature inside and outside the facility.
-1	17 Jul 17	Weighed and allocated 46 animals to treatment groups. Collected duplicate blood and faecal samples. Processed blood samples and stored plasma frozen. Installed a fly trap, verified applicators, stored faecal samples frozen. All animals NAD.
0	18 Jul 17	Mulesed, castrated & tail-docked/ (as appropriate) and treated lambs as per treatment regime. Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 1 to 9 at 15, 30, 60, 120 minutes, then 4 hours post-treatment. Samples processed to extract plasma and plasma stored in triplicate at ~ -20°C. Group 1 animals were sacrificed 4 hours post-treatment. Urine, faeces, loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C. Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 2 to 9 at 6- and 9- hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. Blood samples were collected into 9mL lithium heparin vacutainers from Group 2 at 10 hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. Group 2 animals were sacrificed 10 hours post-treatment. Urine, faeces, loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C. Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 3 to 9 at 12 hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. All animals NAD.

Study Day	Date	Event
1	19 Jul 17	Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 3 to 9 at 18- and 24- hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. Group 3 animals were sacrificed 24 hours post-treatment. Urine, faeces, loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C. Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 4 to 9 at 30- and 36- hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C.
2	20 Jul 17	Blood samples were collected into 9mL lithium heparin vacutainers from Groups 4 to 9 at 48 hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. Group 4 animals were sacrificed 48 hours post-treatment. Urine, faeces, loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C.
3	21 Jul 17	Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 5 to 9 at 72 hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C.
4	22 Jul 17	Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 5 to 9 at 96 hours post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. Group 5 animals were sacrificed 96 hours post-treatment. Urine, faeces, loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C.
7	25 Jul 17	Blood samples were collected into 9 mL lithium heparin vacutainers from Groups 6 to 9, 7 days post-treatment. Samples processed to extract plasma which was stored in triplicate at ~ -20°C. Group 9, 8, 7 & 6 animals were sacrificed 7 days post-treatment. Urine, faeces, loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C.
14	01 Aug 17	Group 10 animals were sacrificed 14 days post-treatment. Loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C.
23	10 Aug 17	Dispatched all blank samples to the analytical laboratory.
28	15 Aug 17	Group 11 animals were sacrificed 28 days post-treatment. Loin and heart muscle, kidney, liver, perirenal fat and mules site were collected. Mules tissue samples were cryoprocessed and all samples were stored at ~ -20°C.
29	16 Aug 17	Group 12 sheep removed from the UNE Animal House Facility.
36	23 Aug 17	Replicate 1 and blank plasma samples sent to the analytical laboratory.
Post-study	24 Sep 17	Dispatched blank faeces and plasma to analytical laboratory.

6 Results

Summary of the animal treatment regime, including animal bodyweight at Day -1, calculated and applied TRI-SOLFEN doses are shown in Table 3.

Table 3: Treatment Regime

Animal ID	Group	Sex	Procedure	Day -1 BWt (kg)	Calc.* Mules/Tail Dock Dose	Calc.* Castration Dose	Applied Castration Dose (mL)	Applied Mules/Tail Dock Dose (mL)
902	1	MC	M/TD, C	17.5	21.0	10.5	11	21
913				22.0	26.4	13.2	14	27
915				23.5	28.2	14.1	15	29
968				20.0	24.0	12.0	12	24
908	2	MC	M/TD, C	25.0	30.0	15.0	15	30
949				22.0	26.4	13.2	14	27
962				18.5	22.2	11.1	12	23
967				19.5	23.4	11.7	12	24
906	3	MC	M/TD, C	23.0	27.6	13.8	14	28
926				20.0	24.0	12.0	12	24
930				24.0	28.8	14.4	15	29
939				17.0	20.4	10.2	11	21
927	4	MC	M/TD, C	23.5	28.2	14.1	15	29
959				20.0	24.0	12.0	12	24
963				18.0	21.6	10.8	11	22
965				22.5	27.0	13.5	14	27
919	5	MC	M/TD, C	25.0	30.0	15.0	15	30
932				20.0	24.0	12.0	12	24
936				18.0	21.6	10.8	11	22
947				23.0	27.6	13.8	14	28
934	6	F	M/TD	22.0	26.4	---	no castration	27
942				23.0	27.6	---	no castration	28
952				22.5	27.0	---	no castration	27
964				23.0	27.6	---	no castration	28
901	7	M	M/TD	17.0	20.4	---	no castration	21
917				23.5	28.2	---	no castration	29
950				19.0	22.8	---	no castration	23
953				20.5	24.6	---	no castration	25
903	8	MC	M/TD, C	18.5	22.2	11.1	12	23
912				18.5	22.2	11.1	12	23
938				24.5	29.4	14.7	15	30
941				21.5	25.8	12.9	13	26
904	9	MC	TD, C	17.5	5.25	10.5	11	6
916				25.5	7.65	15.3	16	8
928				20.0	6.0	12.0	12	6
960				21.0	6.3	12.6	13	7
924	10	MC	M/TD, C	20.0	24.0	12.0	12	24
944				23.5	28.2	14.1	15	29
954				18.5	22.2	11.1	12	23
958				23.5	28.2	14.1	15	29
910	11	MC	M/TD, C	17.5	21.0	10.5	11	21

935				21.0	25.2	12.6	13	26
937				19.0	22.8	11.4	12	23
961				24.5	29.4	14.7	15	30
914	12	MC	M/TD, C	20.5	24.6	12.3	13	25
951				26.5	31.8	15.9	16	32

*In compliance with VICH GL48 the administered dose was the maximum applicable as per final formulation approved label i.e. base dose of 6 mL for a 5 kg lamb or 1.2 mL/kg. For male lambs, the worst case castration dose was added – 3 mL for a 5 kg lamb or 0.6 mL/kg. This gave a total dose of 1.2 + 0.6 = 1.8 mL/kg. Female and non-castrated male lambs were treated at 1.2 mg/kg. Dose volume for the castrated and docked group (Group 9) only was 0.6 + 0.3 = 0.9 mL/kg. M = mules, TD = Tail-Dock, C = Castrate, BWt = Bodyweight.

Plasma, Urine and Faecal Analysis: Group mean concentrations by timepoint of lignocaine and its metabolites, bupivacaine and its metabolites and cetrimide in ovine plasma, urine and faeces are provided in Table 5. The same data reported to the animal level is provided in Tables 6-8 in Appendices 1-3. Table 4 provides the time points at which the metabolite (group mean) peaked in each substrate and the timepoint at which all samples analysed were below LOQ for each metabolite post-treatment (for sheep treated at the combination of mulesing, castration and tail-docking sites). Plasma concentrations of lignocaine and its metabolites and bupivacaine and its metabolites peaked by 4 hours post-treatment. Plasma concentrations of cetrimide peaked by 15 minutes post-treatment.

Tissue Metabolites: Group mean concentrations by timepoint of lignocaine and its metabolites, bupivacaine and its metabolites and cetrimide in ovine skeletal muscle (loin), muscle (heart), kidney, liver, subcutaneous tissue (directly under the application and mulesing site) and perirenal fat tissue are provided in Table 5. The same data reported to the animal level is provided in Tables 6-8 in Appendices1-3. Table 4 provides the timepoints at which the metabolite (group mean) peaked in each tissue and the timepoint at which all samples analysed were below LOQ for each metabolite post-treatment (for sheep treated at the combination of mulesing, castration and tail-docking sites). Levels of cetrimide, lignocaine (and metabolites) and bupivacaine (and metabolites) had peaked in all tissues (except at the mulesing site) by 24 hours post-treatment, except for lignocaine and bupivacaine and metabolites in subcutaneous tissue at the application and mulesing site which had peaked by 28 days. Levels of most metabolites were at peak levels at the last sampling point (28 days) at the mulesing wound indicating that levels concentrated over time which may be associated with wound contraction.

Group mean summary data in the form of cetrimide, lignocaine (and metabolites) and bupivacaine (and metabolites) levels in plasma, urine, faeces, loin muscle, heart muscle, kidney, liver, subcutaneous tissue at the application and mulesing site and perirenal fat tissue samples by timepoint are presented and were used to develop metabolism profiles for each metabolite and substrate (Figures 1-40).

Table 4: Summary of timepoints at which metabolites peak and fall below LOQ by substrate (for sheep undergoing mulesing, tail docking and castration in combination).

	Liver		Kidney		Perirenal Fat		Subcutaneous tissue at mulesing/ application site		Muscle (Heart)	
	Peak	**<LOQ**	**Peak**	**<LOQ**	**Peak**	**<LOQ**	**Peak**	**<LOQ**	**Peak**	**<LOQ**
Lignocaine	4h	28d	4h	>28d	4h	>28d	28d	>28d	4h	>28d
MEGX	4h	28d	4h	>28d	4h	>28d	28d	>28d	4h	>28d
3-OH Lignocaine	10h	>28d	4h	>28d	4h	>28d	28d	>28d	4h	>28d
GX	10h	>28d	10h	>28d	10h	28d	28d	>28d	10h	>28d
Lignocaine N-Oxide	NR	NR	NR	NR	4h	10h	2d	>28d	4h	>28d
2,6-DMA	4h	>28d	4h	28d	4h	>28d	4h	>28d	4h	28d
Bupivacaine	4h	7d	4h	28d	4h	>28d	28d	>28d	4h	7d
3-OH Bupivacaine	10h	14d	4h	7d	4h	2d	14d	>28d	4h	2d
N-Desbutyl Bupivacaine	4h	14d	10h	4d	10h	24h	28d	>28d	4h	24h
Cetrimide	4h	4h	10h	7d	10h	4h	28d	>28d	24h	14d

	Muscle (Loin)		Urine		Faeces		Plasma	
	Peak	**<LOQ**	**Peak**	**<LOQ**	**Peak**	**<LOQ**	**Peak**	**<LOQ ***
Lignocaine	4h	28d	4h	>7d	4h	>7d	15m	>7d
MEGX	4h	14d	4h	>7d	10h	>7d	2h	>7d
3-OH Lignocaine	4h	14d	4h	>7d	10h	>7 d	30m	>7d
GX	10h	>28d	4h	>7d	10h	>7d	4h	>7d
Lignocaine N-Oxide	4h	4h	10h	>7d	4h	24hr	30m	7d
2,6-DMA	4h	14d	4h	>7d	4h	>7d	4h	>7d
Bupivacaine	4h	4d	10h	2d	10h	7d	15m	7d
3-OH Bupivacaine	4h	24h	10h	>7d	24h	>7d	<LOD	15m
N-Desbutyl Bupivacaine	10h	24h	10h	4d	4h	4h	4h	24h
Cetrimide	4d	14d	4h	4h	4d	4h	15m	15m

<LOQ = timepoint at which all samples were below respective LOQ; m = minutes; h = hours; d = days; NR = not reported.

For Cetrimide: LOQ = 10 µg/kg (or L in plasma and urine) (except liver: 15 µg/kg and faeces: 22 µg/kg); For Lignocaine (and metabolites) and Bupivacaine (and metabolites): LOQ = 0.2 µg/kg (or L in plasma and urine) (except GX in plasma: 0.29 µg/L, Lignocaine N-oxide in loin muscle: 0.47 µg/kg, Lignocaine N-oxide in perirenal fat: 0.33 µg/kg, Lignocaine N-oxide in faeces: 0.26 µg/kg, 3-OH Bupivicaine in kidney: 0.25 µg/kg, N-desbutyl Bupivicaine in faeces: 0.87 µg/kg).

Table 5: Group mean lignocaine (and metabolites), bupivacaine (and metabolites) and cetrimide concentrations by tissue and timepoint

Tissue	Timepoint	Group	Sex	Procedure	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6 DMA (µg/kg or L)	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)	Cetrimide (µg/kg or L)
Liver	4 hours	1	MC	Mu/D/C	18.2	20.7	41.8	36.5	NR	47.9	17.7	9.0	2.2	9.5*
	10 hours	2	MC	Mu/D/C	16.53	13.19	45.22	54.94	NR	18.41	10.31	12.46	1.93	6.00*
	24 hours	3	MC	Mu/D/C	1.15	1.09	3.53	13.53	NR	2.28	2.18	3.42	0.32	6.92*
	2 days	4	MC	Mu/D/C	2.34	2.03	2.45	7.08	NR	2.95	1.01	0.48	0.23	4.53*
	4 days	5	MC	Mu/D/C	0.71	0.90	1.27	5.13	NR	0.55	0.35	0.23	<LOD	4.82*
	7 days	6	F	Mu/D	0.21	0.12*	0.39	0.58	NR	0.28	0.12*	0.11*	<LOD	<LOD
	7 days	7	M	Mu/D	0.20*	0.16*	0.39	1.19	NR	0.26	0.10*	0.09*	<LOD	<LOD
	7 days	8	MC	Mu/D/C	0.50	0.53	1.49	3.68	NR	0.50	0.11*	0.16*	<LOD	<LOD
	7 days	9	MC	D/C	0.50	0.30	0.91	2.48	NR	0.56	0.25	0.11*	<LOD	<LOD
	14 days	10	MC	Mu/D/C	0.59	0.22	0.73	1.14	NR	0.49	0.17*	<LOD	<LOD	<LOD
	28 days	11	MC	Mu/D/C	0.11*	<LOD	0.21	0.47	NR	0.15*	0.08*	<LOD	<LOD	<LOD
Kidney	4 hours	1	MC	Mu/D/C	253	120	107	53	NR	48	28	10	3	50
	10 hours	2	MC	Mu/D/C	92.9	62.2	88.8	97.7	NR	14.1	10.8	7.0	3.0	53.5
	24 hours	3	MC	Mu/D/C	12.3	6.3	9.8	14.4	NR	2.5	2.2	4.0	0.5	36.1
	2 days	4	MC	Mu/D/C	6.14	8.11	5.82	16.07	NR	1.50	0.61	0.61	0.25	21.80
	4 days	5	MC	Mu/D/C	2.14	2.25	2.89	4.15	NR	0.41	0.21	0.21*	<LOD	5.95*
	7 days	6	F	Mu/D	1.14	0.69	1.04	0.50	NR	0.27	0.10*	0.09*	<LOD	<LOD
	7 days	7	M	Mu/D	1.04	0.98	0.92	1.08	NR	0.17*	0.09*	<LOD	<LOD	<LOD
	7 days	8	MC	Mu/D/C	2.38	1.85	2.29	3.65	NR	0.49	0.20*	0.11*	<LOD	3.82*
	7 days	9	MC	D/C	2.16	1.91	2.96	2.67	NR	0.35	0.20*	0.17*	0.08*	<LOD
	14 days	10	MC	Mu/D/C	1.22	0.73	1.34	1.01	NR	0.31	0.12*	<LOD	<LOD	<LOD
	28 days	11	MC	Mu/D/C	0.67	0.27	0.39	0.33	NR	0.09*	0.08*	<LOD	<LOD	<LOD

Table 5: Cont.

Tissue	Timepoint	Group	Sex	Procedure	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6 DMA (µg/kg or L)	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)	Cetrimide (µg/kg or L)
Perirenal Fat	4 hours	1	MC	Mu/D/C	264	7	13	7	<LOD	182	54	2	0	6*
	10 hours	2	MC	Mu/D/C	79.1	5.2	11.5	18.6	0.1*	88.3	19.4	1.1	0.4	6.8*
	24 hours	3	MC	Mu/D/C	11.2	0.4	2.4	1.6	0.1*	24.3	5.0	0.6	0.1*	4.0*
	2 days	4	MC	Mu/D/C	7.45	1.11	1.39	2.76	<LOD	12.20	1.19	0.09*	0.08*	<LOD
	4 days	5	MC	Mu/D/C	5.7	0.4	0.5	0.6	0.1*	1.7	0.9	0.1*	0.1*	5.4*
	7 days	6	F	Mu/D	0.705	<LOD	0.135*	<LOD	<LOD	1.165	0.174*	<LOD	<LOD	<LOD
	7 days	7	M	Mu/D	1.18	0.16*	0.21	0.36	<LOD	0.62	0.12*	<LOD	<LOD	<LOD
	7 days	8	MC	Mu/D/C	2.11	0.28	0.75	0.83	<LOD	1.15	0.20*	<LOD	<LOD	3.30*
	7 days	9	MC	D/C	2.09	0.23	0.78	0.49	<LOD	1.30	0.31	0.08*	<LOD	<LOD
	14 days	10	MC	Mu/D/C	1.59	0.14*	0.29	0.17*	<LOD	1.17	0.19*	<LOD	<LOD	<LOD
	28 days	11	MC	Mu/D/C	2.64	0.31	0.15*	0.10*	0.11*	0.42	0.39	<LOD	0.09*	<LOD
Subcutaneous tissue (mulesing tissue)	4 hours	1	MC	Mu/D/C	41865	107	12	19	30	805	6800	321	5	6313
	10 hours	2	MC	Mu/D/C	13603	42	6	32	20	519	2899	73	3	2845
	24 hours	3	MC	Mu/D/C	8270	32	1	7	13	52	1247	45	2	2624
	2 days	4	MC	Mu/D/C	33047	373	7	14	40 [A]	281	9594	801 [A]	26	12331
	4 days	5	MC	Mu/D/C	76471	373	7	31	40 [A]	281	9594	801 [A]	26	12331
	7 days	6	F	Mu/D	50645	430	5	37	37 [A]	35	7180	600	24	9180
	7 days	7	M	Mu/D	26966	233	4	28	40 [A]	41	3977	274	18	6914
	7 days	8	MC	Mu/D/C	6752	77	1	13	29 [A]	15	986	29	7	1384
	7 days	9	MC	D/C	846	19	<LOD	2	10	3	95	5	2	186
	14 days	10	MC	Mu/D/C	134456	802 [A]	15	82	40 [A]	90	20128	1000 [A]	51	23812
	28 days	11	MC	Mu/D/C	145986	1041 [A]	33	177	40 [A]	64	22155	1000 [A]	161	29254
Muscle (Heart)	4 hours	1	MC	Mu/D/C	221	30	13	23	7	25	23	1	1	36
	10 hours	2	MC	Mu/D/C	63.7	12.4	8.5	31.0	0.8	17.8	11.4	0.7	0.7	43.2

Table 5: Cont.

Tissue	Timepoint	Group	Sex	Procedure	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6 DMA (µg/kg or L)	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)	Cetrimide (µg/kg or L)
Muscle (Heart) cont.	24 hours	3	MC	Mu/D/C	6.3	1.3	1.5	4.8	0.1*	2.3	1.6	0.3	0.1*	57.3
	2 days	4	MC	Mu/D/C	3.44	1.99	0.85	4.76	<LOD	1.67	0.48	0.08*	0.09*	33.77
	4 days	5	MC	Mu/D/C	1.47	0.59	0.41	1.41	<LOD	0.41	0.21	<LOD	<LOD	27.29
	7 days	6	F	Mu/D	0.432	0.146*	0.159*	0.188*	<LOD	0.115*	<LOD	<LOD	<LOD	<LOD
	7 days	7	M	Mu/D	0.436	0.227	0.143*	0.413	<LOD	0.101*	<LOD	<LOD	<LOD	<LOD
	7 days	8	MC	Mu/D/C	1.75	0.51	0.34	1.14	<LOD	0.46	0.19*	<LOD	<LOD	8.61*
	7 days	9	MC	D/C	1.26	0.49	0.39	0.83	<LOD	0.30	0.17*	<LOD	<LOD	4.98*
	14 days	10	MC	Mu/D/C	0.610	0.227	0.225	0.387	<LOD	0.229	0.085*	<LOD	<LOD	<LOD
	28 days	11	MC	Mu/D/C	3.0	0.1*	0.1*	0.2*	0.2*	0.1*	0.6	0.1*	0.1*	<LOD
Muscle (Loin)	4 hours	1	MC	Mu/D/C	76.4	23.1	4.7	18.9	0.1*	12.4	7.1	0.4	0.6	8.3*
	10 hours	2	MC	Mu/D/C	22.68	10.68	3.24	29.66	<LOD	5.34	2.81	0.34	0.64	12.37
	24 hours	3	MC	Mu/D/C	2.54	1.14	0.52	4.60	<LOD	0.89	0.38	0.13*	0.12*	21.89
	2 days	4	MC	Mu/D/C	1.668	1.932	0.325	4.472	<LOD	0.490	0.128*	<LOD	0.087*	15.287
	4 days	5	MC	Mu/D/C	0.670	0.710	0.158*	1.673	<LOD	0.121*	<LOD	<LOD	<LOD	22.491
	7 days	6	F	Mu/D	0.205	0.157*	<LOD	0.178*	<LOD	0.071*	<LOD	<LOD	<LOD	<LOD
	7 days	7	M	Mu/D	0.627	0.264	<LOD	0.451	<LOD	<LOD	0.142*	<LOD	<LOD	<LOD
	7 days	8	MC	Mu/D/C	0.948	0.503	0.160*	1.235	<LOD	0.136*	<LOD	<LOD	<LOD	10.241
	7 days	9	MC	D/C	0.57	0.46	0.17*	0.92	<LOD	0.13*	<LOD	<LOD	<LOD	7.89*
	14 days	10	MC	Mu/D/C	0.337	0.270	0.084*	0.454	<LOD	<LOD	<LOD	<LOD	<LOD	3.458*
	28 days	11	MC	Mu/D/C	0.233	0.099*	<LOD	0.185*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Urine	4 hours	1	MC	Mu/D/C	566	40 [A]	1000 [A]	633	10	1334	4	197	3	<LOD
	10 hours	2	MC	Mu/D/C	424	40 [A]	940 [A]	563	43	369	9	309	12	<LOD
	24 hours	3	MC	Mu/D/C	27.2	7.7	272.5	38.6	0.2*	91.3	0.3	95.3	0.6	<LOD
	2 days	4	MC	Mu/D/C	40.9	18.9	269.7	49.5	3.8	47.0	0.1*	16.8	0.3	<LOD
	4 days	5	MC	Mu/D/C	7.5	3.5 [A]	111.6	19.7	0.6	9.0	0.1*	7.7	0.1*	<LOD
	7 days	6	F	Mu/D	1.64	0.54	102.91	2.07	1.09	2.43	<LOD	2.98	<LOD	<LOD
	7 days	7	M	Mu/D	2.117	1.297	106.419	3.451	0.260	4.609	<LOD	1.955	<LOD	<LOD

Table 5: Cont.

Tissue	Timepoint	Group	Sex	Procedure	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6 DMA (µg/kg or L)	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)	Cetrimide (µg/kg or L)
Urine cont.	7 days	8	MC	Mu/D/C	18.03	13.09 [A]	344.49	33.61	0.67	8.85	0.44	11.95	0.16*	<LOD
	7 days	9	MC	D/C	14.85	3.11	241.38	8.83	0.73	6.97	<LOD	7.72	0.10*	<LOD
Faeces	4 hours	1	MC	Mu/D/C	36.6 [A]	13.3	17.0 [A]	5.8	1.1	6.2	6.5	10.3 [A]	0.3*	<LOD
	10 hours	2	MC	Mu/D/C	35.3 [A]	16.2	24.9 [A]	24.0	0.7	5.8	7.1	27.2 [A]	0.2*	9.5*
	24 hours	3	MC	Mu/D/C	4.49	1.60	9.71 [A]	5.98	<LOD	1.83	4.99	37.32 [A]	0.09*	16.10*
	2 days	4	MC	Mu/D/C	2.71	2.35	7.09 [A]	5.28	<LOD	0.81	0.51	24.47 [A]	<LOD	17.05*
	4 days	5	MC	Mu/D/C	0.861	0.797	3.019	1.653	<LOD	0.216	0.241	5.533	<LOD	23.782
	7 days	6	F	Mu/D	0.198*	0.166*	1.117	0.189*	<LOD	<LOD	0.106*	2.442	<LOD	13.825*
	7 days	7	M	Mu/D	0.137*	0.203	0.307	0.306	<LOD	0.085*	0.075*	0.319	<LOD	17.749*
	7 days	8	MC	Mu/D/C	0.389	0.375	1.329	0.822	<LOD	0.218	0.071*	1.958	<LOD	11.365*
	7 days	9	MC	D/C	0.342	0.355	2.109	0.577	<LOD	0.189*	0.115*	2.384	<LOD	25.854
Plasma	Day -1	8	MC	Mu/D/C	0.090*	<LOD	<LOD	0.291	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	15 minutes				40 [A]	13	3	8	8	3	13	<LOD	0	7*
	30 minutes				40 [A]	16	4	14	9	3	9	<LOD	1	4*
	60 minutes				40 [A]	18	3	23	8	4	8	<LOD	0	5*
	2 hours				40 [A]	20	3	33 [A]	8	6	10	<LOD	1	3*
	4 hours				34.9	14.7	2.7	36.1 [A]	5.3	6.3	7.9	0.1*	0.6	<LOD
	6 hours				12.62	7.60	1.79	35.78 [A]	2.56	4.44	3.93	<LOD	0.36	<LOD
	9 hours				6.1	3.8	1.0	29.1	1.4	2.5	2.1	0.1*	0.2*	<LOD
	12 hours				3.28	2.68	0.57	23.35	1.10	1.97	1.38	<LOD	0.17*	<LOD
	18 hours				1.47	1.29	0.29	12.46	0.70	0.92	0.66	<LOD	0.11*	<LOD
	24 hours				1.07	0.95	0.20*	8.12	0.56	0.76	0.56	<LOD	0.10*	<LOD
	30 hours				0.90*	0.65	0.14*	5.29	0.38	0.50	0.43	<LOD	0.09*	<LOD
	36 hours [B]				0.994	0.658	0.116*	4.238	0.340	0.422	0.352	<LOD	<LOD	<LOD

Table 5: Cont.

Tissue	Timepoint	Group	Sex	Procedure	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6 DMA (µg/kg or L)	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)	Cetrimide (µg/kg or L)
Plasma cont.	48 hours	8	MC	Mu/D/C	1.01	0.48	0.10*	2.56	0.30	0.27	0.31	<LOD	<LOD	<LOD
	72 hours				0.61	0.32	0.09*	1.42	0.20*	0.20*	0.19*	<LOD	<LOD	<LOD
	96 hours				0.416	0.273	0.082*	1.247	0.179*	0.196*	0.140*	<LOD	<LOD	<LOD
	7 days				0.265	0.161*	0.072*	0.815	0.132*	0.139*	0.083*	<LOD	<LOD	<LOD

For Lignocaine (and metabolites) and Bupivacaine and metabolites): LOD = 0.07 µg/kg (or L for plasma and urine);
For Lignocaine (and metabolites) and Bupivacaine (and metabolites): LOQ = 0.2 µg/kg (or L in plasma and urine) (except GX in plasma: 0.29 µg/L, Lignocaine N-oxide in loin muscle: 0.47 µg/kg, Lignocaine N-oxide in perirenal fat: 0.33 µg/kg, Lignocaine N-oxide in faeces: 0.26 µg/kg, 3-OH Bupivicaine in kidney: 0.25 µg/kg, N-desbutyl Bupivicaine in faeces: 0.87 µg/kg).
For Cetrimide: LOD = 3 µg/kg (or L for plasma and urine); LOQ = 10 µg/kg (or L in plasma and urine) (except liver: 15 µg/kg and faeces: 22 µg/kg);
[A] Some values exceeded upper limit of quantification; [B] Animal ID 912 plasma data excluded at 36-hour timepoint due to significant outlying data; NR = Not Reported. *Value between LOD and LOQ.

6.1 Metabolism profiles of Cetrimide, Lignocaine, (and Metabolites) and Bupivacaine (and Metabolites) in ovine tissues, urine, faeces and plasma

NB: Data from Groups 1-5, 8 and 10-11 (animals which were mulesed, tail-docked and castrated) were used to graph metabolism profiles.

Figure 1: Cetrimide in Tissues

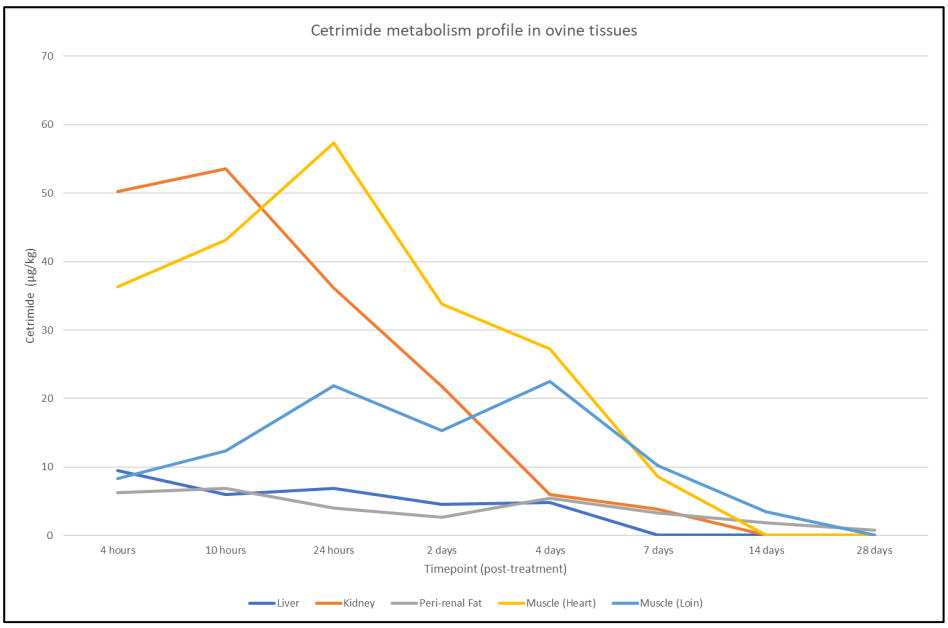


Figure 2: Cetrimide in subcutaneous tissue at the site of mulesing

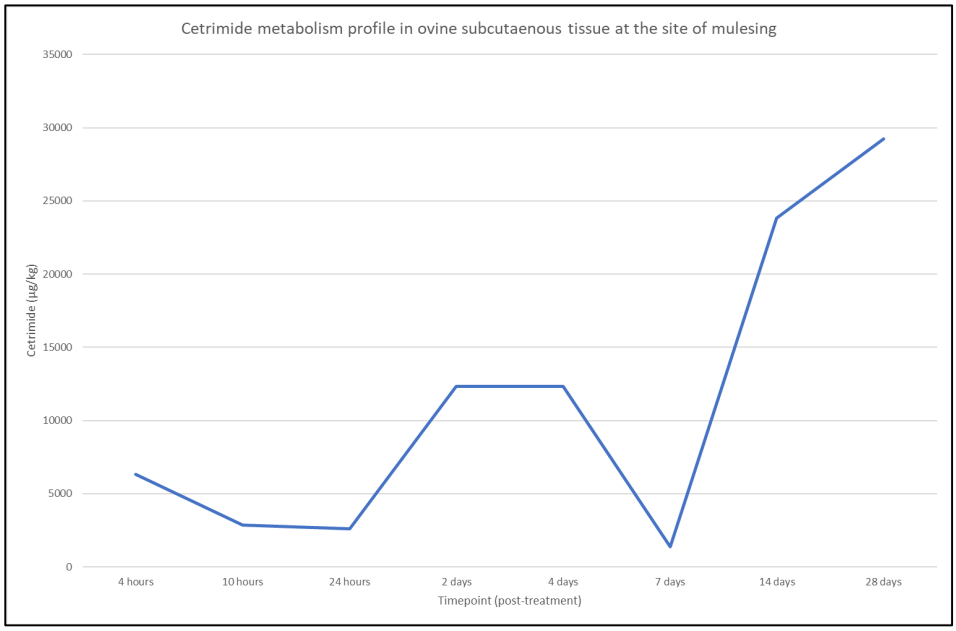


Figure 3: Cetrimide in Urine and Faeces

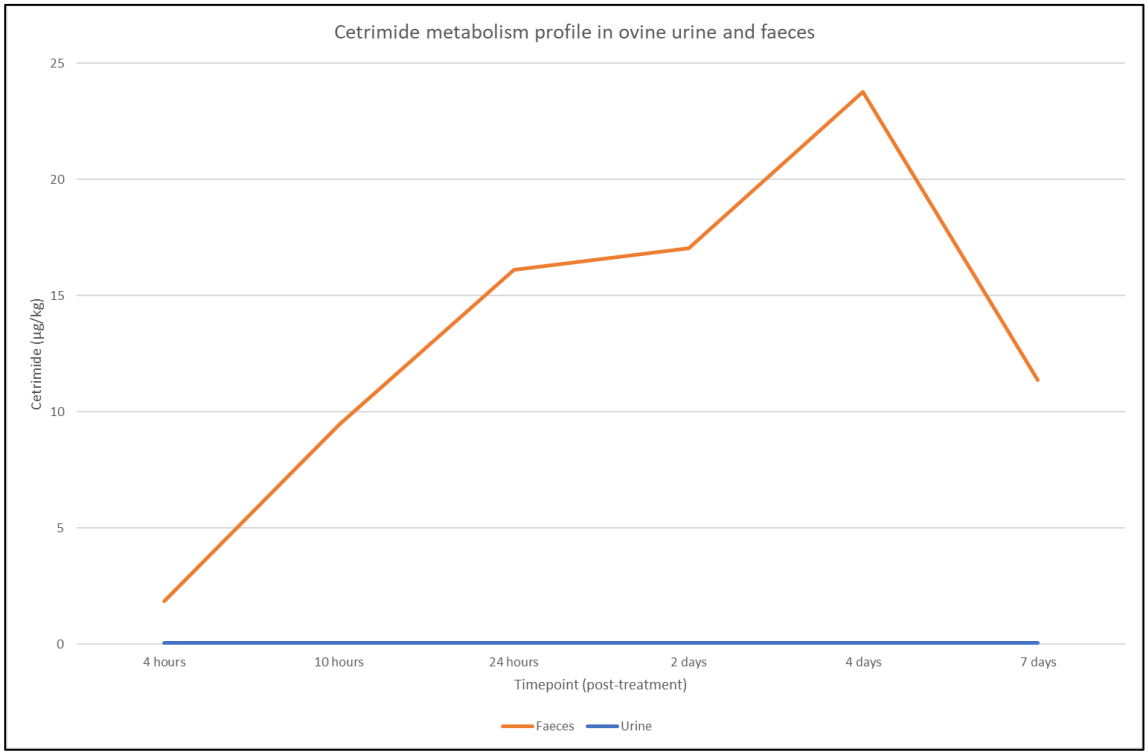
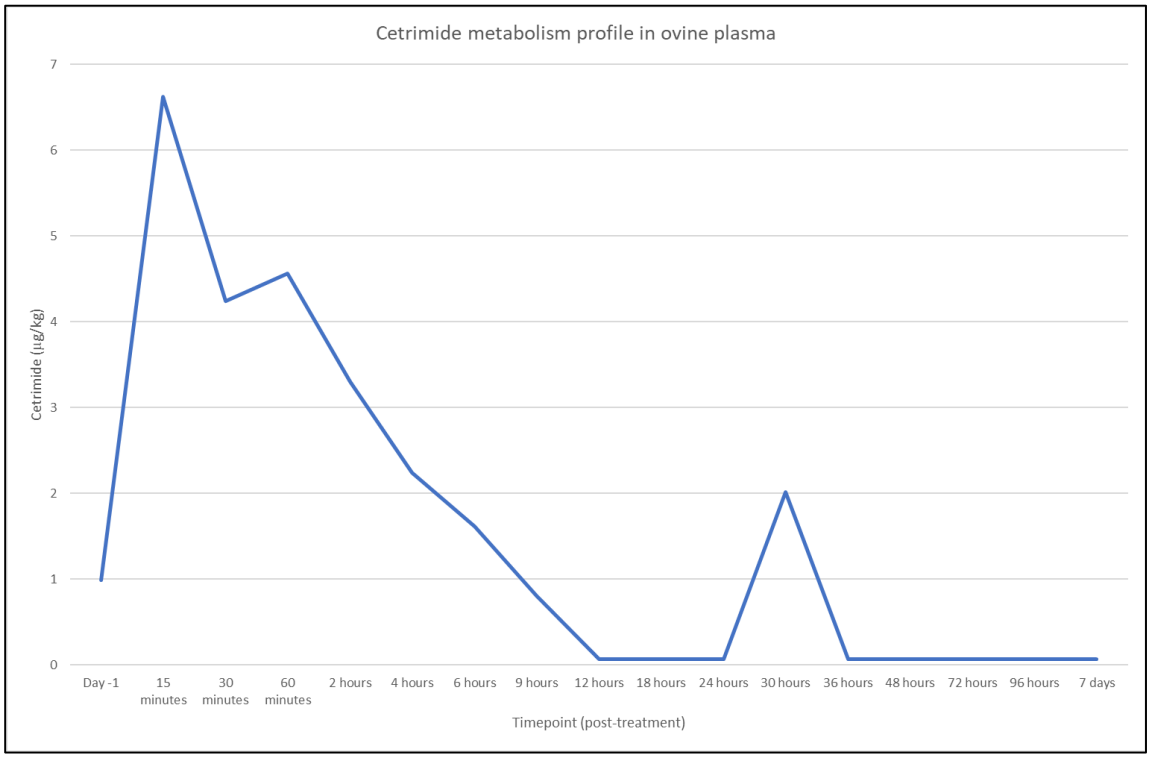


Figure 4: Cetrimide in Plasma*



*ID 912 excluded at 36-hour timepoint due to outlying data

Figure 5: Lignocaine in tissues

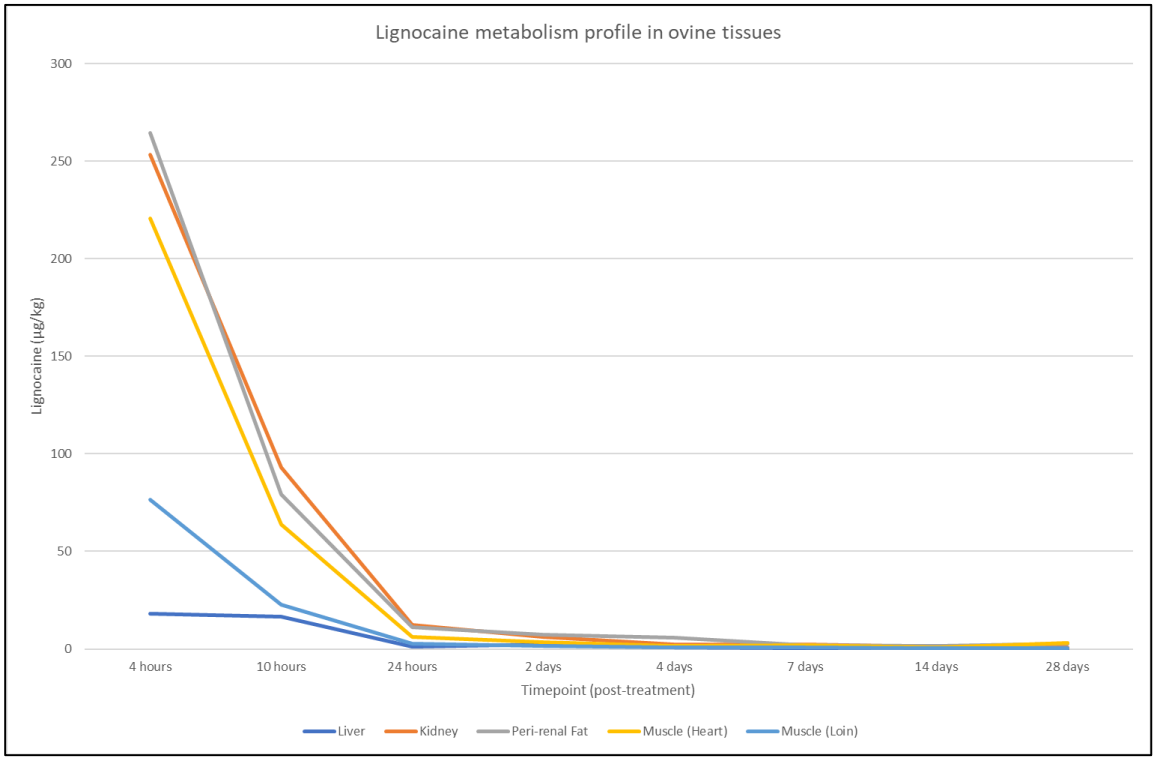


Figure 6: Lignocaine in subcutaneous tissue at the site of mulesing

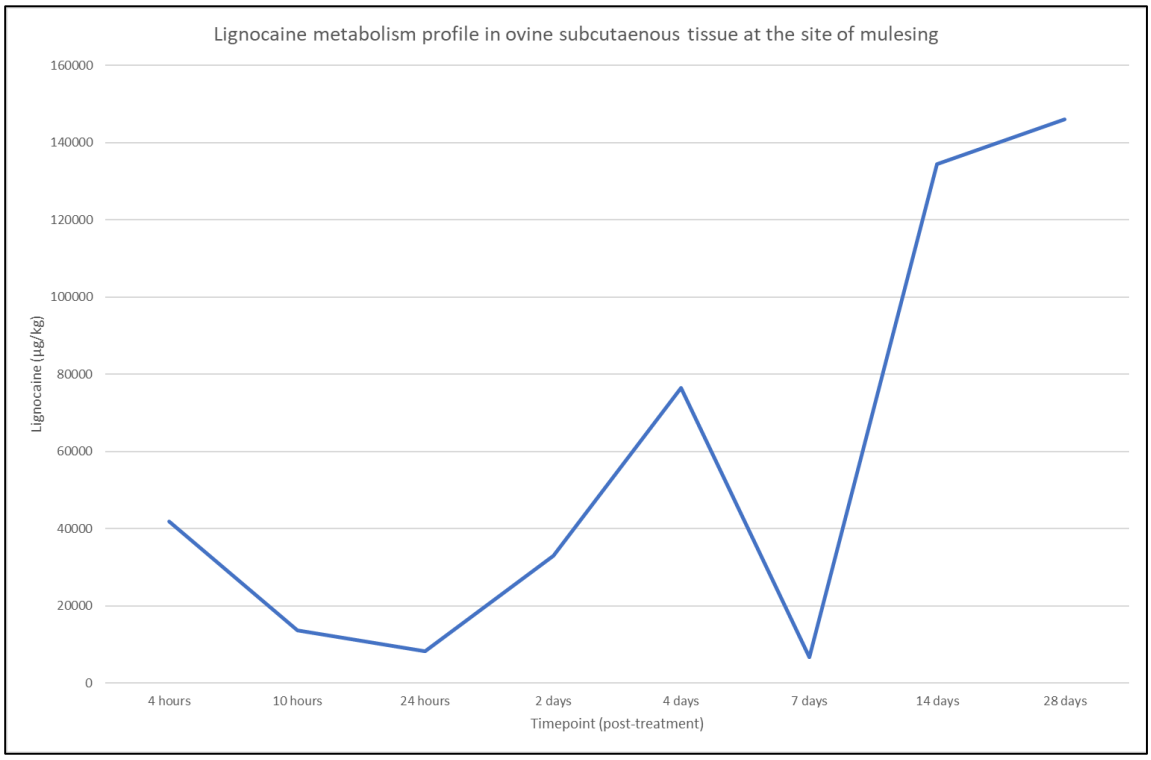


Figure 7: Lignocaine in urine and faeces

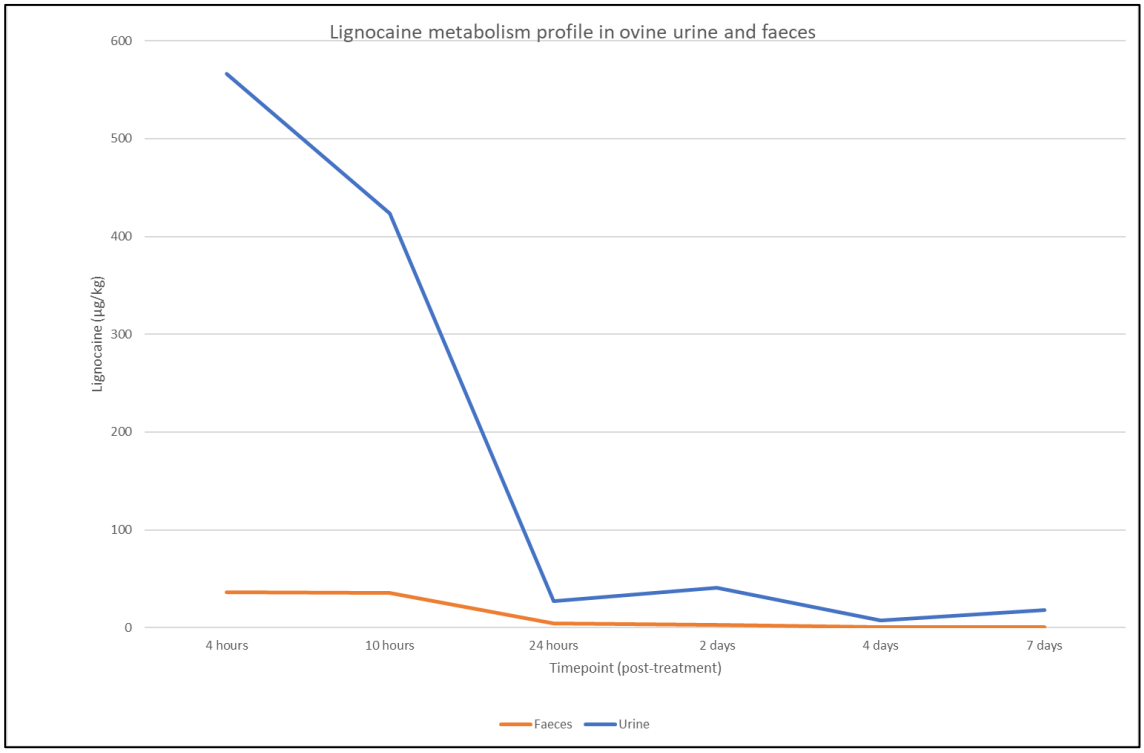
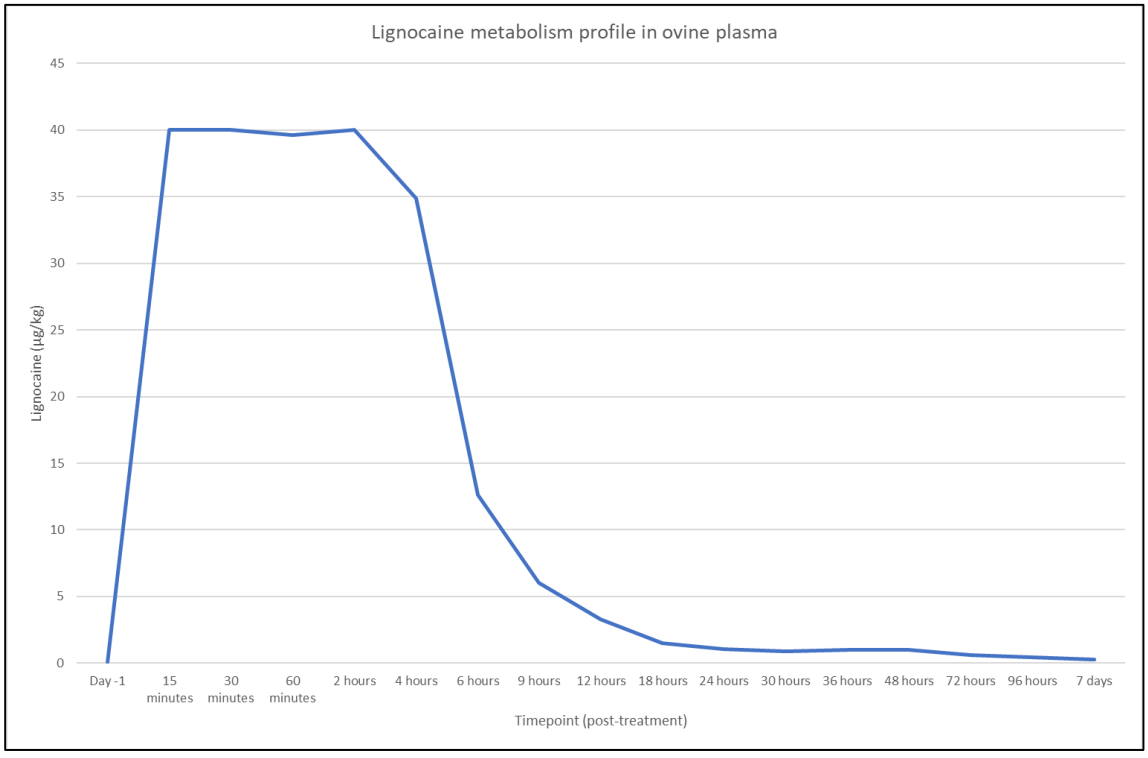


Figure 8: Lignocaine in plasma*



*ID 912 excluded at 36-hour timepoint due to outlying data

Figure 9: MEGX in tissues

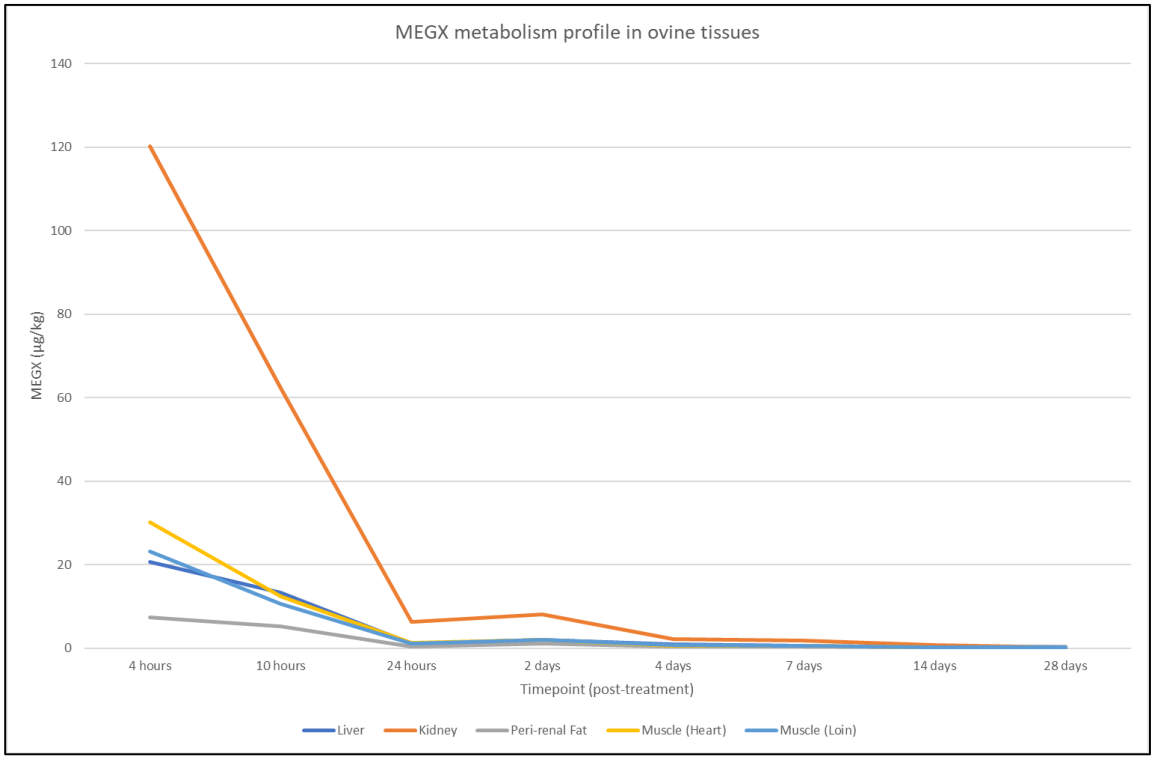
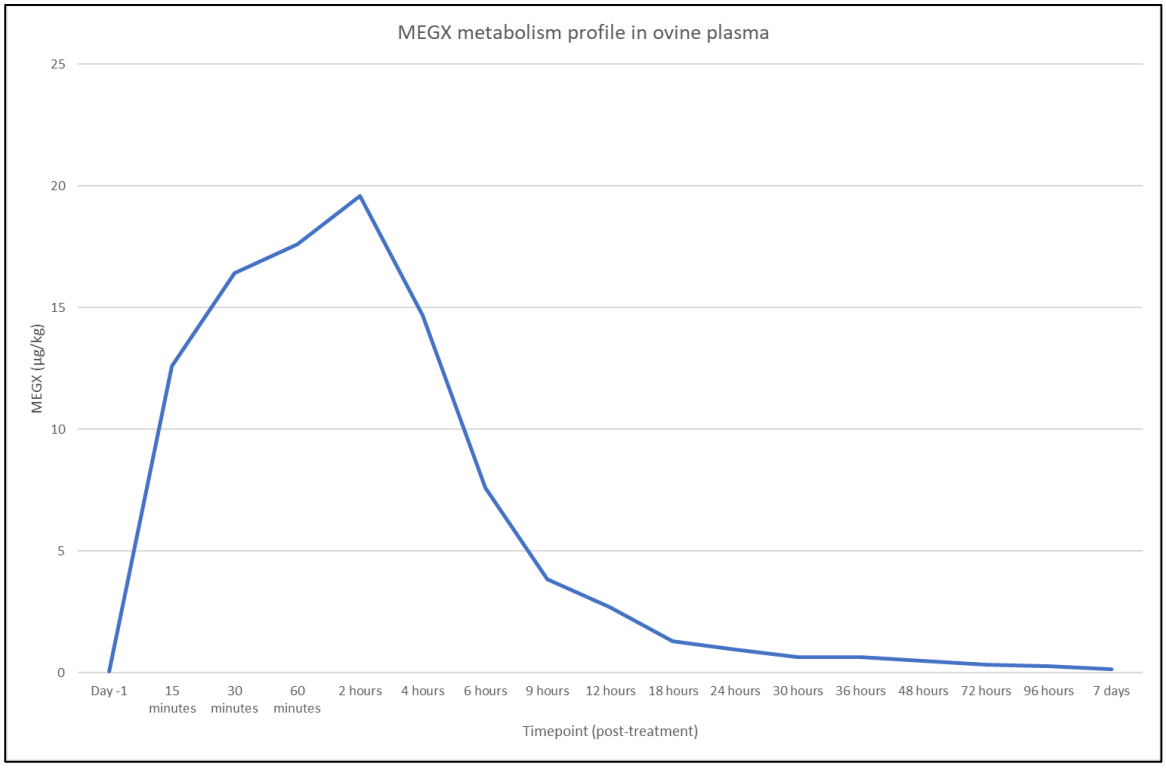


Figure 10: MEGX in subcutaneous tissue at the site of mulesing

Figure 11: MEGX in urine and faeces

Figure 12: MEGX in Plasma*



*ID 912 excluded at 36-hour timepoint due to outlying data

Figure 13: 3-OH Lignocaine in tissues

Figure 14: 3-OH Lignocaine in subcutaneous tissue at the site of mulesing

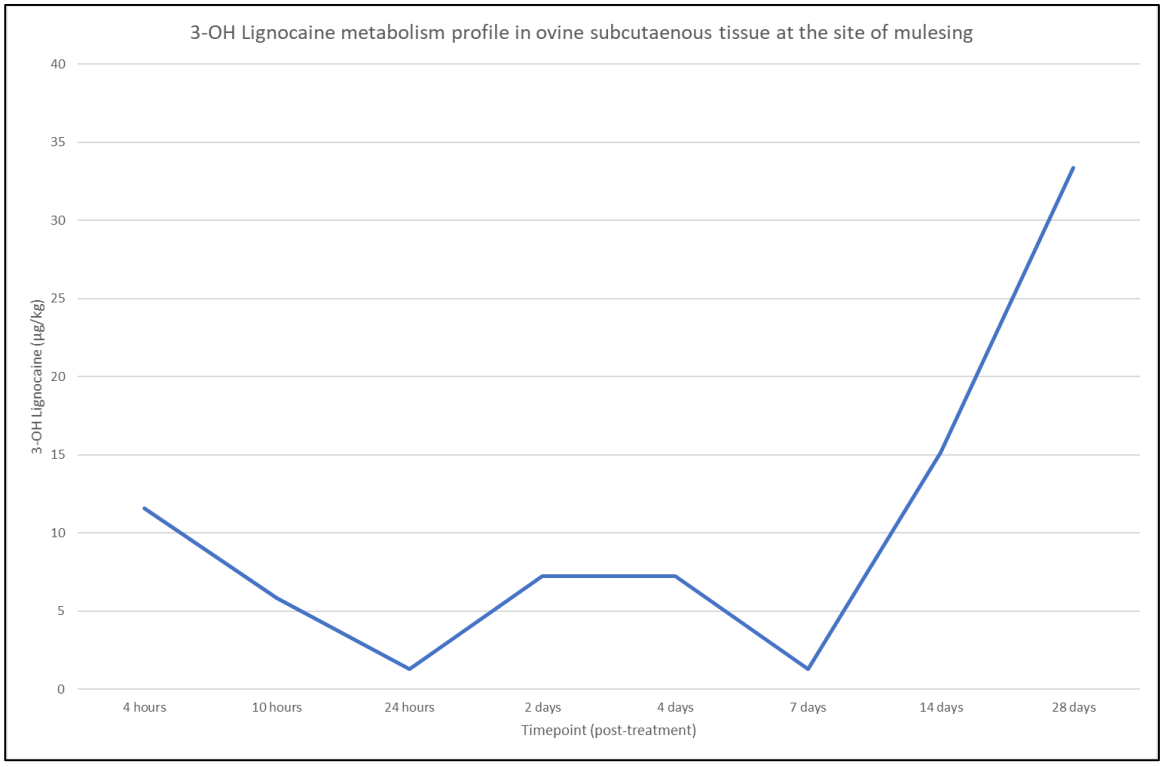


Figure 15: 3-OH Lignocaine in urine and faeces

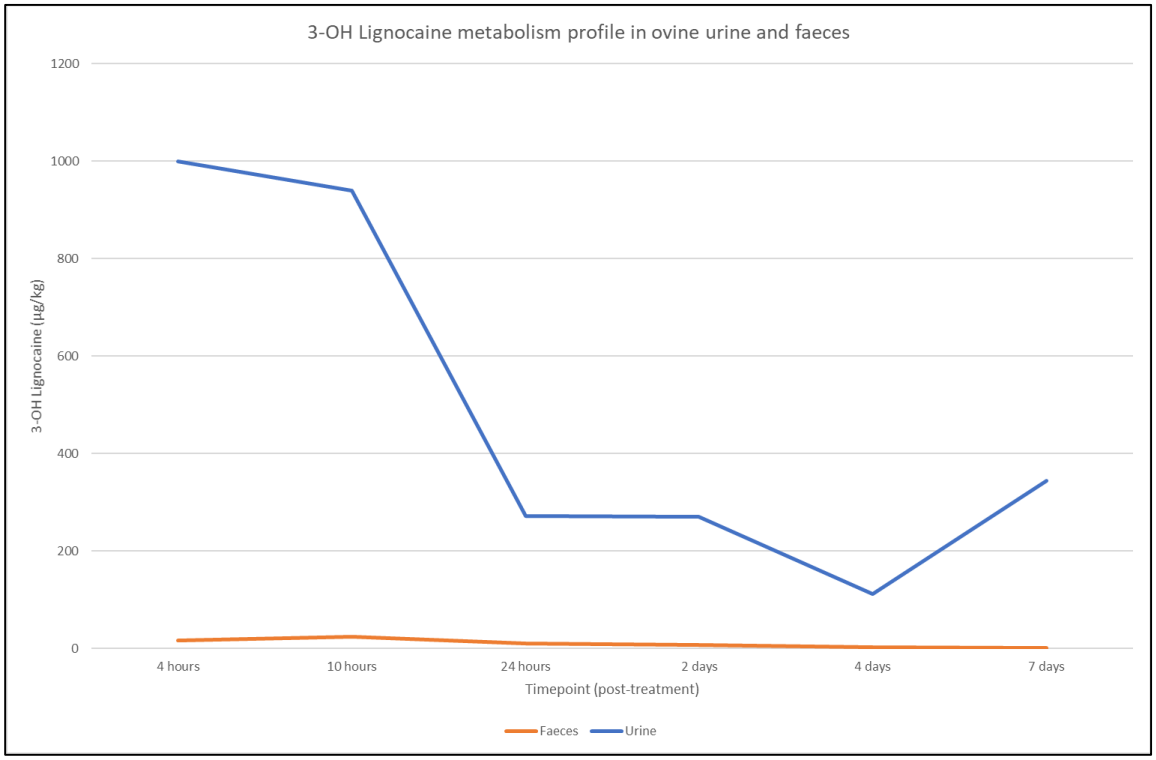
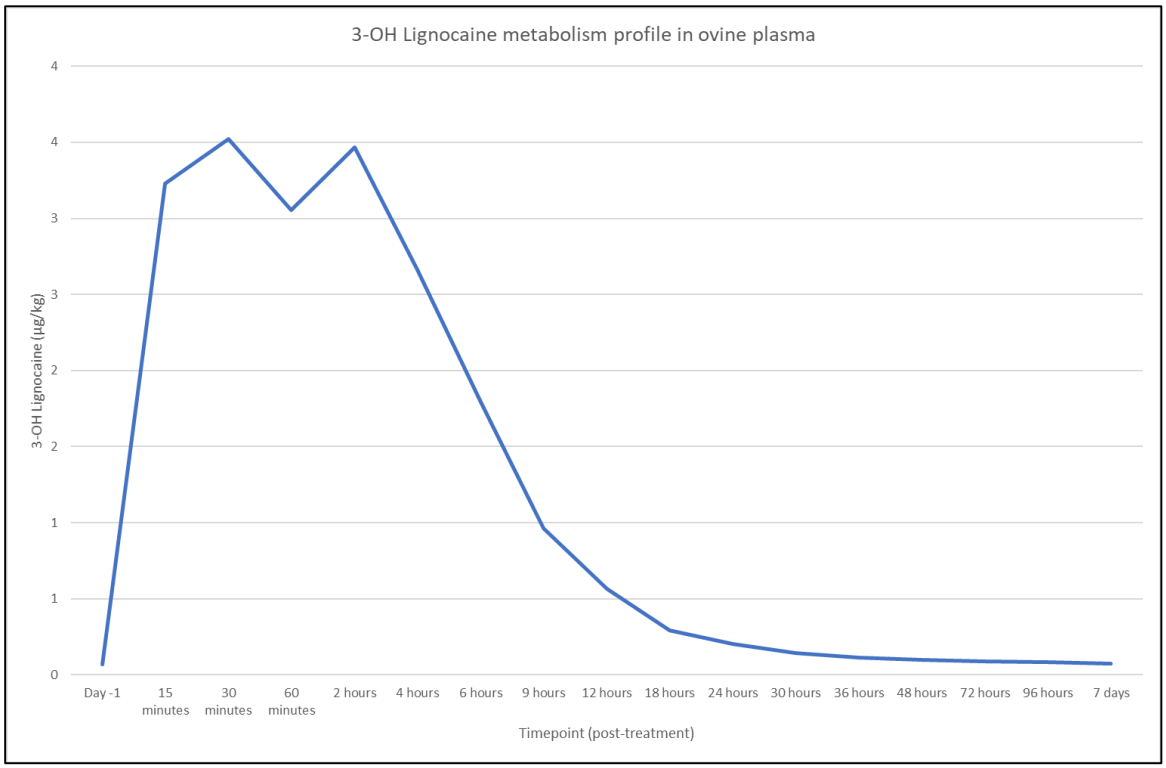


Figure 16: 3-OH Lignocaine in plasma*



* ID 912 excluded at 36-hour timepoint due to outlying data

Figure 17: GX in tissues

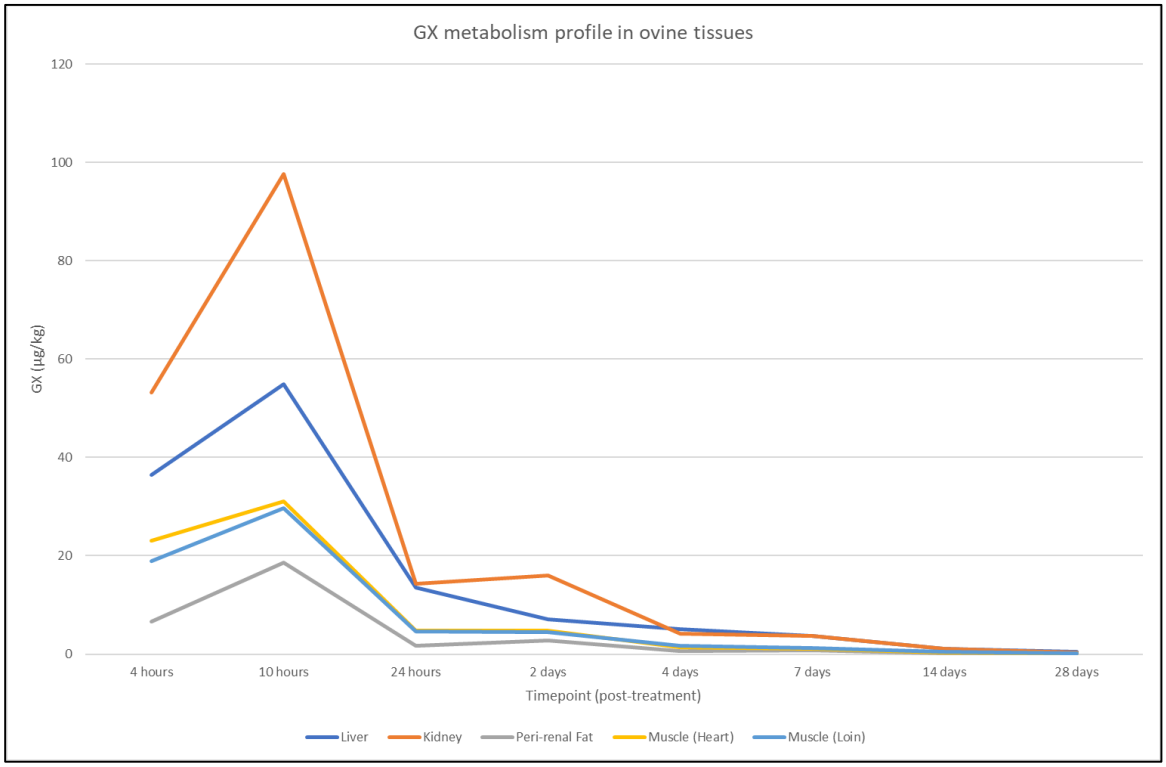


Figure 18: GX in subcutaneous tissue at the site of mulesing

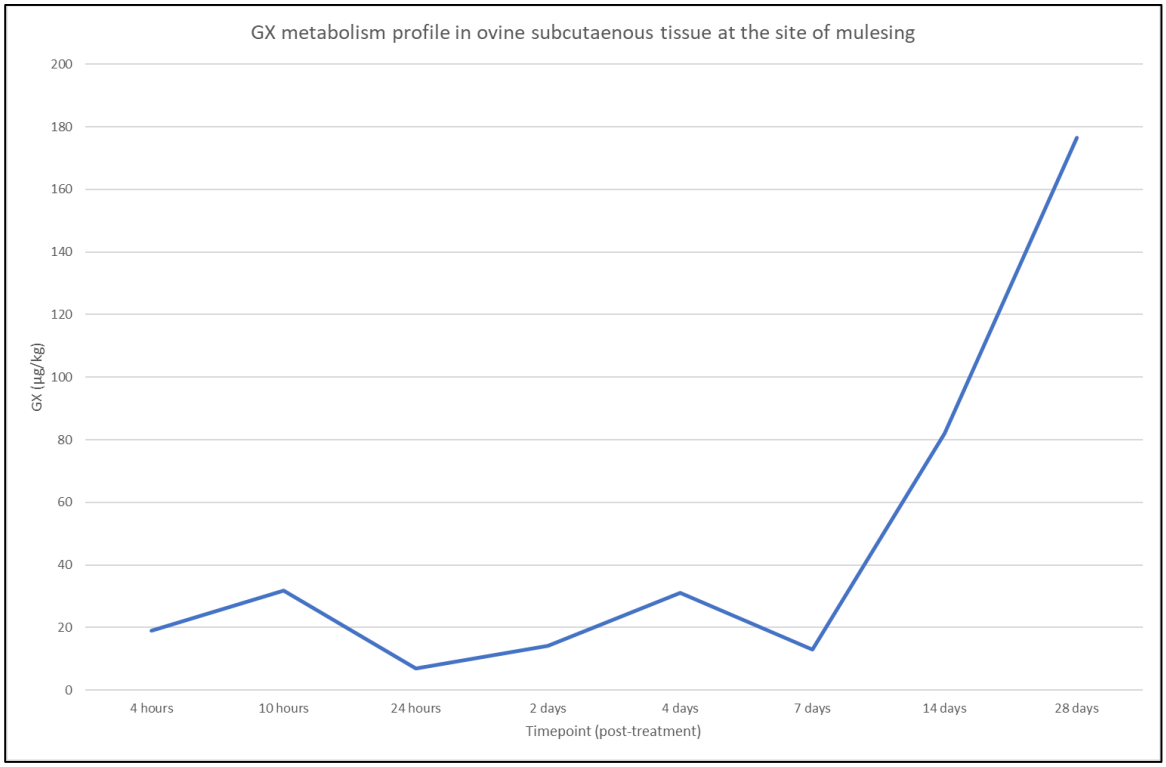


Figure 19: GX in urine and faeces

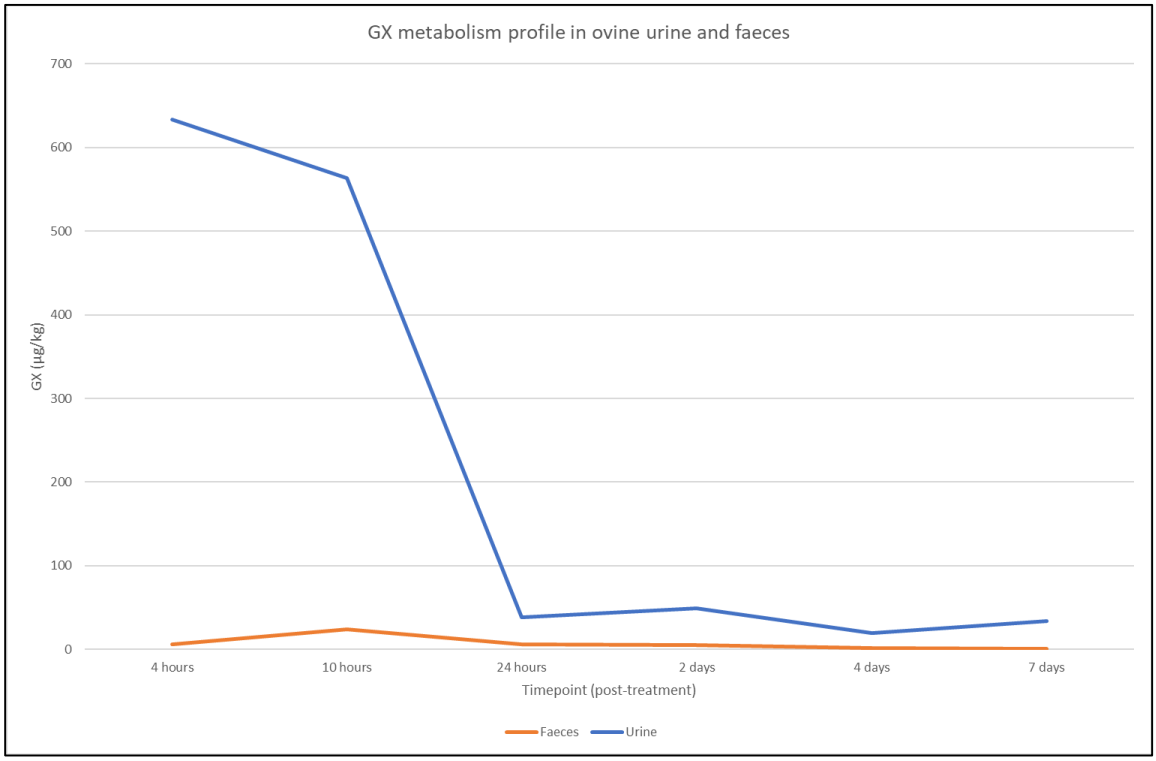
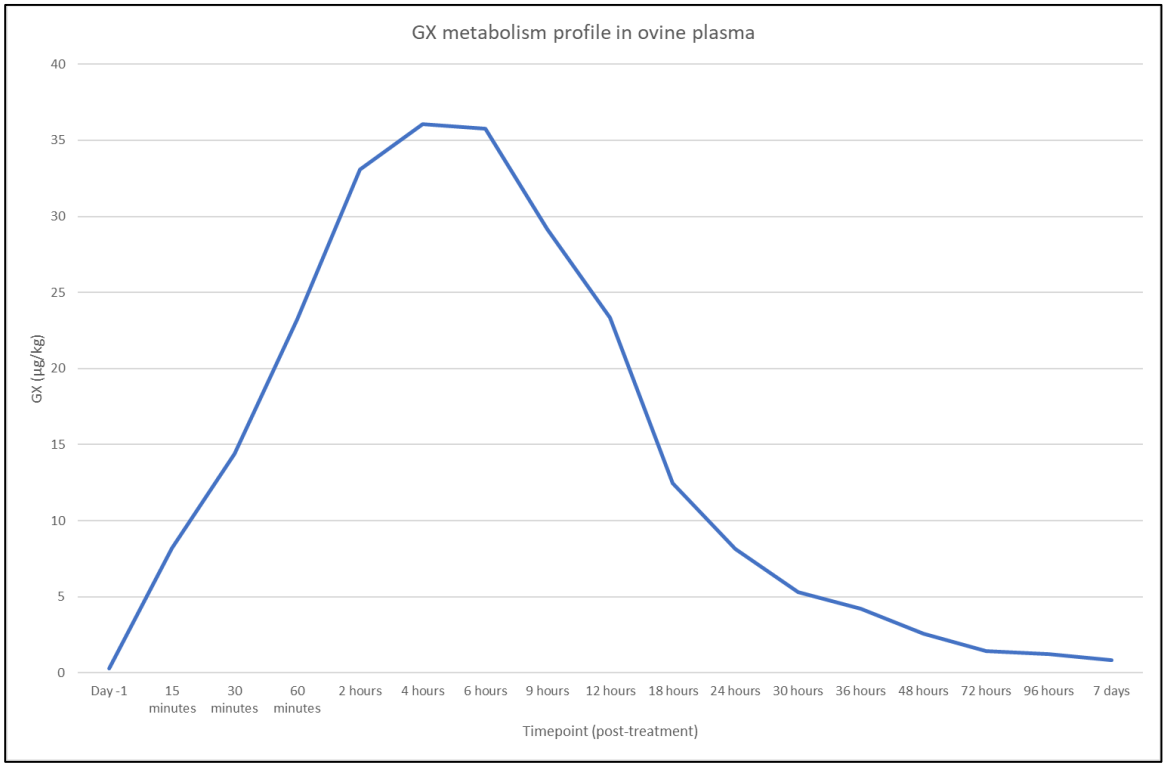


Figure 20: GX in plasma*



* ID 912 excluded at 36-hour timepoint due to outlying data

Figure 21: Lignocaine N-oxide in tissues

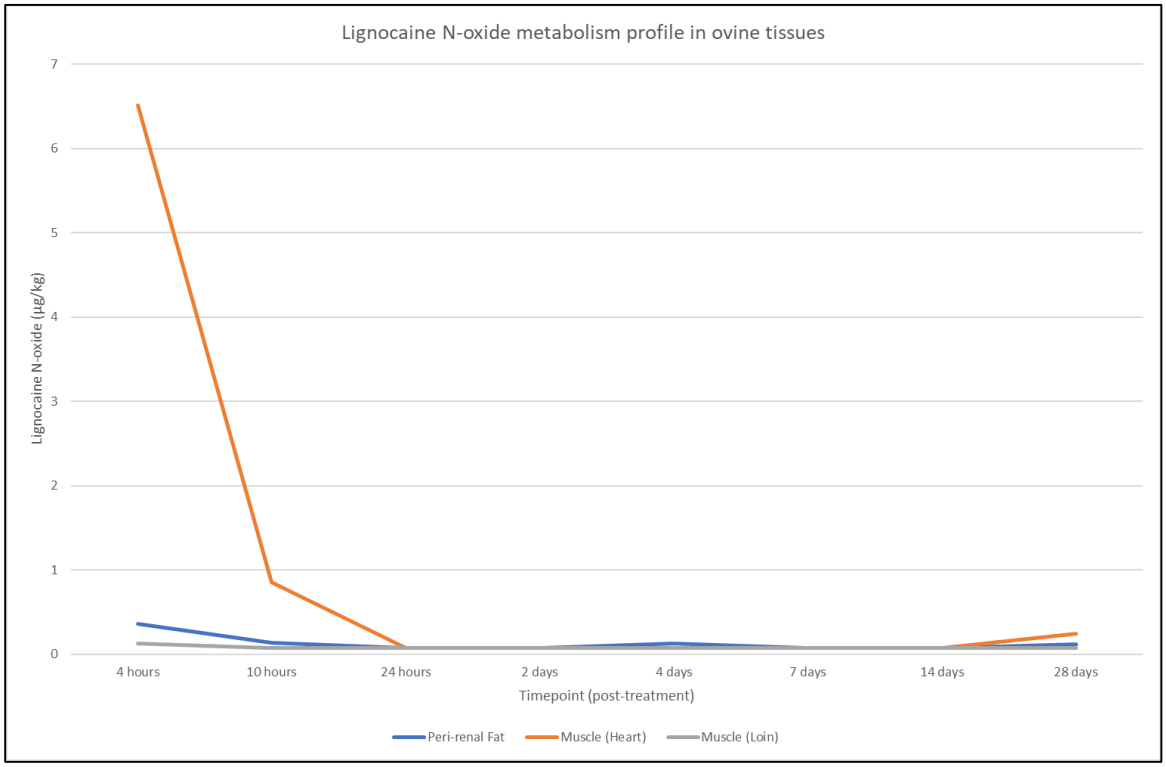


Figure 22: Lignocaine N-oxide in subcutaneous tissue at the site of mulesing

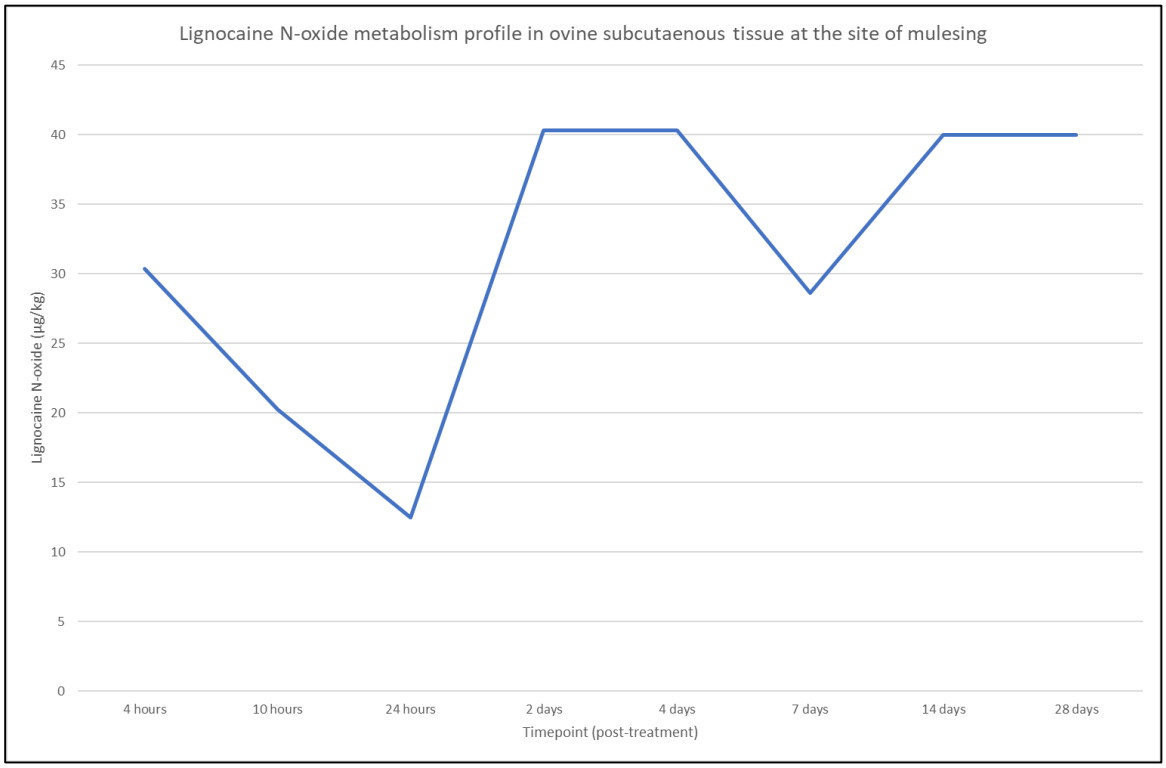


Figure 23: Lignocaine N-oxide in urine and faeces

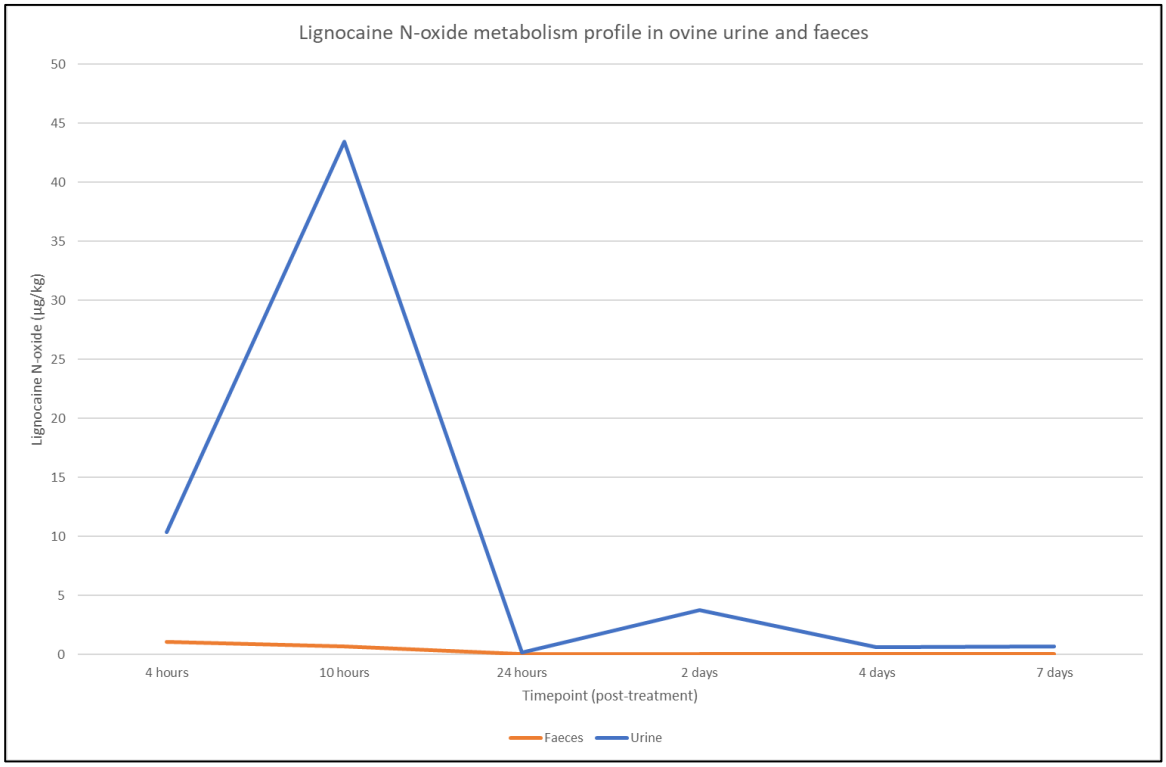
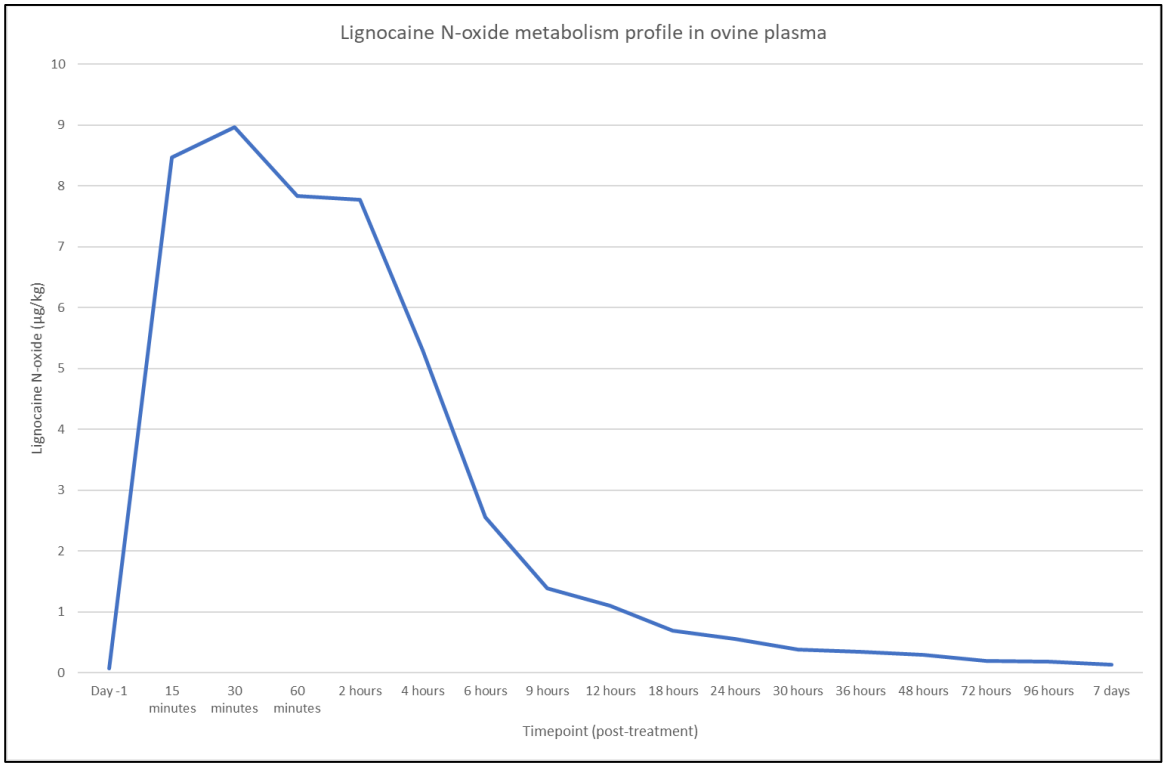


Figure 24: Lignocaine N-oxide in plasma*



* ID 912 excluded at 36-hour timepoint due to outlying data

Figure 25: 2,6 DMA in Tissues

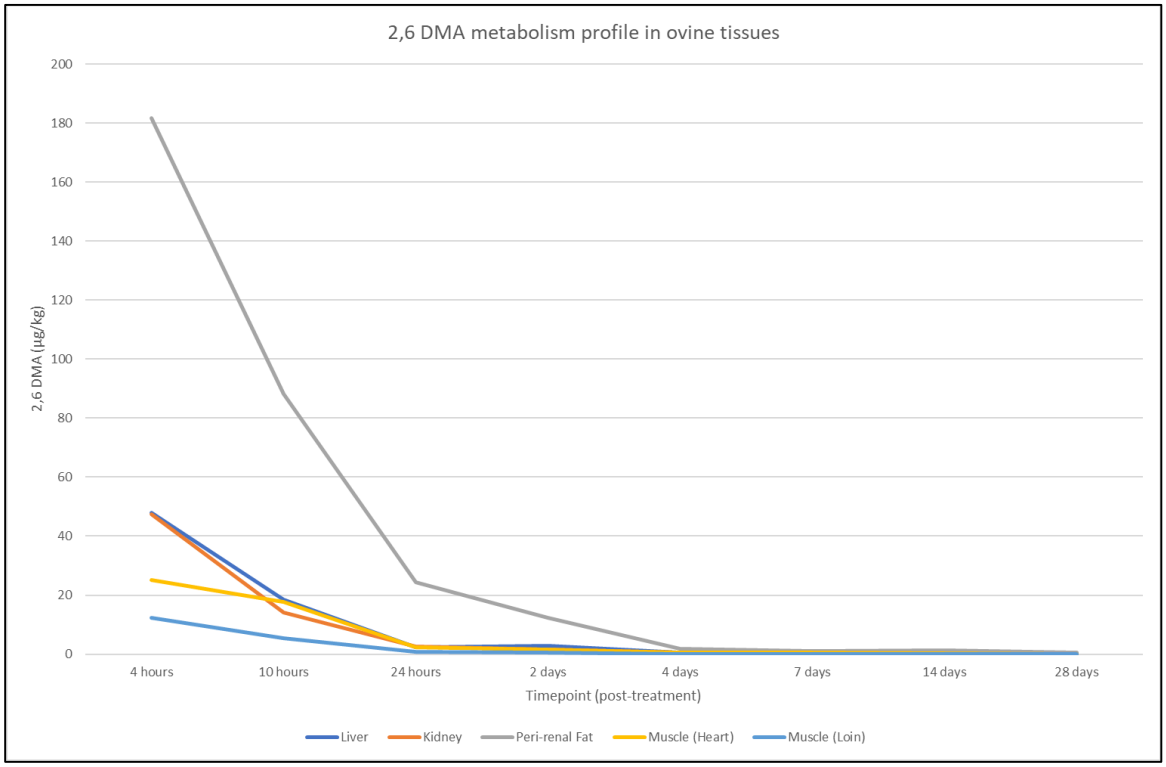


Figure 26: 2,6 DMA in subcutaneous tissue at the site of mulesing

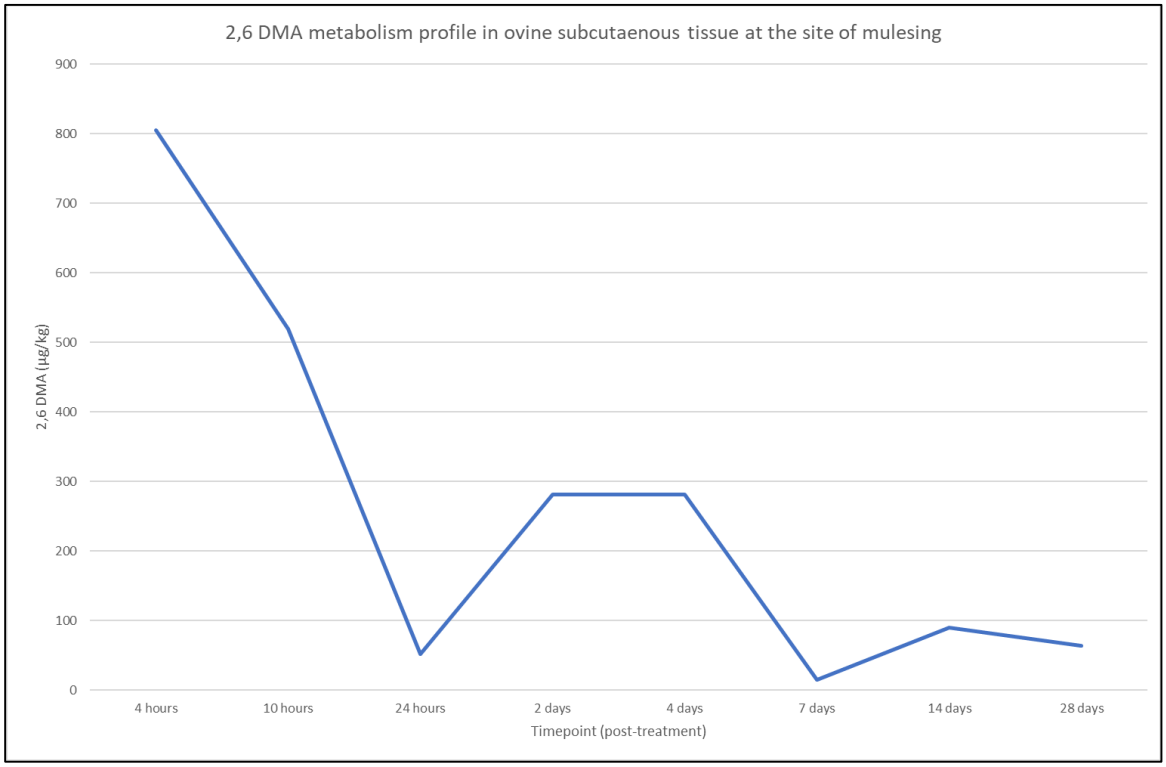
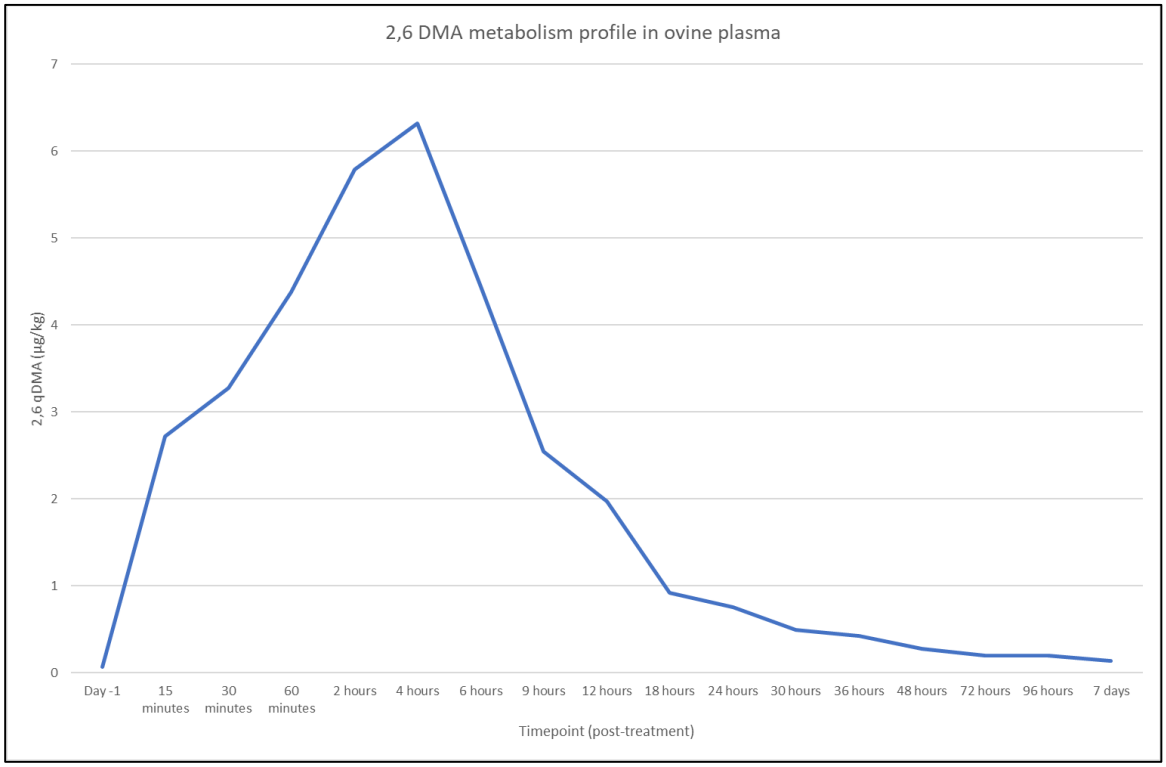


Figure 27: 2,6 DMA in urine and faeces

Figure 28: 2,6 DMA in plasma*



* ID 912 excluded at 36-hour timepoint due to outlying data

Figure 29: Bupivacaine in tissues

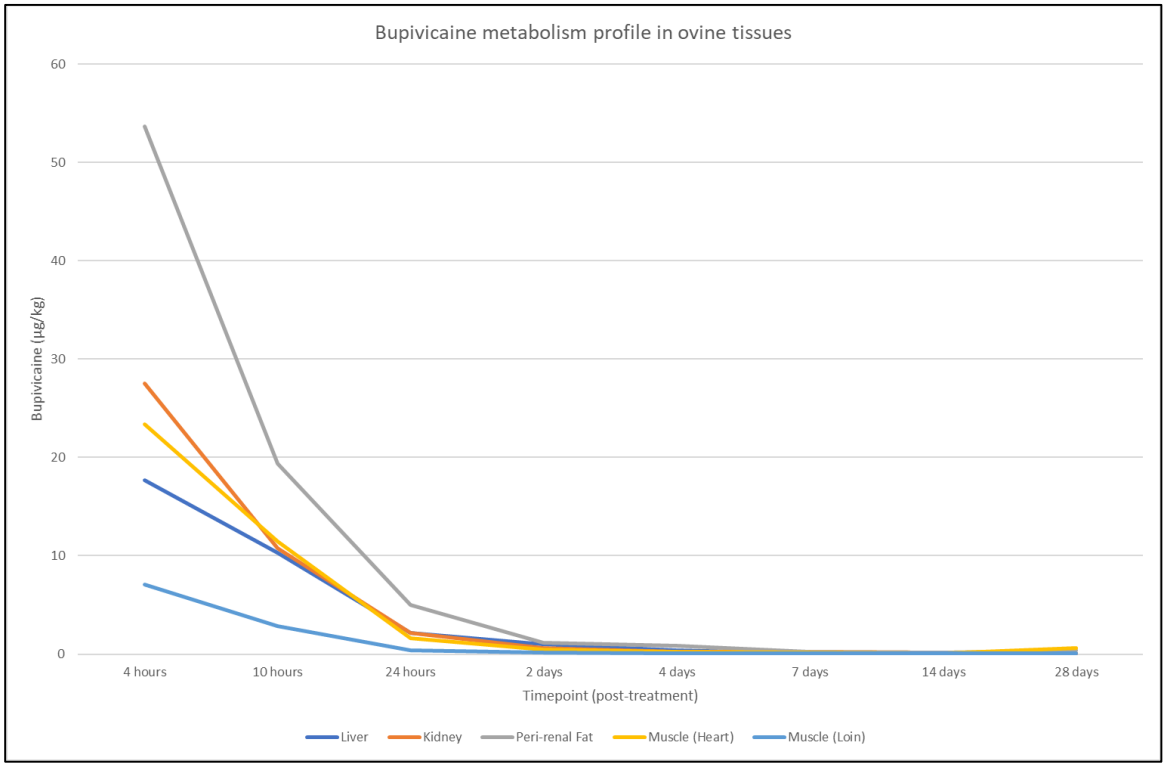


Figure 30: Bupivacaine in subcutaneous tissue at the site of mulesing

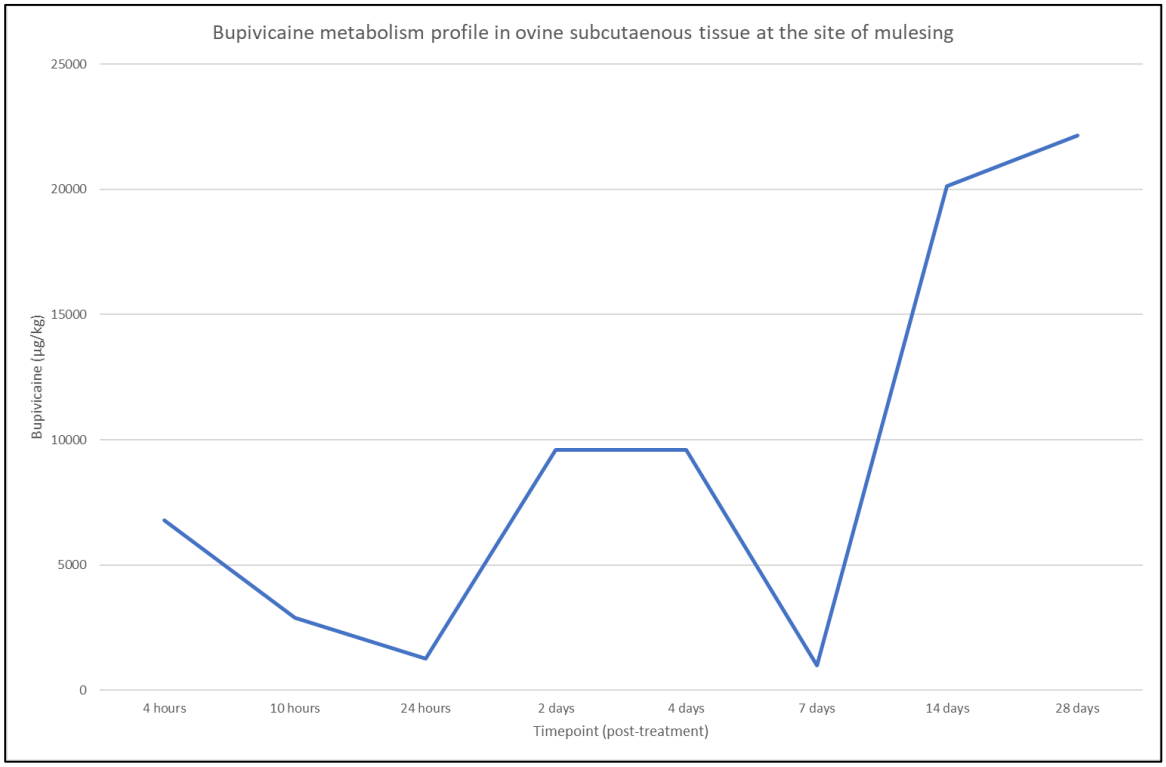


Figure 31: Bupivacaine in urine and faeces

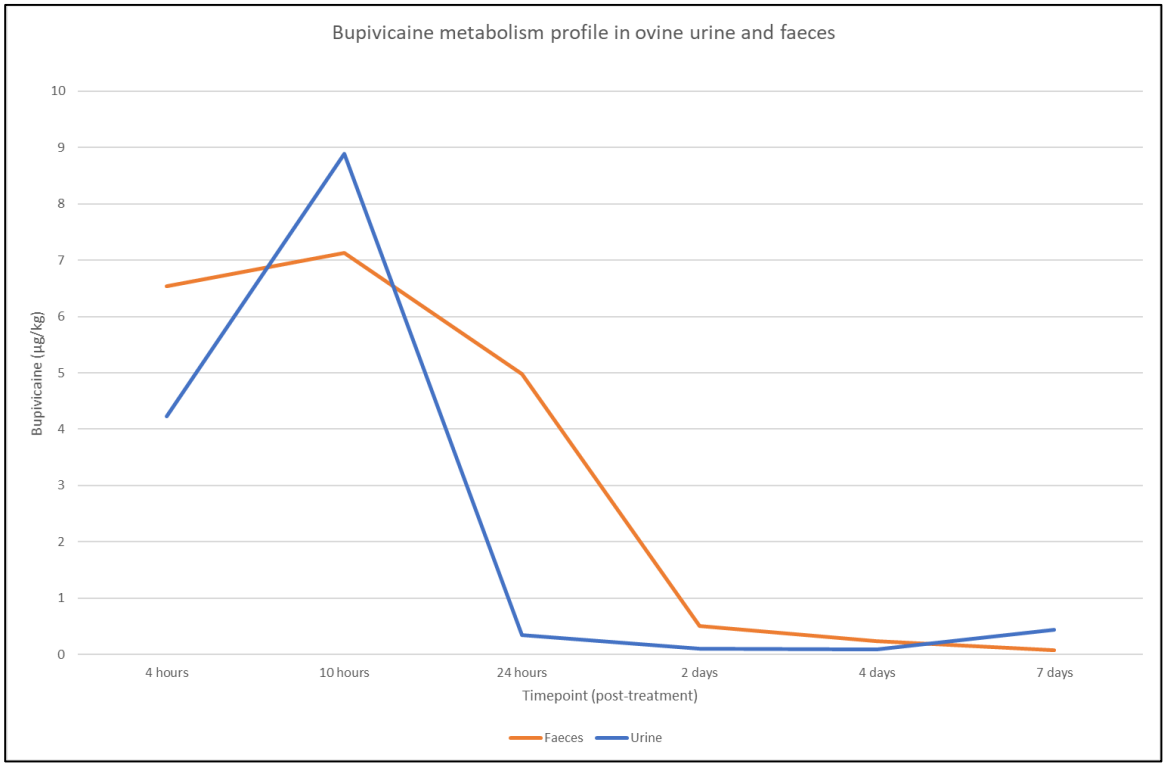
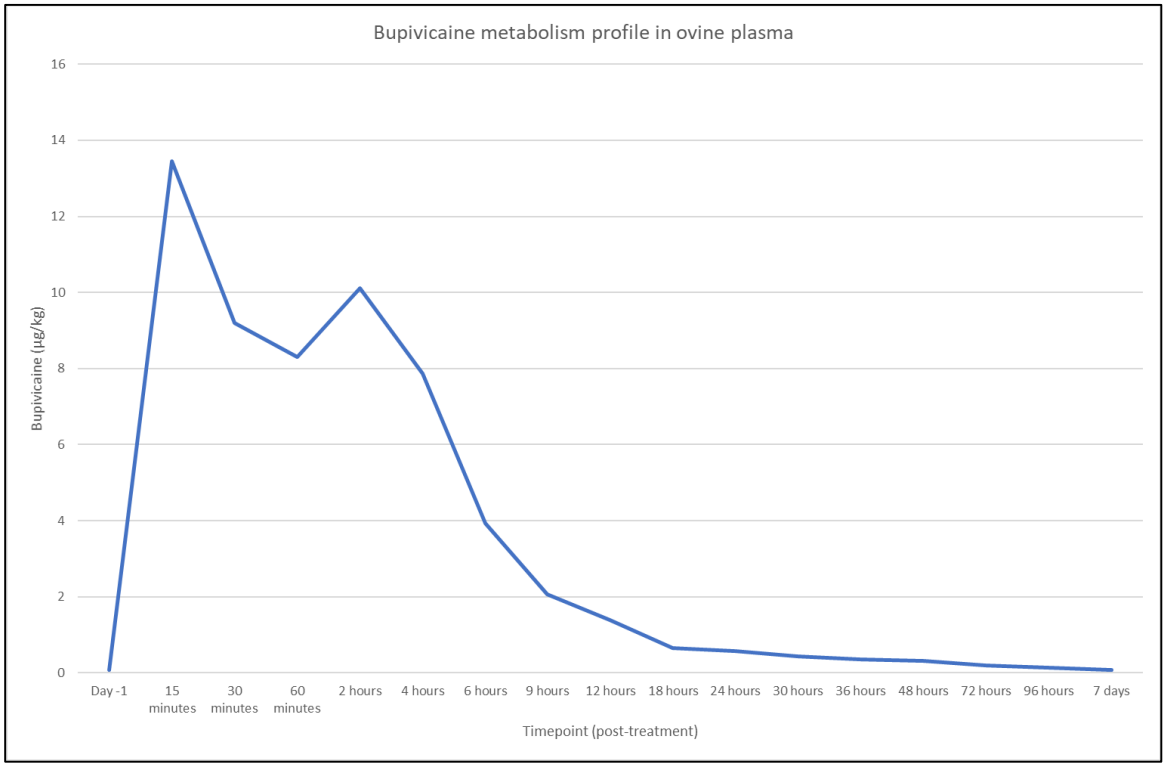


Figure 32: Bupivacaine in plasma*



* ID 912 excluded at 36-hour timepoint due to outlying data

Figure 33: 3-OH Bupivacaine in tissues

Figure 34: 3-OH Bupivacaine in subcutaneous tissues at the site of mulesing

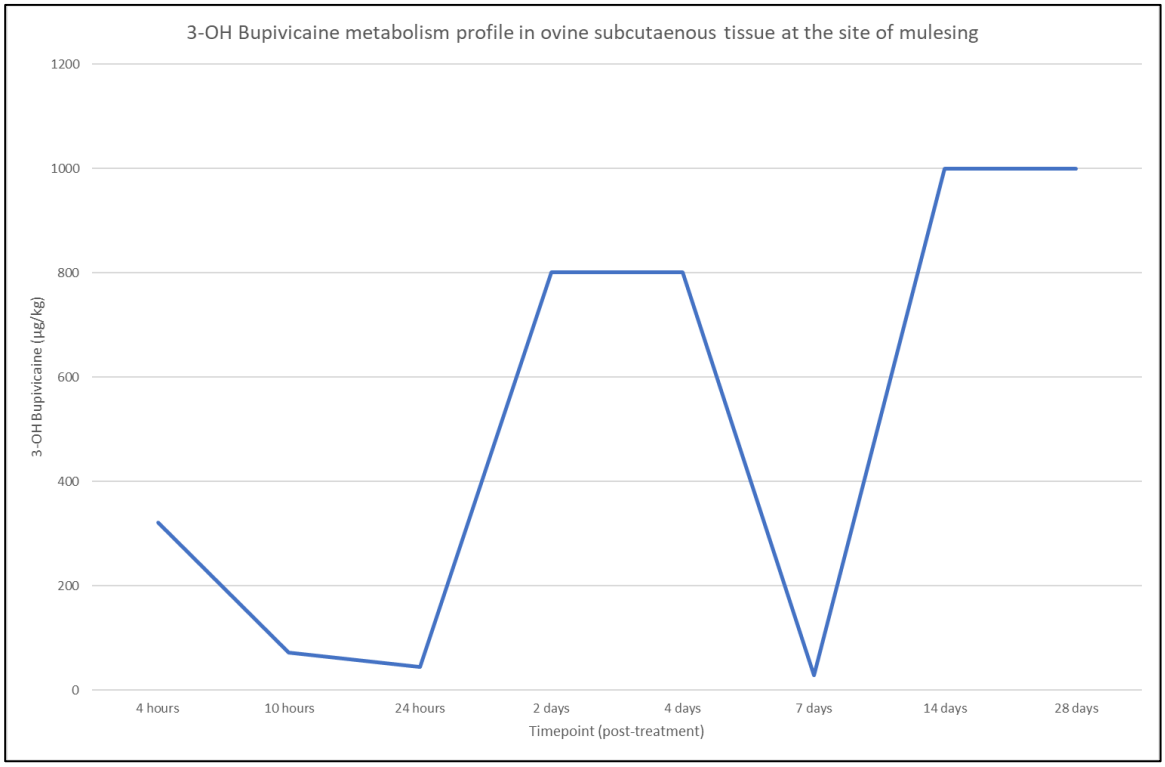


Figure 35: 3-OH Bupivacaine in urine and faeces

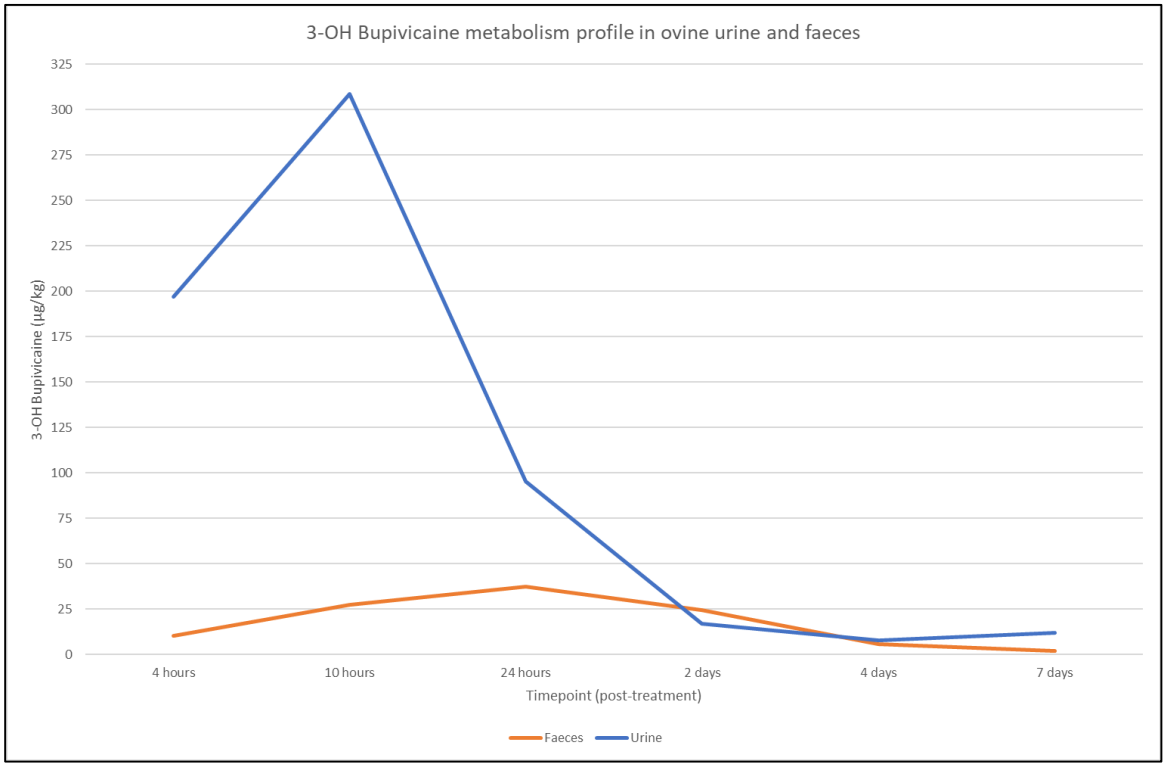
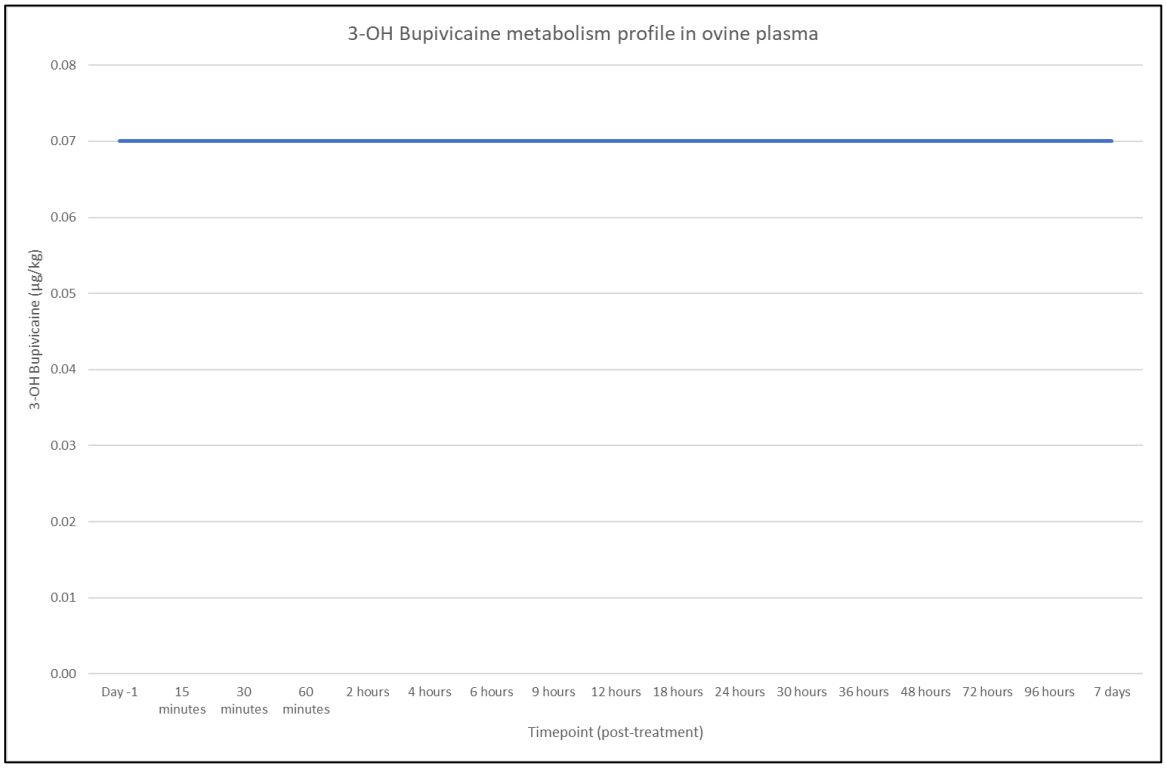


Figure 36: 3-OH Bupivacaine in urine and faeces



* ID 912 excluded at 36-hour timepoint due to outlying data

Figure 37: N-desbutyl bupivacaine in tissues

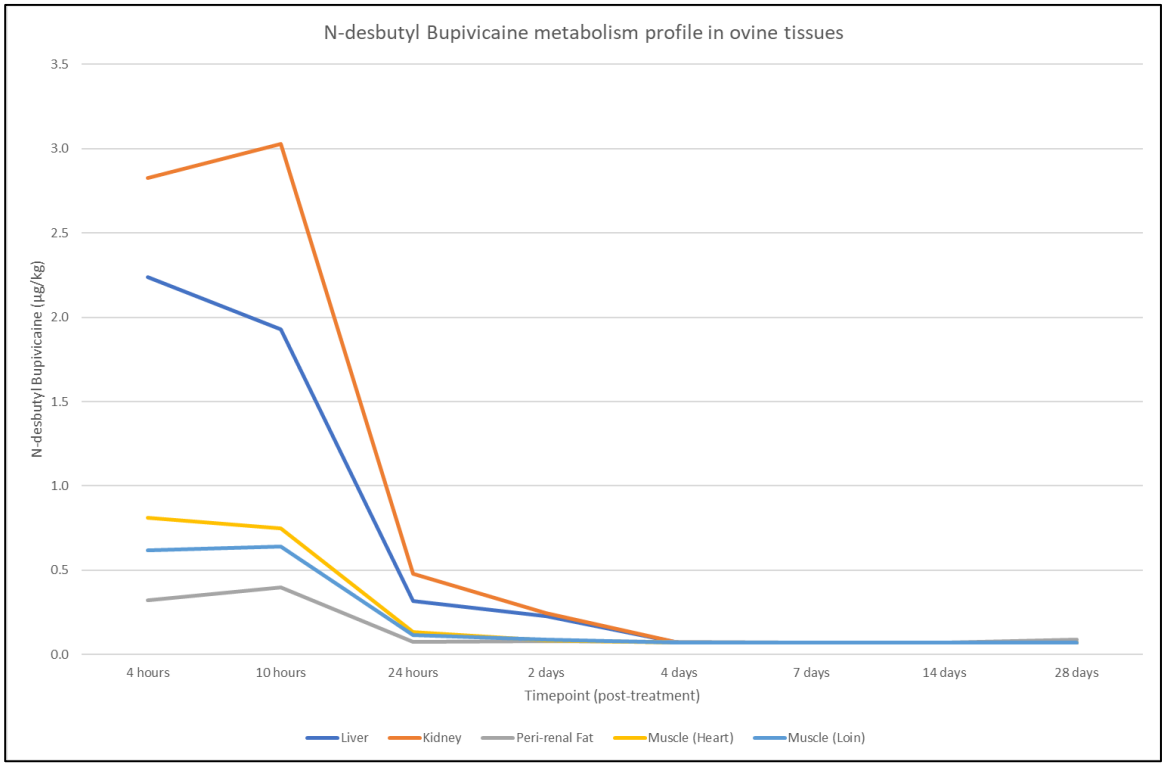


Figure 38: N-desbutyl bupivacaine in subcutaneous tissues at the site of mulesing

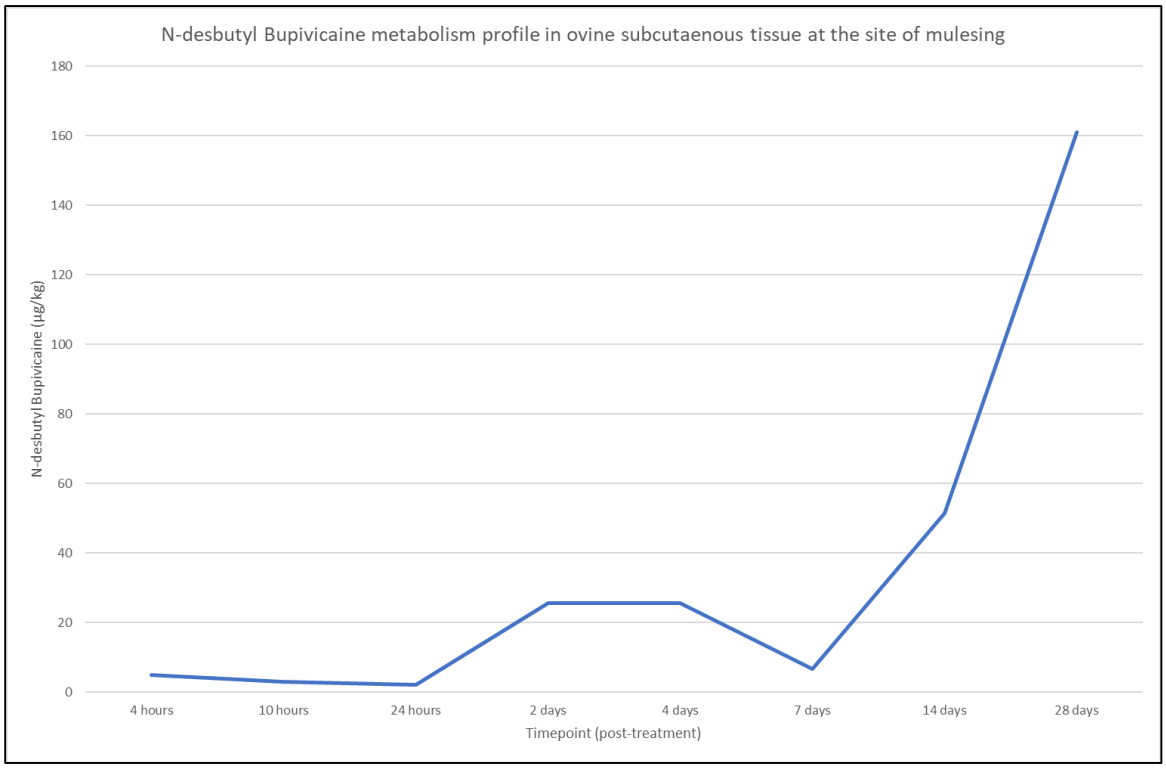


Figure 39: N-desbutyl bupivacaine in urine and faeces

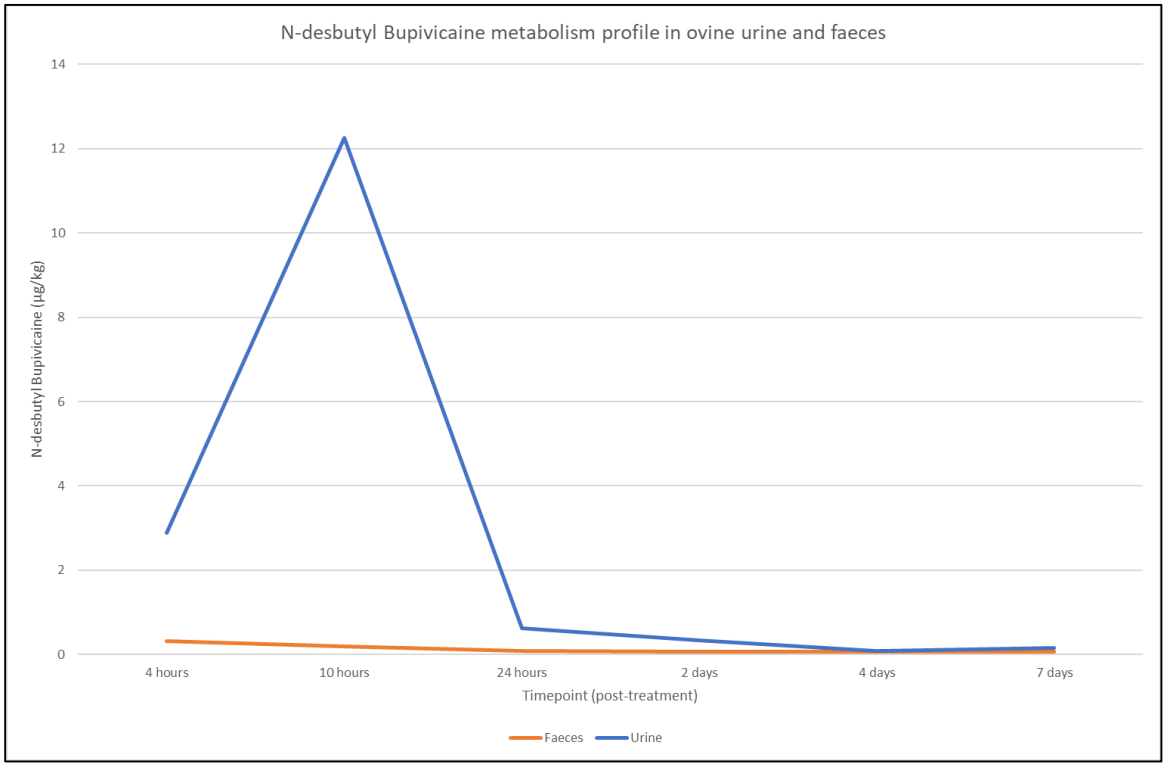
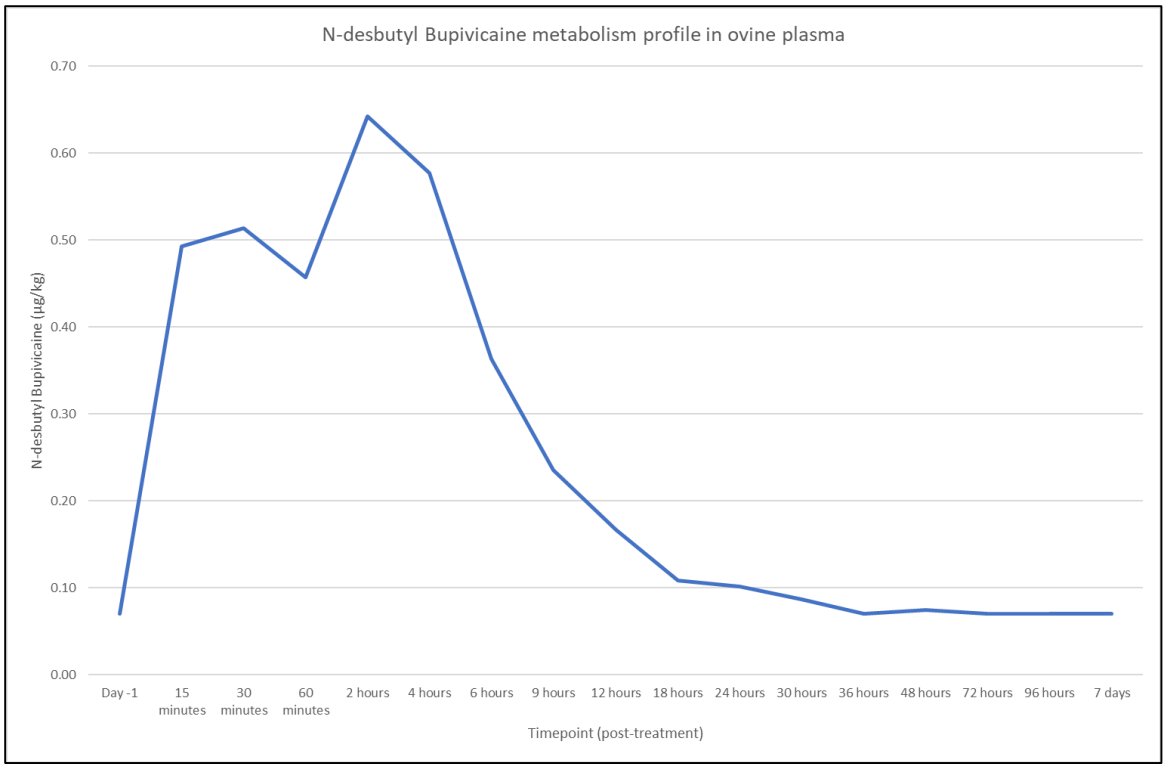


Figure 40: N-desbutyl bupivacaine in plasma*



* ID 912 excluded at 36-hour timepoint due to outlying data

7 Discussion

Lignocaine and its metabolites in MEGX, 3-OH Lignocaine, GX, Lignocaine N-oxide and 2,6-DMA; Bupivacaine and its metabolites 3-OH Bupivacaine and N-desbutyl Bupivacaine; and Cetrimide all reached quantifiable levels (except Lignocaine N-oxide in liver and kidney which was not measured) and had peaked by 10 hours post-treatment in liver, kidney, perirenal fat, muscle (heart and loin), urine and faeces that thus each active and its metabolite could potentially act as residue markers for future studies. Many of the metabolites as outlined in Table 4 had not reach < LOQ by the end of the study at 28 days post-treatment and this would need to be considered in the design of any further tissue residue studies.

The concentration of most of lignocaine (and its metabolites), bupivacaine (and its metabolites) and cetrimide at the treatment site (mules wound) increased through the study and was continuing to rise by the end of the study. This may be associated with concentrating residues associated with a contracting wound and potentially delayed and continued exposure via contaminated fleece.

All of lignocaine and its metabolites, Bupivacaine at its metabolites (except 3-OH Bupivacaine) and Cetrimide reached quantifiable levels in plasma and had peaked by 30-minute post-treatment. Quantifiable levels of various metabolites as indicated in Table 4 continued upon the last sampling timepoint at 7 days post-treatment, and this should be taken into account for any future metabolism work.

8 Impact of Wool Industry – Now & in 5 Years' Time

Mulesing, castration and tail-docking are routine management practices on many properties throughout Australia, aiding in the reduction of flystrike particularly in Merino sheep; however, cause acute pain and stress, resulting in normal behavioural changes following the procedure. Use of TRI-SOLFEN has shown to aid in the healing response and reduction in the pain response. New and additional requirements of the livestock industries required TRI-SOLFEN to be registered for use in mulesing practices.

There is minimal published information available on the pharmacokinetics of TRI-SOLFEN; however, the data obtained from this study has provided characterisation of the metabolism profiles for the individual component parent compounds and metabolites in sheep; determined the residue profiles for each parent compounds in sheep and mapped these profiles through a range of edible tissues and body fluids of sheep. These findings will contribute to the industry in the design and conduct of further pivotal tissue residue studies and guide selection of tissues for any residue monitoring programs that may need to be implemented. Furthermore, these results may assist in tissue residue and metabolism study design that may be required for the extension of the use pattern for TRI-SOLFEN (eg. shearing cuts, foot abscess).

9 Conclusions and Recommendations

This report describes the metabolism profiles of three active components of TRI-SOLFEN (cetrimide, lignocaine and bupivacaine) administered topically to sheep at castration and/or mulesing and/or tail-docking. Group mean data, raw data and metabolism profiles are presented for cetrimide, lignocaine and its metabolites and bupivacaine and its metabolites up to 28 days post-treatment. Data presented may be used to guide further studies in relation to edible tissue residues and compliance for the actives and their metabolites of TRI-SOLFEN applied topically to surgical wounds in lambs.

10 Bibliography

Lomax, S, Sheil, M & Windsor, PA (2008). Impact of topical anaesthesia on pain alleviation and wound healing in lambs after mulesing. Aust Vet J. 86(5); 159-68.

McLean, JG (2014). Use of Tri-Solfen in sheep and other food-producing animals. Aust Vet J. 92(12): N19-20.

11 List of Abbreviations and Glossary

DMA	Dimethylaniline
ESI	Export Slaughter Interval
g	Grams
GLP	Good Laboratory Practice
GX	Glycylxylidide
kg	Kilograms
L	Litres
LOD	Limit of Detection
LOQ	Limit of Quantification
MEGX	Monoethylglycinexylidide
m	Metre
mg	Milligrams
mL	Millilitres
NATA	National Association of Testing Authorities, Australia
OECD	Organisation for Economic Co-operation and Development
OH	Hydroxide
µg	Micrograms
VICH	Veterinary International Committee of Harmonisation
WHP	Withholding Period

12 Appendices

12.1 Appendix 1 – Cetrimide in Ovine Tissues, Urine, Faeces and Plasma

Table 6: Cetrimide in ovine tissues

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/kg)
902	Liver	Group 1	4 hours	9.59*
913	Liver	Group 1	4 hours	9.48*
915	Liver	Group 1	4 hours	6.99*
968	Liver	Group 1	4 hours	12.0*
908	Liver	Group 2	10 hours	<LOD
949	Liver	Group 2	10 hours	7.57*
962	Liver	Group 2	10 hours	6.26*
967	Liver	Group 2	10 hours	10.1*
906	Liver	Group 3	24 hours	3.08*
926	Liver	Group 3	24 hours	14.9*
930	Liver	Group 3	24 hours	5.06*
939	Liver	Group 3	24 hours	4.63*
927	Liver	Group 4	2 days	3.93*
959	Liver	Group 4	2 days	3.45*
963	Liver	Group 4	2 days	6.95*
965	Liver	Group 4	2 days	3.79*
919	Liver	Group 5	4 days	5.36*
932	Liver	Group 5	4 days	3.77*
936	Liver	Group 5	4 days	3.56*
947	Liver	Group 5	4 days	6.57*
934	Liver	Group 6	7 days	<LOD
942	Liver	Group 6	7 days	<LOD
952	Liver	Group 6	7 days	<LOD
964	Liver	Group 6	7 days	<LOD
901	Liver	Group 7	7 days	<LOD
917	Liver	Group 7	7 days	<LOD
950	Liver	Group 7	7 days	<LOD
953	Liver	Group 7	7 days	<LOD
903	Liver	Group 8	7 days	<LOD
912	Liver	Group 8	7 days	<LOD
938	Liver	Group 8	7 days	<LOD
941	Liver	Group 8	7 days	<LOD
904	Liver	Group 9	7 days	<LOD
916	Liver	Group 9	7 days	<LOD
928	Liver	Group 9	7 days	<LOD
960	Liver	Group 9	7 days	<LOD
924	Liver	Group 10	14 days	<LOD
944	Liver	Group 10	14 days	<LOD
954	Liver	Group 10	14 days	<LOD
958	Liver	Group 10	14 days	<LOD
910	Liver	Group 11	28 days	<LOD
935	Liver	Group 11	28 days	<LOD
937	Liver	Group 11	28 days	<LOD
961	Liver	Group 11	28 days	<LOD

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/kg)
902	Kidney	Group 1	4 hours	22.0
913	Kidney	Group 1	4 hours	80.8
915	Kidney	Group 1	4 hours	31.7
968	Kidney	Group 1	4 hours	66.3
908	Kidney	Group 2	10 hours	12.0
949	Kidney	Group 2	10 hours	68.3
962	Kidney	Group 2	10 hours	108
967	Kidney	Group 2	10 hours	25.2
906	Kidney	Group 3	24 hours	49.4
926	Kidney	Group 3	24 hours	31.2
930	Kidney	Group 3	24 hours	28.6
939	Kidney	Group 3	24 hours	35.3
927	Kidney	Group 4	2 days	12.0
959	Kidney	Group 4	2 days	17.2
963	Kidney	Group 4	2 days	6.76*
965	Kidney	Group 4	2 days	51.2
919	Kidney	Group 5	4 days	4.24*
932	Kidney	Group 5	4 days	4.72*
936	Kidney	Group 5	4 days	3.88*
947	Kidney	Group 5	4 days	11.0
934	Kidney	Group 6	7 days	<LOD
942	Kidney	Group 6	7 days	<LOD
952	Kidney	Group 6	7 days	<LOD
964	Kidney	Group 6	7 days	3.35*
901	Kidney	Group 7	7 days	<LOD
917	Kidney	Group 7	7 days	<LOD
950	Kidney	Group 7	7 days	<LOD
953	Kidney	Group 7	7 days	4.15*
903	Kidney	Group 8	7 days	3.64*
912	Kidney	Group 8	7 days	3.20*
938	Kidney	Group 8	7 days	3.24*
941	Kidney	Group 8	7 days	5.19*
904	Kidney	Group 9	7 days	<LOD
916	Kidney	Group 9	7 days	<LOD
928	Kidney	Group 9	7 days	<LOD
960	Kidney	Group 9	7 days	3.72*
924	Kidney	Group 10	14 days	<LOD
944	Kidney	Group 10	14 days	<LOD
954	Kidney	Group 10	14 days	<LOD
958	Kidney	Group 10	14 days	<LOD
910	Kidney	Group 11	28 days	<LOD
935	Kidney	Group 11	28 days	<LOD
937	Kidney	Group 11	28 days	<LOD
961	Kidney	Group 11	28 days	<LOD

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/kg)
902	Perirenal Fat	Group 1	4 hours	4.09*
913	Perirenal Fat	Group 1	4 hours	4.24*
915	Perirenal Fat	Group 1	4 hours	6.89*
968	Perirenal Fat	Group 1	4 hours	9.77*
908	Perirenal Fat	Group 2	10 hours	4.39*
949	Perirenal Fat	Group 2	10 hours	3.60*
962	Perirenal Fat	Group 2	10 hours	6.90*
967	Perirenal Fat	Group 2	10 hours	12.5
906	Perirenal Fat	Group 3	24 hours	<LOD
926	Perirenal Fat	Group 3	24 hours	11.4
930	Perirenal Fat	Group 3	24 hours	<LOD
939	Perirenal Fat	Group 3	24 hours	4.43*
927	Perirenal Fat	Group 4	2 days	<LOD
959	Perirenal Fat	Group 4	2 days	<LOD
963	Perirenal Fat	Group 4	2 days	7.20*
965	Perirenal Fat	Group 4	2 days	3.30*
919	Perirenal Fat	Group 5	4 days	10.6
932	Perirenal Fat	Group 5	4 days	6.63*
936	Perirenal Fat	Group 5	4 days	4.34*
947	Perirenal Fat	Group 5	4 days	<LOD
934	Perirenal Fat	Group 6	7 days	<LOD
942	Perirenal Fat	Group 6	7 days	<LOD
952	Perirenal Fat	Group 6	7 days	<LOD
964	Perirenal Fat	Group 6	7 days	<LOD
901	Perirenal Fat	Group 7	7 days	<LOD
917	Perirenal Fat	Group 7	7 days	<LOD
950	Perirenal Fat	Group 7	7 days	<LOD
953	Perirenal Fat	Group 7	7 days	<LOD
903	Perirenal Fat	Group 8	7 days	<LOD
912	Perirenal Fat	Group 8	7 days	5.55*
938	Perirenal Fat	Group 8	7 days	4.02*
941	Perirenal Fat	Group 8	7 days	3.57*
904	Perirenal Fat	Group 9	7 days	4.42*
916	Perirenal Fat	Group 9	7 days	<LOD
928	Perirenal Fat	Group 9	7 days	<LOD
960	Perirenal Fat	Group 9	7 days	<LOD
924	Perirenal Fat	Group 10	14 days	<LOD
944	Perirenal Fat	Group 10	14 days	<LOD
954	Perirenal Fat	Group 10	14 days	<LOD
958	Perirenal Fat	Group 10	14 days	7.07*
910	Perirenal Fat	Group 11	28 days	3.05*
935	Perirenal Fat	Group 11	28 days	<LOD
937	Perirenal Fat	Group 11	28 days	<LOD
961	Perirenal Fat	Group 11	28 days	<LOD

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/kg)
902	Mules	Group 1	4 hours	4177
913	Mules	Group 1	4 hours	3399
915	Mules	Group 1	4 hours	1449
968	Mules	Group 1	4 hours	16227
908	Mules	Group 2	10 hours	3063
949	Mules	Group 2	10 hours	3695
962	Mules	Group 2	10 hours	3449
967	Mules	Group 2	10 hours	1173
906	Mules	Group 3	24 hours	1663
926	Mules	Group 3	24 hours	997
930	Mules	Group 3	24 hours	7035
939	Mules	Group 3	24 hours	801
927	Mules	Group 4	2 days	681
959	Mules	Group 4	2 days	4778
963	Mules	Group 4	2 days	14177
965	Mules	Group 4	2 days	3422
919	Mules	Group 5	4 days	4875
932	Mules	Group 5	4 days	22237
936	Mules	Group 5	4 days	13488
947	Mules	Group 5	4 days	8725
934	Mules	Group 6	7 days	14763
942	Mules	Group 6	7 days	3586
952	Mules	Group 6	7 days	11900
964	Mules	Group 6	7 days	6472
901	Mules	Group 7	7 days	9890
917	Mules	Group 7	7 days	4564
950	Mules	Group 7	7 days	9270
953	Mules	Group 7	7 days	3932
903	Mules	Group 8	7 days	2429
912	Mules	Group 8	7 days	468
938	Mules	Group 8	7 days	1095
941	Mules	Group 8	7 days	1545
904	Mules	Group 9	7 days	354
916	Mules	Group 9	7 days	263
928	Mules	Group 9	7 days	50.6
960	Mules	Group 9	7 days	75.7
924	Mules	Group 10	14 days	11043
944	Mules	Group 10	14 days	28330
954	Mules	Group 10	14 days	11420
958	Mules	Group 10	14 days	44455
910	Mules	Group 11	28 days	47302
935	Mules	Group 11	28 days	13413
937	Mules	Group 11	28 days	26458
961	Mules	Group 11	28 days	29844

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/kg)
902	Muscle (Heart)	Group 1	4 hours	18.0
913	Muscle (Heart)	Group 1	4 hours	53.5
915	Muscle (Heart)	Group 1	4 hours	25.8
968	Muscle (Heart)	Group 1	4 hours	48.0
908	Muscle (Heart)	Group 2	10 hours	25.4
949	Muscle (Heart)	Group 2	10 hours	35.5
962	Muscle (Heart)	Group 2	10 hours	47.6
967	Muscle (Heart)	Group 2	10 hours	64.3
906	Muscle (Heart)	Group 3	24 hours	32.4
926	Muscle (Heart)	Group 3	24 hours	90.8
930	Muscle (Heart)	Group 3	24 hours	54.9
939	Muscle (Heart)	Group 3	24 hours	51.1
927	Muscle (Heart)	Group 4	2 days	37.6
959	Muscle (Heart)	Group 4	2 days	17.2
963	Muscle (Heart)	Group 4	2 days	34.8
965	Muscle (Heart)	Group 4	2 days	45.5
919	Muscle (Heart)	Group 5	4 days	14.9
932	Muscle (Heart)	Group 5	4 days	26.2
936	Muscle (Heart)	Group 5	4 days	18.2
947	Muscle (Heart)	Group 5	4 days	49.8
934	Muscle (Heart)	Group 6	7 days	<LOD
942	Muscle (Heart)	Group 6	7 days	<LOD
952	Muscle (Heart)	Group 6	7 days	<LOD
964	Muscle (Heart)	Group 6	7 days	<LOD
901	Muscle (Heart)	Group 7	7 days	<LOD
917	Muscle (Heart)	Group 7	7 days	<LOD
950	Muscle (Heart)	Group 7	7 days	<LOD
953	Muscle (Heart)	Group 7	7 days	7.23*
903	Muscle (Heart)	Group 8	7 days	9.95*
912	Muscle (Heart)	Group 8	7 days	5.58*
938	Muscle (Heart)	Group 8	7 days	4.75*
941	Muscle (Heart)	Group 8	7 days	14.2
904	Muscle (Heart)	Group 9	7 days	6.73*
916	Muscle (Heart)	Group 9	7 days	<LOD
928	Muscle (Heart)	Group 9	7 days	5.44*
960	Muscle (Heart)	Group 9	7 days	7.69*
924	Muscle (Heart)	Group 10	14 days	<LOD
944	Muscle (Heart)	Group 10	14 days	<LOD
954	Muscle (Heart)	Group 10	14 days	<LOD
958	Muscle (Heart)	Group 10	14 days	<LOD
910	Muscle (Heart)	Group 11	28 days	<LOD
935	Muscle (Heart)	Group 11	28 days	<LOD
937	Muscle (Heart)	Group 11	28 days	<LOD
961	Muscle (Heart)	Group 11	28 days	<LOD

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/L)
902	Muscle (Loin)	Group 1	4 hours	3.35*
913	Muscle (Loin)	Group 1	4 hours	12.3
915	Muscle (Loin)	Group 1	4 hours	6.59*
968	Muscle (Loin)	Group 1	4 hours	11.1
908	Muscle (Loin)	Group 2	10 hours	5.65*
949	Muscle (Loin)	Group 2	10 hours	12.4
962	Muscle (Loin)	Group 2	10 hours	12.6
967	Muscle (Loin)	Group 2	10 hours	18.9
906	Muscle (Loin)	Group 3	24 hours	14.4
926	Muscle (Loin)	Group 3	24 hours	45.4
930	Muscle (Loin)	Group 3	24 hours	15.1
939	Muscle (Loin)	Group 3	24 hours	12.7
927	Muscle (Loin)	Group 4	2 days	14.7
959	Muscle (Loin)	Group 4	2 days	12.8
963	Muscle (Loin)	Group 4	2 days	14.6
965	Muscle (Loin)	Group 4	2 days	19.0
919	Muscle (Loin)	Group 5	4 days	15.6
932	Muscle (Loin)	Group 5	4 days	25.3
936	Muscle (Loin)	Group 5	4 days	21.3
947	Muscle (Loin)	Group 5	4 days	27.9
934	Muscle (Loin)	Group 6	7 days	4.33*
942	Muscle (Loin)	Group 6	7 days	<LOD
952	Muscle (Loin)	Group 6	7 days	<LOD
964	Muscle (Loin)	Group 6	7 days	3.05*
901	Muscle (Loin)	Group 7	7 days	<LOD
917	Muscle (Loin)	Group 7	7 days	<LOD
950	Muscle (Loin)	Group 7	7 days	3.16*
953	Muscle (Loin)	Group 7	7 days	6.05*
903	Muscle (Loin)	Group 8	7 days	10.6
912	Muscle (Loin)	Group 8	7 days	10.8
938	Muscle (Loin)	Group 8	7 days	8.17*
941	Muscle (Loin)	Group 8	7 days	11.5
904	Muscle (Loin)	Group 9	7 days	13.8
916	Muscle (Loin)	Group 9	7 days	<LOD
928	Muscle (Loin)	Group 9	7 days	8.84*
960	Muscle (Loin)	Group 9	7 days	8.86*
924	Muscle (Loin)	Group 10	14 days	3.93*
944	Muscle (Loin)	Group 10	14 days	6.38*
954	Muscle (Loin)	Group 10	14 days	3.45*
958	Muscle (Loin)	Group 10	14 days	<LOD
910	Muscle (Loin)	Group 11	28 days	<LOD
935	Muscle (Loin)	Group 11	28 days	<LOD
937	Muscle (Loin)	Group 11	28 days	<LOD
961	Muscle (Loin)	Group 11	28 days	<LOD
902	Urine	Group 1	4 hours	<LOD
913	Urine	Group 1	4 hours	<LOD
915	Urine	Group 1	4 hours	<LOD
968	Urine	Group 1	4 hours	<LOD
908	Urine	Group 2	10 hours	<LOD
949	Urine	Group 2	10 hours	<LOD

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/L)
962	Urine	Group 2	10 hours	<LOD
967	Urine	Group 2	10 hours	<LOD
906	Urine	Group 3	24 hours	<LOD
926	Urine	Group 3	24 hours	<LOD
930	Urine	Group 3	24 hours	<LOD
939	Urine	Group 3	24 hours	<LOD
927	Urine	Group 4	2 days	<LOD
959	Urine	Group 4	2 days	NR
963	Urine	Group 4	2 days	<LOD
965	Urine	Group 4	2 days	<LOD
919	Urine	Group 5	4 days	<LOD
932	Urine	Group 5	4 days	NR
936	Urine	Group 5	4 days	<LOD
947	Urine	Group 5	4 days	<LOD
934	Urine	Group 6	7 days	<LOD
942	Urine	Group 6	7 days	<LOD
952	Urine	Group 6	7 days	NR
964	Urine	Group 6	7 days	NR
901	Urine	Group 7	7 days	<LOD
917	Urine	Group 7	7 days	<LOD
950	Urine	Group 7	7 days	<LOD
953	Urine	Group 7	7 days	<LOD
903	Urine	Group 8	7 days	<LOD
912	Urine	Group 8	7 days	<LOD
938	Urine	Group 8	7 days	<LOD
941	Urine	Group 8	7 days	<LOD
904	Urine	Group 9	7 days	<LOD
916	Urine	Group 9	7 days	NR
928	Urine	Group 9	7 days	<LOD
960	Urine	Group 9	7 days	<LOD
902	Faeces	Group 1	4 hours	3.82*
913	Faeces	Group 1	4 hours	3.39*
915	Faeces	Group 1	4 hours	<LOD
968	Faeces	Group 1	4 hours	<LOD
908	Faeces	Group 2	10 hours	<LOD
949	Faeces	Group 2	10 hours	13.2*
962	Faeces	Group 2	10 hours	5.75*
967	Faeces	Group 2	10 hours	18.8*
906	Faeces	Group 3	24 hours	11.6*
926	Faeces	Group 3	24 hours	20.6*
930	Faeces	Group 3	24 hours	17.2*
939	Faeces	Group 3	24 hours	15.0*
927	Faeces	Group 4	2 days	18.7*
959	Faeces	Group 4	2 days	17.1*
963	Faeces	Group 4	2 days	14.5*
965	Faeces	Group 4	2 days	17.9*
919	Faeces	Group 5	4 days	26.8
932	Faeces	Group 5	4 days	22.6
936	Faeces	Group 5	4 days	21.2*
947	Faeces	Group 5	4 days	24.5
934	Faeces	Group 6	7 days	19.9*
942	Faeces	Group 6	7 days	13.3*

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/L)
952	Faeces	Group 6	7 days	10.1*
964	Faeces	Group 6	7 days	12.0*
901	Faeces	Group 7	7 days	10.3*
917	Faeces	Group 7	7 days	14.2*
950	Faeces	Group 7	7 days	26.7
953	Faeces	Group 7	7 days	19.8*
903	Faeces	Group 8	7 days	19.3*
912	Faeces	Group 8	7 days	9.62*
938	Faeces	Group 8	7 days	5.14*
941	Faeces	Group 8	7 days	11.4*
904	Faeces	Group 9	7 days	26.6
916	Faeces	Group 9	7 days	16.7*
928	Faeces	Group 9	7 days	44.0
960	Faeces	Group 9	7 days	16.1*
903	Plasma	Group 8	D-1	<LOD
912	Plasma	Group 8	D-1	<LOD
938	Plasma	Group 8	D-1	<LOD
941	Plasma	Group 8	D-1	3.76*
903	Plasma	Group 8	15 minutes	3.42*
912	Plasma	Group 8	15 minutes	8.40*
938	Plasma	Group 8	15 minutes	7.66*
941	Plasma	Group 8	15 minutes	7.03*
903	Plasma	Group 8	30 minutes	3.39*
912	Plasma	Group 8	30 minutes	6.47*
938	Plasma	Group 8	30 minutes	3.27*
941	Plasma	Group 8	30 minutes	3.85*
903	Plasma	Group 8	60 minutes	3.28*
912	Plasma	Group 8	60 minutes	4.98*
938	Plasma	Group 8	60 minutes	5.44*
941	Plasma	Group 8	60 minutes	4.55*
903	Plasma	Group 8	2 hours	<LOD
912	Plasma	Group 8	2 hours	5.54*
938	Plasma	Group 8	2 hours	3.23*
941	Plasma	Group 8	2 hours	4.37*
903	Plasma	Group 8	4 hours	<LOD
912	Plasma	Group 8	4 hours	3.74*
938	Plasma	Group 8	4 hours	<LOD
941	Plasma	Group 8	4 hours	5.08*
903	Plasma	Group 8	6 hours	3.23*
912	Plasma	Group 8	6 hours	<LOD
938	Plasma	Group 8	6 hours	<LOD
941	Plasma	Group 8	6 hours	3.09*
903	Plasma	Group 8	9 hours	<LOD
912	Plasma	Group 8	9 hours	3.02*
938	Plasma	Group 8	9 hours	<LOD
941	Plasma	Group 8	9 hours	<LOD
903	Plasma	Group 8	12 hours	<LOD
912	Plasma	Group 8	12 hours	<LOD
938	Plasma	Group 8	12 hours	<LOD
941	Plasma	Group 8	12 hours	<LOD
903	Plasma	Group 8	18 hours	<LOD
912	Plasma	Group 8	18 hours	<LOD

Animal ID	Tissue	Group	Timepoint	Cetrimide (µg/L)
938	Plasma	Group 8	18 hours	<LOD
941	Plasma	Group 8	18 hours	<LOD
903	Plasma	Group 8	24 hours	<LOD
912	Plasma	Group 8	24 hours	<LOD
938	Plasma	Group 8	24 hours	<LOD
941	Plasma	Group 8	24 hours	<LOD
903	Plasma	Group 8	30 hours	3.63*
912	Plasma	Group 8	30 hours	4.30*
938	Plasma	Group 8	30 hours	<LOD
941	Plasma	Group 8	30 hours	<LOD
903	Plasma	Group 8	36 hours	<LOD
912	Plasma	Group 8	36 hours	67.9
938	Plasma	Group 8	36 hours	<LOD
941	Plasma	Group 8	36 hours	<LOD
903	Plasma	Group 8	48 hours	<LOD
912	Plasma	Group 8	48 hours	<LOD
938	Plasma	Group 8	48 hours	<LOD
941	Plasma	Group 8	48 hours	<LOD
903	Plasma	Group 8	72 hours	<LOD
912	Plasma	Group 8	72 hours	<LOD
938	Plasma	Group 8	72 hours	<LOD
941	Plasma	Group 8	72 hours	<LOD
903	Plasma	Group 8	96 hours	<LOD
912	Plasma	Group 8	96 hours	<LOD
938	Plasma	Group 8	96 hours	<LOD
941	Plasma	Group 8	96 hours	<LOD
903	Plasma	Group 8	7 days	<LOD
912	Plasma	Group 8	7 days	<LOD
938	Plasma	Group 8	7 days	<LOD
941	Plasma	Group 8	7 days	<LOD

For Cetrimide LOD = 3 µg/kg (or L for plasma and urine); LOQ = 10 µg/kg (or L for plasma and urine) (except liver: 15 µg/kg and faeces: 22 µg/kg); Grp = Group; IS = Insufficient Sample; NR = Not Reported; *Value between LOD and LOQ.

12.2 Appendix 2 – Lignocaine and Metabolites in Ovine Tissues, Urine, Faeces and Plasma

Table 7: Lignocaine and Metabolites in Ovine Tissues, Urine, Faeces and Plasma

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
902	Liver	Group 1	4 hours	13.8	18.2	39.7	63.6	NR	64.0
913	Liver	Group 1	4 hours	19.6	19.2	32.8	19.4	NR	19.9
915	Liver	Group 1	4 hours	28.7	34.6	46.6	24.9	NR	45.8
968	Liver	Group 1	4 hours	10.7	10.8	48.2	38.1	NR	61.9
908	Liver	Group 2	10 hours	4.06	3.81	11.9	18.9	NR	7.77
949	Liver	Group 2	10 hours	16.6	21.1	35.5	42.8	NR	9.60
962	Liver	Group 2	10 hours	9.11	13.3	82.7	83.3	NR	39.6
967	Liver	Group 2	10 hours	36.4	14.6	50.8	74.8	NR	16.6
906	Liver	Group 3	24 hours	1.42	1.16	2.58	9.94	NR	2.95
926	Liver	Group 3	24 hours	1.68	1.37	4.04	14.8	NR	3.06
930	Liver	Group 3	24 hours	0.86	0.901	2.48	10.4	NR	1.22
939	Liver	Group 3	24 hours	0.63	0.929	5.03	18.9	NR	1.90
927	Liver	Group 4	2 days	1.20	0.842	1.51	3.64	NR	0.950
959	Liver	Group 4	2 days	0.964	0.585	1.55	1.43	NR	1.86
963	Liver	Group 4	2 days	3.21	4.92	2.76	20.5	NR	7.78
965	Liver	Group 4	2 days	3.98	1.76	3.99	2.76	NR	1.19
919	Liver	Group 5	4 days	0.674	0.654	1.41	7.85	NR	0.511
932	Liver	Group 5	4 days	0.377	0.349	1.15	2.70	NR	0.461
936	Liver	Group 5	4 days	0.855	1.31	1.67	6.10	NR	0.658
947	Liver	Group 5	4 days	0.914	1.30	0.848	3.87	NR	0.556
934	Liver	Group 6	7 days	0.288	0.138*	0.396	0.549	NR	0.248
942	Liver	Group 6	7 days	0.283	0.169*	0.412	0.392	NR	0.189*
952	Liver	Group 6	7 days	0.115*	0.082*	0.251	0.631	NR	0.199*
964	Liver	Group 6	7 days	0.157*	0.094*	0.487	0.748	NR	0.499
901	Liver	Group 7	7 days	0.220	0.233	1.01	2.75	NR	0.447
917	Liver	Group 7	7 days	0.220	0.097*	0.136*	0.571	NR	0.187*
950	Liver	Group 7	7 days	0.147*	0.119*	0.238	0.464	NR	0.292

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
953	Liver	Group 7	7 days	0.201	0.194*	0.181*	0.987	NR	0.129*
903	Liver	Group 8	7 days	0.559	0.378	3.46	4.73	NR	0.470
912	Liver	Group 8	7 days	0.310	0.355	0.628	3.62	NR	0.777
938	Liver	Group 8	7 days	0.720	0.968	0.893	3.49	NR	0.546
941	Liver	Group 8	7 days	0.430	0.416	0.993	2.88	NR	0.220
904	Liver	Group 9	7 days	0.468	0.546	1.66	6.70	NR	1.11
916	Liver	Group 9	7 days	0.195*	0.277	0.223	1.21	NR	0.305
928	Liver	Group 9	7 days	1.02	0.232	1.30	1.22	NR	0.747
960	Liver	Group 9	7 days	0.329	0.152*	0.459	0.776	NR	<LOD
924	Liver	Group 10	14 days	1.34	0.540	0.967	3.31	NR	0.748
944	Liver	Group 10	14 days	0.473	0.122*	0.428	0.523	NR	0.472
954	Liver	Group 10	14 days	0.284	0.116*	0.723	0.372	NR	0.371
958	Liver	Group 10	14 days	0.278	0.109*	0.806	0.358	NR	0.388
910	Liver	Group 11	28 days	0.114*	<LOD	0.188*	0.887	NR	<LOD
935	Liver	Group 11	28 days	<LOD	<LOD	0.128*	0.196*	NR	<LOD
937	Liver	Group 11	28 days	0.125*	<LOD	0.253	0.166*	NR	<LOD
961	Liver	Group 11	28 days	0.124*	0.087*	0.255	0.615	NR	0.377
902	Kidney	Group 1	4 hours	138	157	77.6	93.8	NR	21.1
913	Kidney	Group 1	4 hours	280	85.8	74.3	27.2	NR	56.3
915	Kidney	Group 1	4 hours	375	141	149	34.4	NR	56.7
968	Kidney	Group 1	4 hours	220	96.5	126	57.3	NR	56.2
908	Kidney	Group 2	10 hours	35.8	28.2	34.4	33.0	NR	14.9
949	Kidney	Group 2	10 hours	68.8	74.4	68.9	81.3	NR	8.54
962	Kidney	Group 2	10 hours	236	117	183	151	NR	29.7
967	Kidney	Group 2	10 hours	31.3	29.6	69.2	126	NR	3.47
906	Kidney	Group 3	24 hours	18.3	9.96	9.46	11.8	NR	4.10
926	Kidney	Group 3	24 hours	15.7	6.51	10.6	18.4	NR	1.84
930	Kidney	Group 3	24 hours	7.07	4.62	5.85	11.8	NR	1.89
939	Kidney	Group 3	24 hours	8.17	3.96	13.4	15.6	NR	2.19
927	Kidney	Group 4	2 days	3.66	3.49	5.71	3.59	NR	0.365
959	Kidney	Group 4	2 days	3.47	2.14	3.28	1.35	NR	0.821
963	Kidney	Group 4	2 days	8.67	21.8	3.79	55.7	NR	3.23

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
965	Kidney	Group 4	2 days	8.74	5.06	10.5	3.59	NR	1.59
919	Kidney	Group 5	4 days	2.72	2.39	3.07	6.86	NR	0.548
932	Kidney	Group 5	4 days	1.42	1.23	2.49	2.33	NR	0.187*
936	Kidney	Group 5	4 days	2.73	2.99	2.77	4.86	NR	0.626
947	Kidney	Group 5	4 days	1.66	2.38	3.21	2.56	NR	0.280
934	Kidney	Group 6	7 days	1.41	0.719	1.08	0.466	NR	0.262
942	Kidney	Group 6	7 days	0.883	0.626	0.888	0.371	NR	0.261
952	Kidney	Group 6	7 days	0.940	0.494	0.807	0.622	NR	0.230
964	Kidney	Group 6	7 days	1.33	0.920	1.38	0.534	NR	0.345
901	Kidney	Group 7	7 days	1.17	1.02	1.30	2.63	NR	0.093*
917	Kidney	Group 7	7 days	1.39	1.12	0.99	0.573	NR	0.257
950	Kidney	Group 7	7 days	0.871	0.729	1.08	0.444	NR	0.111*
953	Kidney	Group 7	7 days	0.752	1.05	0.298	0.653	NR	0.229
903	Kidney	Group 8	7 days	2.69	1.56	3.57	5.08	NR	0.391
912	Kidney	Group 8	7 days	1.79	1.54	2.12	3.57	NR	<LOD
938	Kidney	Group 8	7 days	2.06	2.99	1.30	3.57	NR	0.375
941	Kidney	Group 8	7 days	2.97	1.30	2.18	2.36	NR	1.11
904	Kidney	Group 9	7 days	4.10	4.29	4.88	7.83	NR	0.753
916	Kidney	Group 9	7 days	0.745	1.26	0.547	1.39	NR	<LOD
928	Kidney	Group 9	7 days	2.18	1.57	4.61	0.927	NR	0.263
960	Kidney	Group 9	7 days	1.59	0.519	1.81	0.531	NR	0.316
924	Kidney	Group 10	14 days	1.69	1.41	1.70	3.05	NR	0.183*
944	Kidney	Group 10	14 days	1.15	0.701	1.67	0.505	NR	0.252
954	Kidney	Group 10	14 days	0.695	0.231	0.997	0.202	NR	<LOD
958	Kidney	Group 10	14 days	1.36	0.592	0.989	0.267	NR	0.717
910	Kidney	Group 11	28 days	1.22	0.352	0.452	0.729	NR	0.123*
935	Kidney	Group 11	28 days	0.387	0.141*	0.283	0.090*	NR	0.076*
937	Kidney	Group 11	28 days	0.395	0.127*	0.291	<LOD	NR	<LOD
961	Kidney	Group 11	28 days	0.672	0.447	0.538	0.443	NR	0.090*
902	Perirenal Fat	Group 1	4 hours	124	14.7	22.1	16.8	0.139*	83.2
913	Perirenal Fat	Group 1	4 hours	401	6.02	6.37	2.19	0.457	125
915	Perirenal Fat	Group 1	4 hours	246	5.02	13.8	2.79	0.325*	231

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
968	Perirenal Fat	Group 1	4 hours	287	4.06	11.0	4.63	0.500	288
908	Perirenal Fat	Group 2	10 hours	34.9	1.40	4.77	3.30	<LOD	114
949	Perirenal Fat	Group 2	10 hours	40.4	3.88	14.9	10.8	0.178*	45.3
962	Perirenal Fat	Group 2	10 hours	211	7.07	6.63	16.6	0.234*	187
967	Perirenal Fat	Group 2	10 hours	30.6	8.36	19.6	43.8	<LOD	6.42
906	Perirenal Fat	Group 3	24 hours	15.2	0.400	1.87	0.806	<LOD	28.2
926	Perirenal Fat	Group 3	24 hours	12.6	0.568	3.82	2.62	<LOD	15.9
930	Perirenal Fat	Group 3	24 hours	7.53	0.184*	0.572	0.450	<LOD	36.3
939	Perirenal Fat	Group 3	24 hours	9.46	0.473	3.48	2.69	<LOD	16.9
927	Perirenal Fat	Group 4	2 days	3.99	0.236	0.931	0.365	<LOD	2.72
959	Perirenal Fat	Group 4	2 days	3.57	0.109*	1.08	0.168*	<LOD	7.03
963	Perirenal Fat	Group 4	2 days	13.8	3.93	2.18	10.30	<LOD	13.7
965	Perirenal Fat	Group 4	2 days	8.42	0.162*	1.35	0.194*	<LOD	25.4
919	Perirenal Fat	Group 5	4 days	16.9	0.870	0.753	0.931	0.305*	2.13
932	Perirenal Fat	Group 5	4 days	1.37	0.079*	0.276	0.308	<LOD	1.21
936	Perirenal Fat	Group 5	4 days	2.93	0.512	0.804	0.940	<LOD	2.17
947	Perirenal Fat	Group 5	4 days	1.71	0.239	0.243	0.196*	<LOD	1.42
934	Perirenal Fat	Group 6	7 days	0.894	<LOD	0.194*	<LOD	<LOD	1.01
942	Perirenal Fat	Group 6	7 days	0.545	<LOD	0.183*	<LOD	<LOD	1.17
952	Perirenal Fat	Group 6	7 days	0.560	<LOD	0.080*	<LOD	<LOD	1.07
964	Perirenal Fat	Group 6	7 days	0.822	<LOD	0.083*	<LOD	<LOD	1.41
901	Perirenal Fat	Group 7	7 days	1.84	0.411	0.305	1.14	<LOD	<LOD
917	Perirenal Fat	Group 7	7 days	1.18	<LOD	0.220	<LOD	<LOD	1.02
950	Perirenal Fat	Group 7	7 days	0.928	0.077*	0.236	0.088*	<LOD	0.576
953	Perirenal Fat	Group 7	7 days	0.780	0.080*	0.097*	0.130*	<LOD	0.832
903	Perirenal Fat	Group 8	7 days	2.87	0.376	1.41	1.50	<LOD	0.340
912	Perirenal Fat	Group 8	7 days	1.54	0.334	0.552	0.954	<LOD	0.464
938	Perirenal Fat	Group 8	7 days	1.73	0.265	0.390	0.439	<LOD	0.689
941	Perirenal Fat	Group 8	7 days	2.33	0.131*	0.644	0.416	<LOD	3.10
904	Perirenal Fat	Group 9	7 days	3.96	0.643	2.02	1.63	<LOD	1.71
916	Perirenal Fat	Group 9	7 days	0.644	0.124*	0.110*	0.132*	<LOD	0.434
928	Perirenal Fat	Group 9	7 days	2.30	<LOD	0.684	0.123*	<LOD	1.16

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
960	Perirenal Fat	Group 9	7 days	1.48	<LOD	0.322	<LOD	<LOD	1.92
924	Perirenal Fat	Group 10	14 days	2.20	0.335	0.507	0.462	<LOD	0.814
944	Perirenal Fat	Group 10	14 days	1.38	<LOD	0.247	0.087*	<LOD	1.27
954	Perirenal Fat	Group 10	14 days	1.48	0.076*	0.284	<LOD	<LOD	0.719
958	Perirenal Fat	Group 10	14 days	1.30	<LOD	0.141*	0.077*	<LOD	1.88
910	Perirenal Fat	Group 11	28 days	1.22	0.105*	0.213	0.123*	<LOD	0.429
935	Perirenal Fat	Group 11	28 days	8.20	1.01	0.138*	0.139*	0.245*	0.538
937	Perirenal Fat	Group 11	28 days	0.416	<LOD	0.163*	0.075*	<LOD	0.146*
961	Perirenal Fat	Group 11	28 days	0.716	<LOD	0.081*	<LOD	<LOD	0.584
902	Mules	Group 1	4 hours	30190	92.8	11.8	27.0	35.7	151
913	Mules	Group 1	4 hours	29730	55.0	8.97	9.67	19.2	1059
915	Mules	Group 1	4 hours	23341	76.8	12.8	10.1	18.3	>1000
968	Mules	Group 1	4 hours	84199	204	12.7	28.9	48.3	1011
908	Mules	Group 2	10 hours	10673	40.6	3.64	15.1	17.2	556
949	Mules	Group 2	10 hours	16376	44.9	8.74	24.0	26.0	469
962	Mules	Group 2	10 hours	22994	57.1	5.91	41.0	29.2	>1000
967	Mules	Group 2	10 hours	4369	26.7	4.97	47.3	8.61	52.4
906	Mules	Group 3	24 hours	6256	33.7	1.05	7.17	10.1	41.5
926	Mules	Group 3	24 hours	4269	21.0	1.34	6.98	5.39	14.7
930	Mules	Group 3	24 hours	19812	60.4	1.69	7.80	30.7	140
939	Mules	Group 3	24 hours	2742	14.4	1.08	5.92	3.82	11.9
927	Mules	Group 4	2 days	1620	9.07	5.64	2.12	4.55	10.4
959	Mules	Group 4	2 days	43158	126	2.59	9.01	>40	99.8
963	Mules	Group 4	2 days	79555	321	5.76	38.8	>40	34.2
965	Mules	Group 4	2 days	7854	51.9	1.63	6.99	20.77	73.0
919	Mules	Group 5	4 days	26163	87.9	2.55	26.3	41.29	64.5
932	Mules	Group 5	4 days	120069	458	9.04	30.1	>40	90.1
936	Mules	Group 5	4 days	92943	365	6.55	37.6	>40	311
947	Mules	Group 5	4 days	66710	581	10.8	30.1	>40	660
934	Mules	Group 6	7 days	97270	721	7.95	57.2	>40	76.8
942	Mules	Group 6	7 days	9723	72.0	1.10	17.2	28.6	16.2
952	Mules	Group 6	7 days	61226	644	6.39	47.5	>40	26.4

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964	Mules	Group 6	7 days	34361	281	3.66	25.4	>40	19.5
901	Mules	Group 7	7 days	44005	538	10.1	51.0	>40	31.2
917	Mules	Group 7	7 days	21124	120	2.34	26.6	>40	50.1
950	Mules	Group 7	7 days	31319	189	2.95	23.5	>40	47.9
953	Mules	Group 7	7 days	11418	85.9	1.08	9.14	38.8	35.8
903	Mules	Group 8	7 days	10793	132	2.28	23.5	>40	9.41
912	Mules	Group 8	7 days	2945	60.1	0.825	9.56	17.0	2.75
938	Mules	Group 8	7 days	5423	44.7	0.842	6.99	17.5	6.88
941	Mules	Group 8	7 days	7848	70.6	1.30	11.9	>40	40.6
904	Mules	Group 9	7 days	1014	35.7	0.575	5.11	25.0	1.97
916	Mules	Group 9	7 days	2054	31.4	0.358	1.70	11.6	7.68
928	Mules	Group 9	7 days	148	6.25	0.226	0.628	2.81	0.583
960	Mules	Group 9	7 days	169	4.23	0.141*	0.343	2.30	1.89
924	Mules	Group 10	14 days	80690	663	9.34	41.8	>40	39.2
944	Mules	Group 10	14 days	131441	>1000	17.5	114	>40	46.4
954	Mules	Group 10	14 days	60225	544	7.27	37.4	>40	64.5
958	Mules	Group 10	14 days	265469	>1000	26.6	135	>40	210
910	Mules	Group 11	28 days	250726	>1000	55.5	373	>40	112
935	Mules	Group 11	28 days	119889	1089	18.4	91.8	>40	60.4
937	Mules	Group 11	28 days	57558	1075	21.3	131	>40	39.6
961	Mules	Group 11	28 days	155769	>1000	38.3	111	>40	41.9
902	Muscle (Heart)	Group 1	4 hours	133	42.2	9.30	40.7	0.699	13.0
913	Muscle (Heart)	Group 1	4 hours	291	26.0	13.1	12.5	11.02	28.2
915	Muscle (Heart)	Group 1	4 hours	218	27.2	15.2	13.3	4.83	35.0
968	Muscle (Heart)	Group 1	4 hours	241	25.2	12.5	25.7	9.50	24.4
908	Muscle (Heart)	Group 2	10 hours	30.2	7.80	5.97	15.6	0.405*	11.1
949	Muscle (Heart)	Group 2	10 hours	41.2	13.4	8.54	26.9	0.358*	10.9
962	Muscle (Heart)	Group 2	10 hours	164	19.5	9.11	43.3	2.56	45.4
967	Muscle (Heart)	Group 2	10 hours	19.1	8.71	10.3	38.2	<LOD	3.64
906	Muscle (Heart)	Group 3	24 hours	10.1	1.78	1.14	3.27	<LOD	2.26
926	Muscle (Heart)	Group 3	24 hours	6.80	1.40	1.96	6.32	<LOD	2.08
930	Muscle (Heart)	Group 3	24 hours	3.28	1.20	0.869	4.25	<LOD	1.30

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939	Muscle (Heart)	Group 3	24 hours	5.15	0.952	1.89	5.45	<LOD	3.72
927	Muscle (Heart)	Group 4	2 days	1.76	0.849	0.549	1.12	<LOD	<LOD
959	Muscle (Heart)	Group 4	2 days	2.69	0.411	0.646	0.380	<LOD	2.28
963	Muscle (Heart)	Group 4	2 days	5.39	5.62	0.784	16.5	<LOD	2.25
965	Muscle (Heart)	Group 4	2 days	3.92	1.08	1.44	0.998	<LOD	2.09
919	Muscle (Heart)	Group 5	4 days	1.87	0.569	0.687	2.32	<LOD	0.540
932	Muscle (Heart)	Group 5	4 days	0.580	0.325	0.300	0.725	<LOD	0.107*
936	Muscle (Heart)	Group 5	4 days	2.43	0.771	0.388	1.62	<LOD	0.773
947	Muscle (Heart)	Group 5	4 days	0.997	0.709	0.268	0.988	<LOD	0.227
934	Muscle (Heart)	Group 6	7 days	0.434	0.164*	0.141*	0.169*	<LOD	0.136*
942	Muscle (Heart)	Group 6	7 days	0.499	0.161*	0.262	0.171*	<LOD	0.131*
952	Muscle (Heart)	Group 6	7 days	0.275	0.076*	0.105*	0.224	<LOD	<LOD
964	Muscle (Heart)	Group 6	7 days	0.520	0.184*	0.127*	0.188*	<LOD	0.122*
901	Muscle (Heart)	Group 7	7 days	0.444	0.311	0.168*	0.979	<LOD	<LOD
917	Muscle (Heart)	Group 7	7 days	0.512	0.145*	0.164*	0.199*	<LOD	0.188*
950	Muscle (Heart)	Group 7	7 days	0.527	0.212	0.168*	0.208	<LOD	0.075*
953	Muscle (Heart)	Group 7	7 days	0.260	0.238	<LOD	0.267	<LOD	<LOD
903	Muscle (Heart)	Group 8	7 days	2.20	0.428	0.468	1.47	<LOD	0.201
912	Muscle (Heart)	Group 8	7 days	1.09	0.496	0.322	1.27	<LOD	0.176*
938	Muscle (Heart)	Group 8	7 days	1.24	0.730	0.222	1.07	<LOD	<LOD
941	Muscle (Heart)	Group 8	7 days	2.48	0.389	0.356	0.746	<LOD	1.41
904	Muscle (Heart)	Group 9	7 days	2.21	1.00	0.704	2.35	<LOD	0.650
916	Muscle (Heart)	Group 9	7 days	0.355	0.313	0.099*	0.383	<LOD	<LOD
928	Muscle (Heart)	Group 9	7 days	1.56	0.530	0.496	0.402	<LOD	0.116*
960	Muscle (Heart)	Group 9	7 days	0.920	0.126*	0.279	0.181*	<LOD	0.355
924	Muscle (Heart)	Group 10	14 days	0.683	0.488	0.292	1.02	<LOD	0.240
944	Muscle (Heart)	Group 10	14 days	0.798	0.187*	0.289	0.186*	<LOD	<LOD
954	Muscle (Heart)	Group 10	14 days	0.308	0.082*	0.193*	0.184*	<LOD	<LOD
958	Muscle (Heart)	Group 10	14 days	0.652	0.150*	0.128*	0.160*	<LOD	0.538
910	Muscle (Heart)	Group 11	28 days	11.0	0.217	0.089*	0.310	0.746	<LOD
935	Muscle (Heart)	Group 11	28 days	0.122*	<LOD	<LOD	<LOD	<LOD	<LOD
937	Muscle (Heart)	Group 11	28 days	0.516	<LOD	0.112*	0.142*	<LOD	<LOD

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961	Muscle (Heart)	Group 11	28 days	0.357	0.186*	0.095*	0.147*	<LOD	0.088*
902	Muscle (Loin)	Group 1	4 hours	51.4	31.0	3.99	28.8	0.095*	4.26
913	Muscle (Loin)	Group 1	4 hours	104	18.2	5.32	11.4	0.130*	13.4
915	Muscle (Loin)	Group 1	4 hours	76.6	23.9	5.22	12.3	0.168*	16.4
968	Muscle (Loin)	Group 1	4 hours	73.3	19.2	4.20	23.1	0.123*	15.6
908	Muscle (Loin)	Group 2	10 hours	7.34	4.58	1.90	12.9	<LOD	5.20
949	Muscle (Loin)	Group 2	10 hours	18.2	12.9	3.35	26.8	<LOD	2.59
962	Muscle (Loin)	Group 2	10 hours	54.5	16.5	4.15	41.7	<LOD	11.9
967	Muscle (Loin)	Group 2	10 hours	10.7	8.74	3.55	37.3	<LOD	1.65
906	Muscle (Loin)	Group 3	24 hours	3.65	1.47	0.423	3.11	<LOD	0.940
926	Muscle (Loin)	Group 3	24 hours	2.90	1.33	0.573	5.92	<LOD	0.567
930	Muscle (Loin)	Group 3	24 hours	1.45	0.991	0.267	3.79	<LOD	1.00
939	Muscle (Loin)	Group 3	24 hours	2.15	0.776	0.799	5.59	<LOD	1.06
927	Muscle (Loin)	Group 4	2 days	0.776	0.661	0.220	1.11	<LOD	0.097*
959	Muscle (Loin)	Group 4	2 days	0.969	0.493	0.218	0.422	<LOD	0.175*
963	Muscle (Loin)	Group 4	2 days	2.62	5.43	0.386	15.46	<LOD	1.19
965	Muscle (Loin)	Group 4	2 days	2.30	1.14	0.476	0.900	<LOD	0.495
919	Muscle (Loin)	Group 5	4 days	0.676	0.654	0.233	2.53	<LOD	0.113*
932	Muscle (Loin)	Group 5	4 days	0.478	0.331	0.124*	0.806	<LOD	<LOD
936	Muscle (Loin)	Group 5	4 days	0.990	1.20	0.192*	2.56	<LOD	0.206
947	Muscle (Loin)	Group 5	4 days	0.534	0.649	0.084*	0.801	<LOD	0.097*
934	Muscle (Loin)	Group 6	7 days	0.254	0.154*	<LOD	0.155*	<LOD	<LOD
942	Muscle (Loin)	Group 6	7 days	0.183*	0.148*	<LOD	0.135*	<LOD	<LOD
952	Muscle (Loin)	Group 6	7 days	0.160*	0.129*	<LOD	0.197*	<LOD	<LOD
964	Muscle (Loin)	Group 6	7 days	0.224	0.196*	<LOD	0.225	<LOD	0.073*
901	Muscle (Loin)	Group 7	7 days	0.474	0.309	0.070*	1.11	<LOD	<LOD
917	Muscle (Loin)	Group 7	7 days	1.21	0.267	<LOD	0.229	<LOD	<LOD
950	Muscle (Loin)	Group 7	7 days	0.333	0.184*	<LOD	0.137*	<LOD	<LOD
953	Muscle (Loin)	Group 7	7 days	0.489	0.296	<LOD	0.326	<LOD	<LOD
903	Muscle (Loin)	Group 8	7 days	0.784	0.437	0.237	1.65	<LOD	0.080*
912	Muscle (Loin)	Group 8	7 days	1.01	0.510	0.161*	1.32	<LOD	<LOD
938	Muscle (Loin)	Group 8	7 days	1.28	0.757	0.106*	1.10	<LOD	<LOD

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941	Muscle (Loin)	Group 8	7 days	0.719	0.309	0.136*	0.867	<LOD	0.323
904	Muscle (Loin)	Group 9	7 days	1.19	1.08	0.341	2.83	<LOD	0.270
916	Muscle (Loin)	Group 9	7 days	0.148*	0.241	<LOD	0.277	<LOD	<LOD
928	Muscle (Loin)	Group 9	7 days	0.640	0.417	0.182*	0.386	<LOD	<LOD
960	Muscle (Loin)	Group 9	7 days	0.292	0.087*	0.084*	0.196*	<LOD	0.097*
924	Muscle (Loin)	Group 10	14 days	0.528	0.627	0.121*	1.43	<LOD	<LOD
944	Muscle (Loin)	Group 10	14 days	0.215	0.146*	<LOD	0.125*	<LOD	<LOD
954	Muscle (Loin)	Group 10	14 days	0.290	0.153*	0.076*	0.175*	<LOD	<LOD
958	Muscle (Loin)	Group 10	14 days	0.315	0.155*	<LOD	0.088*	<LOD	<LOD
910	Muscle (Loin)	Group 11	28 days	0.181*	0.123*	<LOD	0.323	<LOD	<LOD
935	Muscle (Loin)	Group 11	28 days	0.293	<LOD	<LOD	0.083*	<LOD	<LOD
937	Muscle (Loin)	Group 11	28 days	0.129*	<LOD	<LOD	0.126*	<LOD	<LOD
961	Muscle (Loin)	Group 11	28 days	0.329	0.133*	<LOD	0.208	<LOD	<LOD
902	Urine	Group 1	4 hours	190	>40	>1000	653	4.64	301
913	Urine	Group 1	4 hours	638	>40	>1000	666	13.4	1348
915	Urine	Group 1	4 hours	711	>40	>1000	354	2.55	1020
968	Urine	Group 1	4 hours	726	>40	>1000	860	20.9	2668
908	Urine	Group 2	10 hours	152	>40	761	121	<LOD	476
949	Urine	Group 2	10 hours	675	>40	>1000	658	50.5	243
962	Urine	Group 2	10 hours	473	>40	>1000	781	97.6	564
967	Urine	Group 2	10 hours	395	>40	>1000	691	25.5	192
906	Urine	Group 3	24 hours	38.2	9.50	300	40.2	<LOD	180
926	Urine	Group 3	24 hours	46.1	8.28	198	56.4	0.101*	54.6
930	Urine	Group 3	24 hours	15.5	6.72	254	31.4	0.392	72.2
939	Urine	Group 3	24 hours	9.15	6.18	338	26.5	0.085*	58.2
927	Urine	Group 4	2 days	12.0	8.46	351	36.3	0.463	12.5
959	Urine	Group 4	2 days	IS	IS	IS	IS	IS	IS
963	Urine	Group 4	2 days	16.2	>40	78.4	88.5	7.12	69.5
965	Urine	Group 4	2 days	94.4	8.19	380	23.6	3.80	59.1
919	Urine	Group 5	4 days	10.9	2.64	94.4	13.7	0.352	11.3
932	Urine	Group 5	4 days	IS	IS	IS	IS	IS	IS
936	Urine	Group 5	4 days	3.61	3.11	105	20.4	0.368	8.52

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947	Urine	Group 5	4 days	7.93	4.80	135	25.0	1.13	7.17
934	Urine	Group 6	7 days	1.27	0.489	33.4	2.15	0.410	2.16
942	Urine	Group 6	7 days	2.01	0.588	172	1.99	1.77	2.70
952	Urine	Group 6	7 days	IS	IS	IS	IS	IS	IS
964	Urine	Group 6	7 days	IS	IS	IS	IS	IS	IS
901	Urine	Group 7	7 days	0.276	1.07	264	5.97	0.405	0.952
917	Urine	Group 7	7 days	4.57	1.67	71.7	3.40	0.144*	8.90
950	Urine	Group 7	7 days	2.19	1.24	69.1	2.52	0.318	2.16
953	Urine	Group 7	7 days	1.43	1.21	21.0	1.92	0.173*	6.42
903	Urine	Group 8	7 days	1.71	2.48	496	16.5	0.398	4.15
912	Urine	Group 8	7 days	2.34	5.71	278	29.7	0.788	2.09
938	Urine	Group 8	7 days	62.3	>40	351	84.6	1.19	3.25
941	Urine	Group 8	7 days	5.72	4.17	252	3.68	0.327	25.9
904	Urine	Group 9	7 days	4.74	2.70	157	19.1	0.790	8.42
916	Urine	Group 9	7 days	IS	IS	IS	IS	IS	IS
928	Urine	Group 9	7 days	36.8	5.61	410	5.87	0.900	7.71
902	Urine	Group 1	4 hours	190	>40	>1000	653	4.64	301
913	Urine	Group 1	4 hours	638	>40	>1000	666	13.4	1348
915	Urine	Group 1	4 hours	711	>40	>1000	354	2.55	1020
968	Urine	Group 1	4 hours	726	>40	>1000	860	20.9	2668
908	Urine	Group 2	10 hours	152	>40	761	121	<LOD	476
949	Urine	Group 2	10 hours	675	>40	>1000	658	50.5	243
962	Urine	Group 2	10 hours	473	>40	>1000	781	97.6	564
967	Urine	Group 2	10 hours	395	>40	>1000	691	25.5	192
906	Urine	Group 3	24 hours	38.2	9.50	300	40.2	<LOD	180
926	Urine	Group 3	24 hours	46.1	8.28	198	56.4	0.101*	54.6
930	Urine	Group 3	24 hours	15.5	6.72	254	31.4	0.392	72.2
939	Urine	Group 3	24 hours	9.15	6.18	338	26.5	0.085*	58.2
927	Urine	Group 4	2 days	12.0	8.46	351	36.3	0.463	12.5
959	Urine	Group 4	2 days	IS	IS	IS	IS	IS	IS
963	Urine	Group 4	2 days	16.2	>40	78.4	88.5	7.12	69.5
965	Urine	Group 4	2 days	94.4	8.19	380	23.6	3.80	59.1

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
919	Urine	Group 5	4 days	10.9	2.64	94.4	13.7	0.352	11.3
932	Urine	Group 5	4 days	IS	IS	IS	IS	IS	IS
936	Urine	Group 5	4 days	3.61	3.11	105	20.4	0.368	8.52
947	Urine	Group 5	4 days	7.93	4.80	135	25.0	1.13	7.17
934	Urine	Group 6	7 days	1.27	0.489	33.4	2.15	0.410	2.16
942	Urine	Group 6	7 days	2.01	0.588	172	1.99	1.77	2.70
952	Urine	Group 6	7 days	IS	IS	IS	IS	IS	IS
964	Urine	Group 6	7 days	IS	IS	IS	IS	IS	IS
901	Urine	Group 7	7 days	0.276	1.07	264	5.97	0.405	0.952
917	Urine	Group 7	7 days	4.57	1.67	71.7	3.40	0.144*	8.90
950	Urine	Group 7	7 days	2.19	1.24	69.1	2.52	0.318	2.16
953	Urine	Group 7	7 days	1.43	1.21	21.0	1.92	0.173*	6.42
903	Urine	Group 8	7 days	1.71	2.48	496	16.5	0.398	4.15
912	Urine	Group 8	7 days	2.34	5.71	278	29.7	0.788	2.09
938	Urine	Group 8	7 days	62.3	>40	351	84.6	1.19	3.25
941	Urine	Group 8	7 days	5.72	4.17	252	3.68	0.327	25.9
904	Urine	Group 9	7 days	4.74	2.70	157	19.1	0.790	8.42
916	Urine	Group 9	7 days	IS	IS	IS	IS	IS	IS
928	Urine	Group 9	7 days	36.8	5.61	410	5.87	0.900	7.71
960	Urine	Group 9	7 days	2.99	1.01	158	1.56	0.502	4.78
902	Faeces	Group 1	4 hours	26.5	21.4	>40	10.5	0.654	1.94
913	Faeces	Group 1	4 hours	>40	7.10	7.76	3.97	0.979	6.03
915	Faeces	Group 1	4 hours	>40	15.2	11.0	3.64	1.19	9.20
968	Faeces	Group 1	4 hours	>40	9.32	9.42	5.12	1.57	7.65
908	Faeces	Group 2	10 hours	21.2	8.49	>40	12.2	0.268	4.56
949	Faeces	Group 2	10 hours	>40	13.2	>40	12.9	0.598	3.00
962	Faeces	Group 2	10 hours	>40	28.4	9.57	35.1	1.35	12.8
967	Faeces	Group 2	10 hours	>40	14.7	>10	35.8	0.513	3.02
906	Faeces	Group 3	24 hours	6.40	1.66	8.85	3.47	0.089*	1.56
926	Faeces	Group 3	24 hours	5.19	1.88	>10	9.28	<LOD	1.94
930	Faeces	Group 3	24 hours	2.78	1.68	>10	4.91	<LOD	1.86
939	Faeces	Group 3	24 hours	3.60	1.19	>10	6.26	0.070*	1.98

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
927	Faeces	Group 4	2 days	1.12	0.684	6.03	1.24	<LOD	0.167*
959	Faeces	Group 4	2 days	1.36	0.596	2.32	0.771	<LOD	0.433
963	Faeces	Group 4	2 days	5.07	6.19	>10	17.3	<LOD	1.80
965	Faeces	Group 4	2 days	3.28	1.93	>10	1.79	<LOD	0.825
919	Faeces	Group 5	4 days	0.760	0.657	3.30	2.26	<LOD	0.209
932	Faeces	Group 5	4 days	0.570	0.356	0.234	1.24	<LOD	<LOD
936	Faeces	Group 5	4 days	0.931	0.816	7.23	1.21	<LOD	0.166*
947	Faeces	Group 5	4 days	1.18	1.36	1.31	1.91	<LOD	0.421
934	Faeces	Group 6	7 days	0.267	0.240	0.945	0.188*	<LOD	<LOD
942	Faeces	Group 6	7 days	0.213	0.137*	3.20	0.254	<LOD	<LOD
952	Faeces	Group 6	7 days	0.089*	0.117*	<LOD	0.127*	<LOD	<LOD
964	Faeces	Group 6	7 days	0.222	0.172*	0.256	0.188*	<LOD	<LOD
901	Faeces	Group 7	7 days	0.119*	0.251	0.100*	0.642	<LOD	0.080*
917	Faeces	Group 7	7 days	0.189*	0.192*	0.988	0.145*	<LOD	<LOD
950	Faeces	Group 7	7 days	0.144*	0.186*	<LOD	0.358	<LOD	<LOD
953	Faeces	Group 7	7 days	0.097*	0.185*	<LOD	0.080*	<LOD	0.118*
903	Faeces	Group 8	7 days	0.340	0.349	0.885	1.14	<LOD	0.107*
912	Faeces	Group 8	7 days	0.222	0.335	0.530	0.714	<LOD	0.171*
938	Faeces	Group 8	7 days	0.700	0.582	0.115*	0.781	<LOD	0.076*
941	Faeces	Group 8	7 days	0.294	0.233	3.79	0.656	<LOD	0.519
904	Faeces	Group 9	7 days	0.868	0.764	7.62	1.27	<LOD	0.376
916	Faeces	Group 9	7 days	<LOD	0.205	<LOD	0.289	<LOD	0.101*
928	Faeces	Group 9	7 days	0.258	0.320	0.332	0.512	<LOD	0.078*
960	Faeces	Group 9	7 days	0.174*	0.132*	0.414	0.238	<LOD	0.200*
903	Plasma	Group 8	D-1	0.080*	<LOD	<LOD	0.335	<LOD	<LOD
912	Plasma	Group 8	D-1	0.078*	<LOD	<LOD	0.308	<LOD	<LOD
938	Plasma	Group 8	D-1	0.097*	<LOD	<LOD	0.244*	<LOD	<LOD
941	Plasma	Group 8	D-1	0.106*	<LOD	<LOD	0.276*	<LOD	<LOD
903	Plasma	Group 8	15 minutes	>40	11.9	5.17	11.4	8.50	3.07
912	Plasma	Group 8	15 minutes	>40	15.8	3.93	11.8	11.5	3.44
938	Plasma	Group 8	15 minutes	>40	17.7	1.72	6.88	9.05	2.93
941	Plasma	Group 8	15 minutes	>40	4.93	2.09	2.80	4.86	1.43

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
903	Plasma	Group 8	30 minutes	>40	15.1	5.28	18.8	7.48	3.28
912	Plasma	Group 8	30 minutes	>40	20.1	4.25	21.0	12.3	4.32
938	Plasma	Group 8	30 minutes	>40	23.3	2.05	12.5	10.1	3.40
941	Plasma	Group 8	30 minutes	>40	7.31	2.52	5.29	6.03	2.11
903	Plasma	Group 8	60 minutes	>40	13.5	3.77	26.4	5.95	3.77
912	Plasma	Group 8	60 minutes	>40	20.4	3.92	32.7	10.6	5.48
938	Plasma	Group 8	60 minutes	>40	27.8	1.92	23.8	9.07	4.82
941	Plasma	Group 8	60 minutes	38.4	8.74	2.61	9.90	5.70	3.44
903	Plasma	Group 8	2 hours	>40	13.0	3.64	33.6	4.55	4.21
912	Plasma	Group 8	2 hours	>40	22.0	4.57	>40	10.8	6.91
938	Plasma	Group 8	2 hours	>40	32.2	2.12	>40	8.71	5.35
941	Plasma	Group 8	2 hours	>40	11.1	3.54	18.8	7.05	6.69
903	Plasma	Group 8	4 hours	30.8	8.97	2.54	39.0	3.01	4.67
912	Plasma	Group 8	4 hours	30.4	13.7	2.25	>40	5.09	5.04
938	Plasma	Group 8	4 hours	38.4	26.1	2.19	>40	6.79	5.25
941	Plasma	Group 8	4 hours	>40	10.0	3.67	25.3	6.33	10.3
903	Plasma	Group 8	6 hours	9.74	4.02	2.41	32.9	1.28	2.65
912	Plasma	Group 8	6 hours	9.41	7.01	1.26	>40	2.37	3.03
938	Plasma	Group 8	6 hours	9.53	12.8	0.923	>40	2.64	2.60
941	Plasma	Group 8	6 hours	21.8	6.60	2.58	30.2	3.95	9.47
903	Plasma	Group 8	9 hours	11.4	2.28	1.21	23.5	0.953	1.59
912	Plasma	Group 8	9 hours	3.54	3.42	0.868	31.5	1.28	1.72
938	Plasma	Group 8	9 hours	4.26	6.47	0.351	38.2	1.45	1.74
941	Plasma	Group 8	9 hours	5.03	3.21	1.42	23.3	1.87	5.12
903	Plasma	Group 8	12 hours	3.77	1.79	0.737	21.2	0.691	1.52
912	Plasma	Group 8	12 hours	2.70	2.34	0.366	23.0	1.09	1.431
938	Plasma	Group 8	12 hours	2.74	4.26	0.221	30.8	1.02	0.96
941	Plasma	Group 8	12 hours	3.91	2.35	0.945	18.4	1.60	3.98
903	Plasma	Group 8	18 hours	2.31	1.19	0.519	13.1	0.587	0.954
912	Plasma	Group 8	18 hours	1.12	1.14	0.199*	11.6	0.731	0.629
938	Plasma	Group 8	18 hours	1.18	1.65	0.088*	13.4	0.534	0.434
941	Plasma	Group 8	18 hours	1.28	1.19	0.369	11.7	0.945	1.66

Animal ID	Tissue	Grp.	Timepoint	Lignocaine (µg/kg or L)	MEGX (µg/kg or L)	3-OH Lignocaine (µg/kg or L)	GX (µg/kg or L)	Lignocaine N-oxide (µg/kg or L)	2,6-DMA (µg/kg or L)
903	Plasma	Group 8	24 hours	1.89	1.18	0.404	10.5	0.614	0.830
912	Plasma	Group 8	24 hours	0.943	0.830	0.095*	6.61	0.606	0.417
938	Plasma	Group 8	24 hours	0.592	0.983	<LOD	7.16	0.363	0.263
941	Plasma	Group 8	24 hours	0.860	0.791	0.232	8.17	0.650	1.52
903	Plasma	Group 8	30 hours	1.19	0.661	0.243	7.62	0.365	0.444
912	Plasma	Group 8	30 hours	0.630	0.477	<LOD	3.93	0.382	0.269
938	Plasma	Group 8	30 hours	1.03	0.957	<LOD	4.68	0.350	0.202
941	Plasma	Group 8	30 hours	0.760	0.495	0.184*	4.94	0.442	1.08
903	Plasma	Group 8	36 hours	0.958	0.483	0.165*	5.26	0.286	0.279
912	Plasma	Group 8	36 hours	>40	2.02	0.104*	2.70	2.68	4.77
938	Plasma	Group 8	36 hours	0.993	1.13	<LOD	3.87	0.393	0.140*
941	Plasma	Group 8	36 hours	1.03	0.359	0.112*	3.59	0.341	0.847
903	Plasma	Group 8	48 hours	1.29	0.488	0.153*	3.81	0.300	0.261
912	Plasma	Group 8	48 hours	1.61	0.437	<LOD	1.94	0.348	0.161*
938	Plasma	Group 8	48 hours	0.715	0.712	<LOD	2.17	0.283	0.097*
941	Plasma	Group 8	48 hours	0.435	0.295	0.107*	2.32	0.266	0.578
903	Plasma	Group 8	72 hours	1.11	0.419	0.138*	2.16	0.255	0.173*
912	Plasma	Group 8	72 hours	0.744	0.293	<LOD	1.15	0.220	0.090*
938	Plasma	Group 8	72 hours	0.274	0.391	<LOD	1.26	0.154*	<LOD
941	Plasma	Group 8	72 hours	0.312	0.184*	<LOD	1.13	0.177*	0.470
903	Plasma	Group 8	96 hours	0.759	0.371	0.116*	1.90	0.202	0.208
912	Plasma	Group 8	96 hours	0.374	0.270	<LOD	1.19	0.193*	0.085*
938	Plasma	Group 8	96 hours	0.241	0.283	<LOD	0.942	0.131*	<LOD
941	Plasma	Group 8	96 hours	0.290	0.168*	<LOD	0.955	0.190*	0.421
903	Plasma	Group 8	7 days	0.368	0.150*	0.078*	1.01	0.117*	0.110*
912	Plasma	Group 8	7 days	0.234	0.165*	<LOD	0.830	0.158*	<LOD
938	Plasma	Group 8	7 days	0.199	0.229	<LOD	0.743	0.113*	<LOD
941	Plasma	Group 8	7 days	0.258	0.099*	<LOD	0.680	0.139*	0.304

For Lignocaine (and metabolites): LOD = 0.07 µg/kg (or L for plasma and urine); LOQ = 0.2 µg/kg (or L for plasma and urine) (except GX in plasma: 0.29 µg/L, Lignocaine N-oxide in loin muscle: 0.47 µg/kg, Lignocaine N-oxide in perirenal fat: 0.33 µg/kg, Lignocaine N-oxide in faeces: 0.26 µg/kg); Grp = Group; IS = Insufficient Sample; NR = Not Reported; *Value between LOD and LOQ; > = value above upper limit of quantification

12.3 Appendix 3 – Bupivacaine and Metabolites in Ovine Tissues, Urine, Faeces and Plasma

Table 8: Bupivacaine and Metabolites in Ovine Tissues, Urine, Faeces and Plasma

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
902	Liver	Group 1	4 hours	19.7	7.02	3.50
913	Liver	Group 1	4 hours	10.2	5.54	0.860
915	Liver	Group 1	4 hours	22.4	16.0	3.49
968	Liver	Group 1	4 hours	18.5	7.46	1.11
908	Liver	Group 2	10 hours	6.70	7.15	0.704
949	Liver	Group 2	10 hours	15.1	13.8	2.54
962	Liver	Group 2	10 hours	10.7	12.5	3.35
967	Liver	Group 2	10 hours	8.75	16.3	1.12
906	Liver	Group 3	24 hours	3.01	1.71	0.305
926	Liver	Group 3	24 hours	1.63	3.49	0.372
930	Liver	Group 3	24 hours	2.86	4.27	0.411
939	Liver	Group 3	24 hours	1.23	4.22	0.179*
927	Liver	Group 4	2 days	0.348	0.280	0.083*
959	Liver	Group 4	2 days	0.702	0.455	0.197*
963	Liver	Group 4	2 days	1.26	0.381	0.383
965	Liver	Group 4	2 days	1.73	0.809	0.249
919	Liver	Group 5	4 days	0.278	0.291	<LOD
932	Liver	Group 5	4 days	0.295	0.163*	<LOD
936	Liver	Group 5	4 days	0.249	0.206	<LOD
947	Liver	Group 5	4 days	0.585	0.277	0.074*
934	Liver	Group 6	7 days	0.127*	0.139*	<LOD
942	Liver	Group 6	7 days	0.113*	0.097*	<LOD
952	Liver	Group 6	7 days	0.110*	0.105*	<LOD
964	Liver	Group 6	7 days	0.128*	0.117*	<LOD
901	Liver	Group 7	7 days	0.090*	0.139*	<LOD
917	Liver	Group 7	7 days	0.077*	<LOD	<LOD
950	Liver	Group 7	7 days	0.088*	0.083*	<LOD
953	Liver	Group 7	7 days	0.133*	<LOD	<LOD
903	Liver	Group 8	7 days	0.168*	0.204	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
912	Liver	Group 8	7 days	0.094*	0.110*	<LOD
938	Liver	Group 8	7 days	0.075*	0.127*	<LOD
941	Liver	Group 8	7 days	0.116*	0.207	<LOD
904	Liver	Group 9	7 days	0.269	0.228	<LOD
916	Liver	Group 9	7 days	0.086*	<LOD	<LOD
928	Liver	Group 9	7 days	0.560	0.083*	<LOD
960	Liver	Group 9	7 days	0.073*	<LOD	<LOD
924	Liver	Group 10	14 days	0.298	0.078*	<LOD
944	Liver	Group 10	14 days	0.160*	<LOD	<LOD
954	Liver	Group 10	14 days	0.110*	<LOD	<LOD
958	Liver	Group 10	14 days	0.114*	<LOD	<LOD
910	Liver	Group 11	28 days	<LOD	<LOD	<LOD
935	Liver	Group 11	28 days	<LOD	<LOD	<LOD
937	Liver	Group 11	28 days	<LOD	<LOD	<LOD
961	Liver	Group 11	28 days	0.114*	<LOD	<LOD
902	Kidney	Group 1	4 hours	9.70	4.60	4.27
913	Kidney	Group 1	4 hours	56.4	9.45	1.11
915	Kidney	Group 1	4 hours	22.3	11.1	4.53
968	Kidney	Group 1	4 hours	21.7	13.1	1.39
908	Kidney	Group 2	10 hours	5.98	6.75	1.23
949	Kidney	Group 2	10 hours	17.9	7.72	3.92
962	Kidney	Group 2	10 hours	15.4	7.21	5.42
967	Kidney	Group 2	10 hours	3.81	6.50	1.53
906	Kidney	Group 3	24 hours	3.03	2.47	0.472
926	Kidney	Group 3	24 hours	2.04	4.86	0.587
930	Kidney	Group 3	24 hours	2.13	3.15	0.555
939	Kidney	Group 3	24 hours	1.43	5.46	0.310
927	Kidney	Group 4	2 days	0.278	0.401	0.081*
959	Kidney	Group 4	2 days	0.389	0.435	0.157*
963	Kidney	Group 4	2 days	0.677	0.329	0.425
965	Kidney	Group 4	2 days	1.11	1.28	0.325
919	Kidney	Group 5	4 days	0.241	0.226*	0.073*
932	Kidney	Group 5	4 days	0.176*	0.120*	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
936	Kidney	Group 5	4 days	0.169*	0.072*	<LOD
947	Kidney	Group 5	4 days	0.262	0.436	0.071*
934	Kidney	Group 6	7 days	0.150*	0.121*	<LOD
942	Kidney	Group 6	7 days	0.086*	<LOD	<LOD
952	Kidney	Group 6	7 days	0.079*	<LOD	<LOD
964	Kidney	Group 6	7 days	0.090*	0.093*	<LOD
901	Kidney	Group 7	7 days	0.134*	<LOD	<LOD
917	Kidney	Group 7	7 days	<LOD	<LOD	<LOD
950	Kidney	Group 7	7 days	<LOD	<LOD	<LOD
953	Kidney	Group 7	7 days	0.083*	<LOD	<LOD
903	Kidney	Group 8	7 days	0.197*	0.122*	<LOD
912	Kidney	Group 8	7 days	0.076*	0.100*	<LOD
938	Kidney	Group 8	7 days	0.116*	<LOD	<LOD
941	Kidney	Group 8	7 days	0.407	0.129*	<LOD
904	Kidney	Group 9	7 days	0.285	0.266	0.108*
916	Kidney	Group 9	7 days	0.075*	<LOD	<LOD
928	Kidney	Group 9	7 days	0.317	0.218*	<LOD
960	Kidney	Group 9	7 days	0.104*	0.137*	<LOD
924	Kidney	Group 10	14 days	0.235	0.089*	<LOD
944	Kidney	Group 10	14 days	0.087*	0.070*	<LOD
954	Kidney	Group 10	14 days	<LOD	<LOD	<LOD
958	Kidney	Group 10	14 days	0.086*	<LOD	<LOD
910	Kidney	Group 11	28 days	0.114*	<LOD	<LOD
935	Kidney	Group 11	28 days	<LOD	<LOD	<LOD
937	Kidney	Group 11	28 days	<LOD	<LOD	<LOD
961	Kidney	Group 11	28 days	<LOD	<LOD	<LOD
902	Perirenal Fat	Group 1	4 hours	16.5	1.64	0.612
913	Perirenal Fat	Group 1	4 hours	89.8	2.34	0.154*
915	Perirenal Fat	Group 1	4 hours	46.4	1.45	0.346
968	Perirenal Fat	Group 1	4 hours	62.0	1.81	0.171*
908	Perirenal Fat	Group 2	10 hours	18.0	0.716	0.160*
949	Perirenal Fat	Group 2	10 hours	19.1	1.06	0.385
962	Perirenal Fat	Group 2	10 hours	36.8	1.04	0.609

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
967	Perirenal Fat	Group 2	10 hours	3.65	1.48	0.432
906	Perirenal Fat	Group 3	24 hours	7.44	0.303	<LOD
926	Perirenal Fat	Group 3	24 hours	4.02	0.939	0.099*
930	Perirenal Fat	Group 3	24 hours	5.89	0.190*	<LOD
939	Perirenal Fat	Group 3	24 hours	2.70	0.812	<LOD
927	Perirenal Fat	Group 4	2 days	0.432	<LOD	<LOD
959	Perirenal Fat	Group 4	2 days	0.835	0.104*	<LOD
963	Perirenal Fat	Group 4	2 days	1.23	0.088*	0.111*
965	Perirenal Fat	Group 4	2 days	2.27	0.106*	<LOD
919	Perirenal Fat	Group 5	4 days	2.51	<LOD	0.099*
932	Perirenal Fat	Group 5	4 days	0.230	<LOD	<LOD
936	Perirenal Fat	Group 5	4 days	0.236	<LOD	<LOD
947	Perirenal Fat	Group 5	4 days	0.458	<LOD	<LOD
934	Perirenal Fat	Group 6	7 days	0.152*	<LOD	<LOD
942	Perirenal Fat	Group 6	7 days	0.196*	<LOD	<LOD
952	Perirenal Fat	Group 6	7 days	0.136*	<LOD	<LOD
964	Perirenal Fat	Group 6	7 days	0.213	<LOD	<LOD
901	Perirenal Fat	Group 7	7 days	0.167*	<LOD	<LOD
917	Perirenal Fat	Group 7	7 days	0.092*	<LOD	<LOD
950	Perirenal Fat	Group 7	7 days	0.084*	<LOD	<LOD
953	Perirenal Fat	Group 7	7 days	0.150*	<LOD	<LOD
903	Perirenal Fat	Group 8	7 days	0.148*	0.089*	<LOD
912	Perirenal Fat	Group 8	7 days	0.079*	<LOD	<LOD
938	Perirenal Fat	Group 8	7 days	0.112*	<LOD	<LOD
941	Perirenal Fat	Group 8	7 days	0.450	<LOD	<LOD
904	Perirenal Fat	Group 9	7 days	0.304	0.125*	<LOD
916	Perirenal Fat	Group 9	7 days	0.070*	<LOD	<LOD
928	Perirenal Fat	Group 9	7 days	0.552	<LOD	<LOD
960	Perirenal Fat	Group 9	7 days	0.307	<LOD	<LOD
924	Perirenal Fat	Group 10	14 days	0.288	<LOD	<LOD
944	Perirenal Fat	Group 10	14 days	0.156*	<LOD	<LOD
954	Perirenal Fat	Group 10	14 days	0.138*	<LOD	<LOD
958	Perirenal Fat	Group 10	14 days	0.161*	<LOD	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
910	Perirenal Fat	Group 11	28 days	0.111*	<LOD	<LOD
935	Perirenal Fat	Group 11	28 days	1.28	<LOD	0.136*
937	Perirenal Fat	Group 11	28 days	<LOD	<LOD	<LOD
961	Perirenal Fat	Group 11	28 days	0.106*	<LOD	<LOD
902	Mules	Group 1	4 hours	2637	165	2.91
913	Mules	Group 1	4 hours	3948	171	2.08
915	Mules	Group 1	4 hours	4620	99	4.17
968	Mules	Group 1	4 hours	15994	850	10.9
908	Mules	Group 2	10 hours	2421	46.9	3.45
949	Mules	Group 2	10 hours	3756	43.1	3.43
962	Mules	Group 2	10 hours	4946	175	3.69
967	Mules	Group 2	10 hours	474	26.2	1.29
906	Mules	Group 3	24 hours	1211	36.5	1.73
926	Mules	Group 3	24 hours	651	26.0	1.59
930	Mules	Group 3	24 hours	2622	101	4.33
939	Mules	Group 3	24 hours	504	16.6	1.08
927	Mules	Group 4	2 days	301	10.2	0.715
959	Mules	Group 4	2 days	4275	555	5.01
963	Mules	Group 4	2 days	8797	>1000	17.0
965	Mules	Group 4	2 days	1267	43.4	3.39
919	Mules	Group 5	4 days	3352	204	6.09
932	Mules	Group 5	4 days	16322	>1000	37.4
936	Mules	Group 5	4 days	10017	>1000	22.5
947	Mules	Group 5	4 days	8686	>1000	36.0
934	Mules	Group 6	7 days	14886	>1000	42.3
942	Mules	Group 6	7 days	1900	49.6	4.57
952	Mules	Group 6	7 days	8050	968	34.3
964	Mules	Group 6	7 days	3883	381	15.5
901	Mules	Group 7	7 days	6229	600	37.8
917	Mules	Group 7	7 days	2842	138	7.66
950	Mules	Group 7	7 days	5081	333	19.7
953	Mules	Group 7	7 days	1754	24.7	6.81
903	Mules	Group 8	7 days	2027	26.0	11.1

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
912	Mules	Group 8	7 days	306	18.0	7.02
938	Mules	Group 8	7 days	627	31.6	2.75
941	Mules	Group 8	7 days	986	41.2	6.22
904	Mules	Group 9	7 days	132	7.74	3.90
916	Mules	Group 9	7 days	205	12.1	2.87
928	Mules	Group 9	7 days	21.3	0.466	0.684
960	Mules	Group 9	7 days	22.5	0.609	0.471
924	Mules	Group 10	14 days	14417	>1000	40.0
944	Mules	Group 10	14 days	21572	>1000	66.7
954	Mules	Group 10	14 days	7891	>1000	36.5
958	Mules	Group 10	14 days	36630	>1000	62.8
910	Mules	Group 11	28 days	36685	>1000	376
935	Mules	Group 11	28 days	17341	>1000	53.3
937	Mules	Group 11	28 days	10844	>1000	107
961	Mules	Group 11	28 days	23749	>1000	107
902	Muscle (Heart)	Group 1	4 hours	8.33	0.487	1.32
913	Muscle (Heart)	Group 1	4 hours	38.5	0.757	0.340
915	Muscle (Heart)	Group 1	4 hours	21.1	0.634	1.09
968	Muscle (Heart)	Group 1	4 hours	25.7	0.740	0.491
908	Muscle (Heart)	Group 2	10 hours	6.94	0.817	0.426
949	Muscle (Heart)	Group 2	10 hours	15.1	0.997	0.829
962	Muscle (Heart)	Group 2	10 hours	21.0	0.440	1.26
967	Muscle (Heart)	Group 2	10 hours	2.65	0.629	0.468
906	Muscle (Heart)	Group 3	24 hours	2.28	0.212	0.086*
926	Muscle (Heart)	Group 3	24 hours	1.35	0.437	0.145*
930	Muscle (Heart)	Group 3	24 hours	1.52	0.305	0.199*
939	Muscle (Heart)	Group 3	24 hours	1.20	0.395	0.104*
927	Muscle (Heart)	Group 4	2 days	0.229	<LOD	<LOD
959	Muscle (Heart)	Group 4	2 days	0.455	<LOD	<LOD
963	Muscle (Heart)	Group 4	2 days	0.462	<LOD	0.134*
965	Muscle (Heart)	Group 4	2 days	0.793	0.110*	<LOD
919	Muscle (Heart)	Group 5	4 days	0.218	<LOD	<LOD
932	Muscle (Heart)	Group 5	4 days	0.117*	<LOD	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
936	Muscle (Heart)	Group 5	4 days	0.258	<LOD	<LOD
947	Muscle (Heart)	Group 5	4 days	0.253	<LOD	<LOD
934	Muscle (Heart)	Group 6	7 days	<LOD	<LOD	<LOD
942	Muscle (Heart)	Group 6	7 days	<LOD	<LOD	<LOD
952	Muscle (Heart)	Group 6	7 days	<LOD	<LOD	<LOD
964	Muscle (Heart)	Group 6	7 days	<LOD	<LOD	<LOD
901	Muscle (Heart)	Group 7	7 days	<LOD	<LOD	<LOD
917	Muscle (Heart)	Group 7	7 days	<LOD	<LOD	<LOD
950	Muscle (Heart)	Group 7	7 days	<LOD	<LOD	<LOD
953	Muscle (Heart)	Group 7	7 days	<LOD	<LOD	<LOD
903	Muscle (Heart)	Group 8	7 days	0.137*	<LOD	<LOD
912	Muscle (Heart)	Group 8	7 days	<LOD	<LOD	<LOD
938	Muscle (Heart)	Group 8	7 days	0.132*	<LOD	<LOD
941	Muscle (Heart)	Group 8	7 days	0.411	<LOD	<LOD
904	Muscle (Heart)	Group 9	7 days	0.175*	<LOD	<LOD
916	Muscle (Heart)	Group 9	7 days	<LOD	<LOD	<LOD
928	Muscle (Heart)	Group 9	7 days	0.334	<LOD	<LOD
960	Muscle (Heart)	Group 9	7 days	0.090*	<LOD	<LOD
924	Muscle (Heart)	Group 10	14 days	0.122*	<LOD	<LOD
944	Muscle (Heart)	Group 10	14 days	0.078*	<LOD	<LOD
954	Muscle (Heart)	Group 10	14 days	<LOD	<LOD	<LOD
958	Muscle (Heart)	Group 10	14 days	0.070*	<LOD	<LOD
910	Muscle (Heart)	Group 11	28 days	2.20	<LOD	<LOD
935	Muscle (Heart)	Group 11	28 days	<LOD	<LOD	<LOD
937	Muscle (Heart)	Group 11	28 days	<LOD	<LOD	<LOD
961	Muscle (Heart)	Group 11	28 days	0.085*	<LOD	<LOD
902	Muscle (Loin)	Group 1	4 hours	3.40	0.326	1.00
913	Muscle (Loin)	Group 1	4 hours	13.9	0.597	0.247
915	Muscle (Loin)	Group 1	4 hours	5.39	0.394	0.876
968	Muscle (Loin)	Group 1	4 hours	5.63	0.389	0.352
908	Muscle (Loin)	Group 2	10 hours	1.47	0.337	0.311
949	Muscle (Loin)	Group 2	10 hours	3.85	0.461	0.748
962	Muscle (Loin)	Group 2	10 hours	4.83	0.260	1.03

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
967	Muscle (Loin)	Group 2	10 hours	1.10	0.284	0.465
906	Muscle (Loin)	Group 3	24 hours	0.538	<LOD	0.102*
926	Muscle (Loin)	Group 3	24 hours	0.265	0.154*	0.147*
930	Muscle (Loin)	Group 3	24 hours	0.429	0.109*	0.144*
939	Muscle (Loin)	Group 3	24 hours	0.303	0.199*	0.077*
927	Muscle (Loin)	Group 4	2 days	<LOD	<LOD	<LOD
959	Muscle (Loin)	Group 4	2 days	<LOD	<LOD	<LOD
963	Muscle (Loin)	Group 4	2 days	0.162*	<LOD	0.121*
965	Muscle (Loin)	Group 4	2 days	0.209	<LOD	0.088*
919	Muscle (Loin)	Group 5	4 days	<LOD	<LOD	<LOD
932	Muscle (Loin)	Group 5	4 days	<LOD	<LOD	<LOD
936	Muscle (Loin)	Group 5	4 days	<LOD	<LOD	<LOD
947	Muscle (Loin)	Group 5	4 days	<LOD	<LOD	<LOD
934	Muscle (Loin)	Group 6	7 days	<LOD	<LOD	<LOD
942	Muscle (Loin)	Group 6	7 days	<LOD	<LOD	<LOD
952	Muscle (Loin)	Group 6	7 days	<LOD	<LOD	<LOD
964	Muscle (Loin)	Group 6	7 days	<LOD	<LOD	<LOD
901	Muscle (Loin)	Group 7	7 days	<LOD	<LOD	<LOD
917	Muscle (Loin)	Group 7	7 days	0.358	<LOD	<LOD
950	Muscle (Loin)	Group 7	7 days	<LOD	<LOD	<LOD
953	Muscle (Loin)	Group 7	7 days	<LOD	<LOD	<LOD
903	Muscle (Loin)	Group 8	7 days	<LOD	<LOD	<LOD
912	Muscle (Loin)	Group 8	7 days	0.071*	<LOD	<LOD
938	Muscle (Loin)	Group 8	7 days	<LOD	<LOD	<LOD
941	Muscle (Loin)	Group 8	7 days	<LOD	<LOD	<LOD
904	Muscle (Loin)	Group 9	7 days	<LOD	<LOD	<LOD
916	Muscle (Loin)	Group 9	7 days	<LOD	<LOD	<LOD
928	Muscle (Loin)	Group 9	7 days	<LOD	<LOD	<LOD
960	Muscle (Loin)	Group 9	7 days	<LOD	<LOD	<LOD
924	Muscle (Loin)	Group 10	14 days	<LOD	<LOD	<LOD
944	Muscle (Loin)	Group 10	14 days	<LOD	<LOD	<LOD
954	Muscle (Loin)	Group 10	14 days	<LOD	<LOD	<LOD
958	Muscle (Loin)	Group 10	14 days	<LOD	<LOD	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
910	Muscle (Loin)	Group 11	28 days	<LOD	<LOD	<LOD
935	Muscle (Loin)	Group 11	28 days	<LOD	<LOD	<LOD
937	Muscle (Loin)	Group 11	28 days	<LOD	<LOD	<LOD
961	Muscle (Loin)	Group 11	28 days	<LOD	<LOD	<LOD
902	Urine	Group 1	4 hours	0.824	80.9	3.04
913	Urine	Group 1	4 hours	8.89	277	2.09
915	Urine	Group 1	4 hours	2.85	137	3.96
968	Urine	Group 1	4 hours	4.35	294	2.43
908	Urine	Group 2	10 hours	1.25	84.0	3.03
949	Urine	Group 2	10 hours	19.2	433	20.3
962	Urine	Group 2	10 hours	1.87	69.3	8.27
967	Urine	Group 2	10 hours	13.2	648	17.4
906	Urine	Group 3	24 hours	0.501	74.0	0.493
926	Urine	Group 3	24 hours	0.319	78.0	0.893
930	Urine	Group 3	24 hours	0.339	125	0.748
939	Urine	Group 3	24 hours	0.211	105	0.365
927	Urine	Group 4	2 days	<LOD	19.2	0.125*
959	Urine	Group 4	2 days	IS	IS	IS
963	Urine	Group 4	2 days	<LOD	3.21	0.298
965	Urine	Group 4	2 days	0.155*	28.0	0.582
919	Urine	Group 5	4 days	<LOD	4.06	0.100*
932	Urine	Group 5	4 days	IS	IS	IS
936	Urine	Group 5	4 days	<LOD	2.91	<LOD
947	Urine	Group 5	4 days	0.124*	16.2	<LOD
934	Urine	Group 6	7 days	<LOD	1.82	<LOD
942	Urine	Group 6	7 days	<LOD	4.14	<LOD
952	Urine	Group 6	7 days	IS	IS	IS
964	Urine	Group 6	7 days	IS	IS	IS
901	Urine	Group 7	7 days	<LOD	2.15	<LOD
917	Urine	Group 7	7 days	<LOD	2.05	<LOD
950	Urine	Group 7	7 days	<LOD	2.58	<LOD
953	Urine	Group 7	7 days	<LOD	1.04	<LOD
903	Urine	Group 8	7 days	<LOD	6.73	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
912	Urine	Group 8	7 days	<LOD	9.62	0.101*
938	Urine	Group 8	7 days	1.41	18.7	0.391
941	Urine	Group 8	7 days	0.187*	12.7	<LOD
904	Urine	Group 9	7 days	<LOD	3.16	<LOD
916	Urine	Group 9	7 days	IS	IS	IS
928	Urine	Group 9	7 days	<LOD	10.4	0.159*
902	Urine	Group 1	4 hours	<LOD	9.58	<LOD
913	Urine	Group 1	4 hours	0.824	80.9	3.04
915	Urine	Group 1	4 hours	8.89	277	2.09
968	Urine	Group 1	4 hours	2.85	137	3.96
908	Urine	Group 2	10 hours	4.35	294	2.43
949	Urine	Group 2	10 hours	1.25	84.0	3.03
962	Urine	Group 2	10 hours	19.2	433	20.3
967	Urine	Group 2	10 hours	1.87	69.3	8.27
906	Urine	Group 3	24 hours	13.2	648	17.4
926	Urine	Group 3	24 hours	0.501	74.0	0.493
930	Urine	Group 3	24 hours	0.319	78.0	0.893
939	Urine	Group 3	24 hours	0.339	125	0.748
927	Urine	Group 4	2 days	0.211	105	0.365
959	Urine	Group 4	2 days	<LOD	19.2	0.125*
963	Urine	Group 4	2 days	IS	IS	IS
965	Urine	Group 4	2 days	<LOD	3.21	0.298
919	Urine	Group 5	4 days	0.155*	28.0	0.582
932	Urine	Group 5	4 days	<LOD	4.06	0.100*
936	Urine	Group 5	4 days	IS	IS	IS
947	Urine	Group 5	4 days	<LOD	2.91	<LOD
934	Urine	Group 6	7 days	0.124*	16.2	<LOD
942	Urine	Group 6	7 days	<LOD	1.82	<LOD
952	Urine	Group 6	7 days	<LOD	4.14	<LOD
964	Urine	Group 6	7 days	IS	IS	IS
901	Urine	Group 7	7 days	IS	IS	IS
917	Urine	Group 7	7 days	<LOD	2.15	<LOD
950	Urine	Group 7	7 days	<LOD	2.05	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
953	Urine	Group 7	7 days	<LOD	2.58	<LOD
903	Urine	Group 8	7 days	<LOD	1.04	<LOD
912	Urine	Group 8	7 days	<LOD	6.73	<LOD
938	Urine	Group 8	7 days	<LOD	9.62	0.101*
941	Urine	Group 8	7 days	1.41	18.7	0.391
904	Urine	Group 9	7 days	0.187*	12.7	<LOD
916	Urine	Group 9	7 days	<LOD	3.16	<LOD
928	Urine	Group 9	7 days	IS	IS	IS
960	Urine	Group 9	7 days	<LOD	10.4	0.159*
902	Faeces	Group 1	4 hours	0.834	>40	0.795*
913	Faeces	Group 1	4 hours	8.12	0.213	<LOD
915	Faeces	Group 1	4 hours	10.2	0.502	0.318*
968	Faeces	Group 1	4 hours	7.00	0.648	<LOD
908	Faeces	Group 2	10 hours	6.91	>40	<LOD
949	Faeces	Group 2	10 hours	9.08	28.6	0.388*
962	Faeces	Group 2	10 hours	4.67	0.287	0.140*
967	Faeces	Group 2	10 hours	7.88	>40	0.177*
906	Faeces	Group 3	24 hours	5.79	29.3	<LOD
926	Faeces	Group 3	24 hours	4.11	>40	<LOD
930	Faeces	Group 3	24 hours	4.89	>40	0.142*
939	Faeces	Group 3	24 hours	5.16	>40	<LOD
927	Faeces	Group 4	2 days	0.214	18.8	<LOD
959	Faeces	Group 4	2 days	0.249	6.10	<LOD
963	Faeces	Group 4	2 days	0.500	33.0	<LOD
965	Faeces	Group 4	2 days	1.06	>40	<LOD
919	Faeces	Group 5	4 days	0.245	9.05	<LOD
932	Faeces	Group 5	4 days	0.091*	0.100*	<LOD
936	Faeces	Group 5	4 days	0.230	9.95	<LOD
947	Faeces	Group 5	4 days	0.399	3.03	<LOD
934	Faeces	Group 6	7 days	0.156*	2.02	<LOD
942	Faeces	Group 6	7 days	0.123*	6.77	<LOD
952	Faeces	Group 6	7 days	<LOD	<LOD	<LOD
964	Faeces	Group 6	7 days	0.073*	0.90	<LOD

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
901	Faeces	Group 7	7 days	<LOD	<LOD	<LOD
917	Faeces	Group 7	7 days	<LOD	1.05	<LOD
950	Faeces	Group 7	7 days	<LOD	0.077*	<LOD
953	Faeces	Group 7	7 days	0.090*	0.079*	<LOD
903	Faeces	Group 8	7 days	<LOD	0.719	<LOD
912	Faeces	Group 8	7 days	<LOD	0.543	<LOD
938	Faeces	Group 8	7 days	<LOD	0.116*	<LOD
941	Faeces	Group 8	7 days	0.075*	6.45	<LOD
904	Faeces	Group 9	7 days	0.233	7.62	<LOD
916	Faeces	Group 9	7 days	<LOD	<LOD	<LOD
928	Faeces	Group 9	7 days	0.085*	0.743	<LOD
960	Faeces	Group 9	7 days	<LOD	1.10	<LOD
903	Plasma	Group 8	D-1	<LOD	<LOD	<LOD
912	Plasma	Group 8	D-1	<LOD	<LOD	<LOD
938	Plasma	Group 8	D-1	<LOD	<LOD	<LOD
941	Plasma	Group 8	D-1	<LOD	<LOD	<LOD
903	Plasma	Group 8	15 minutes	19.1	<LOD	0.554
912	Plasma	Group 8	15 minutes	20.5	<LOD	1.14
938	Plasma	Group 8	15 minutes	10.6	<LOD	0.212
941	Plasma	Group 8	15 minutes	3.59	<LOD	<LOD
903	Plasma	Group 8	30 minutes	12.1	<LOD	0.541
912	Plasma	Group 8	30 minutes	15.4	<LOD	1.20
938	Plasma	Group 8	30 minutes	6.20	<LOD	0.229
941	Plasma	Group 8	30 minutes	3.03	<LOD	0.08674*
903	Plasma	Group 8	60 minutes	9.90	<LOD	0.456
912	Plasma	Group 8	60 minutes	15.2	<LOD	1.04
938	Plasma	Group 8	60 minutes	5.01	<LOD	0.236
941	Plasma	Group 8	60 minutes	3.07	<LOD	0.100*
903	Plasma	Group 8	2 hours	9.59	<LOD	0.531
912	Plasma	Group 8	2 hours	20.6	<LOD	1.54
938	Plasma	Group 8	2 hours	5.25	<LOD	0.347
941	Plasma	Group 8	2 hours	4.95	<LOD	0.150*
903	Plasma	Group 8	4 hours	7.44	<LOD	0.388

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
912	Plasma	Group 8	4 hours	10.8	<LOD	1.33
938	Plasma	Group 8	4 hours	5.51	<LOD	0.392
941	Plasma	Group 8	4 hours	7.70	<LOD	0.199*
903	Plasma	Group 8	6 hours	2.83	<LOD	0.211
912	Plasma	Group 8	6 hours	4.65	<LOD	0.766
938	Plasma	Group 8	6 hours	2.43	<LOD	0.273
941	Plasma	Group 8	6 hours	5.80	<LOD	0.204
903	Plasma	Group 8	9 hours	2.38	<LOD	0.131*
912	Plasma	Group 8	9 hours	1.79	<LOD	0.432
938	Plasma	Group 8	9 hours	1.30	<LOD	0.212
941	Plasma	Group 8	9 hours	2.79	<LOD	0.167*
903	Plasma	Group 8	12 hours	1.18	<LOD	0.102*
912	Plasma	Group 8	12 hours	1.35	<LOD	0.294
938	Plasma	Group 8	12 hours	0.812	<LOD	0.144*
941	Plasma	Group 8	12 hours	2.20	<LOD	0.128*
903	Plasma	Group 8	18 hours	0.851	<LOD	0.074*
912	Plasma	Group 8	18 hours	0.720	<LOD	0.210
938	Plasma	Group 8	18 hours	0.353	<LOD	<LOD
941	Plasma	Group 8	18 hours	0.709	<LOD	0.082*
903	Plasma	Group 8	24 hours	0.839	<LOD	0.098*
912	Plasma	Group 8	24 hours	0.586	<LOD	0.168*
938	Plasma	Group 8	24 hours	0.233	<LOD	<LOD
941	Plasma	Group 8	24 hours	0.601	<LOD	<LOD
903	Plasma	Group 8	30 hours	0.589	<LOD	0.096*
912	Plasma	Group 8	30 hours	0.447	<LOD	0.111*
938	Plasma	Group 8	30 hours	0.264	<LOD	<LOD
941	Plasma	Group 8	30 hours	0.423	<LOD	<LOD
903	Plasma	Group 8	36 hours	0.445	<LOD	<LOD
912	Plasma	Group 8	36 hours	>40	<LOD	0.150*
938	Plasma	Group 8	36 hours	0.219	<LOD	<LOD
941	Plasma	Group 8	36 hours	0.391	<LOD	<LOD
903	Plasma	Group 8	48 hours	0.426	<LOD	<LOD
912	Plasma	Group 8	48 hours	0.444	<LOD	0.087*

Animal ID	Tissue	Group	Timepoint	Bupivacaine (µg/kg or L)	3-OH Bupivacaine (µg/kg or L)	N-desbutyl Bupivacaine (µg/kg or L)
938	Plasma	Group 8	48 hours	0.151*	<LOD	<LOD
941	Plasma	Group 8	48 hours	0.200	<LOD	<LOD
903	Plasma	Group 8	72 hours	0.350	<LOD	<LOD
912	Plasma	Group 8	72 hours	0.203	<LOD	<LOD
938	Plasma	Group 8	72 hours	<LOD	<LOD	<LOD
941	Plasma	Group 8	72 hours	0.153*	<LOD	<LOD
903	Plasma	Group 8	96 hours	0.265	<LOD	<LOD
912	Plasma	Group 8	96 hours	0.118*	<LOD	<LOD
938	Plasma	Group 8	96 hours	<LOD	<LOD	<LOD
941	Plasma	Group 8	96 hours	0.107*	<LOD	<LOD
903	Plasma	Group 8	7 days	0.121*	<LOD	<LOD
912	Plasma	Group 8	7 days	<LOD	<LOD	<LOD
938	Plasma	Group 8	7 days	<LOD	<LOD	<LOD
941	Plasma	Group 8	7 days	<LOD	<LOD	<LOD

12.4 Appendix 4 – AWI Communication Report Template

Name of project
Metabolism study to determine the quantity and identify the nature of parent compounds and metabolites in ovine tissue and fluids following the topical administration of TRI-SOLFEN (lignocaine, bupivacaine plus cetrimide).
Name of research body
Invetus Pty Ltd.
Name(s) of any other project co-funding bodies and funding split
Australian Wool Innovation (AWI) and Animal Ethics Pty Ltd.
Name(s) of any organisations involved (and specify how they are involved)
As above.
Project start date
Start date of the animal phase: 18 July 2017
Project end date
End date of the animal phase: 15 August 2017 End date of the laboratory phase: 22 March 2019
Other key dates (eg key milestones report(s), events , product launch)
Milestone 4002261 – 0190 - Trial Protocol Finalised – 21 June 2017 (submitted) Milestone 4002261 - 0200 – Commencement of Animal Phase – 21 June 2017 (submitted) Milestone 4002261-0210 – Completion of Animal Phase – 31 July 2017 (submitted) Milestone 4002261-0240 – Completion of Analytical Phase and Change of Study Director – 01 April 2019 (submitted)
Main objectives of the project (approx. 150 words)
The study aimed to provide data for each of the three active components of TRI-SOLFEN Topical Anaesthetic and Antiseptic Solution for Pain Relief in Lambs and Calves with the purpose of identifying and quantifying the individual component parent compound and metabolites that potentially comprise the residues of concern in edible tissues; determine the residue(s) for each compound that can serve as a marker for analytical methods intended for compliance purposes; identify target tissue or tissues, as applicable to residue monitoring programmes as well as provide some indication of the depletion of the identified residues of concern (target residues) in edible tissues).
Project description (approx. 250 words)
The basic process of the study was to apply treatment with TRI-SOLFEN and harvest plasma, edible tissues, urine and faeces from treated sheep at various time intervals through until 28 days post-treatment. These harvested samples were used to measure the levels of the actives (and their metabolites) of TRI-SOLFEN. 46 weaned Merino lambs (4 females, 42 male) were allocated to 1 of

12 groups. Blood samples were collected on Day -1. On Day 0, animals were mulesed and/or tail docked and/or castrated and TRI-SOLFEN applied to the surgical site according to the treatment regime, with dose amount based on animal bodyweight. Mulesing was completed by a certified contract surgical muleser. Other procedures were performed by and treatment was administered by Invetus Veterinarians. From time of treatment until each group was sacrificed (up until 7 days post-treatment), blood samples were collected from all animals at repeat and frequent intervals and processed for plasma collection. At sacrifice, tissues and fluid collected up until 28 days post-treatment included; urine, faeces, kidney, loin and heart muscle, liver, perirenal fat and subcutaneous tissue at the mulesing site. Tissue, fluid and plasma samples were sent frozen to Eurofins Agroscience Testing (Lane Cove, NSW) for analysis of lignocaine, bupivacaine and cetrimide including metabolites. Following receipt of the analytical results containing the tissue residue data, the metabolism profiles were graphed, and final report compiled.

Project (and key milestones) outcomes and outputs (approx. 250 words)

Key outputs from the project and their relevance include:

- Metabolism of the actives (and their metabolites) in ovine plasma, urine and faeces – relevant to pharmacokinetics of TRI-SOLFEN;
- Metabolism of the actives (and their metabolites) in edible ovine tissue – relevant to pharmacokinetics and residues in edible tissue.

The results of this study can be used in designing future pivotal tissue residue studies for determination of withholding periods and export slaughter intervals if required.

Benefits for woolgrowers and wool industry (approx. 150 words) Is the project related to other AWI-funded or other past/present research

Mulesing, on many properties throughout Australia is a routine management practice that aids in the reduction of flystrike; however, is a painful procedure. Use of TRI-SOLFEN has shown to aid in the healing response and reduction in the pain response. New and additional requirements of the livestock industries required TRI-SOLFEN to be registered for use in mulesing practices. Specifically, there has not been any metabolism or residue data reported for TRI-SOLFEN use in sheep. This study was designed to provide supportive data for the WHP associated with the product.

There is minimal published information available on the pharmacokinetics of TRI-SOLFEN; however, the data obtained from this study will allow better characterisation of residues in edible tissues and as markers for analytical methods, and therefore the definition of the WHP and ESI according to more recent compliance purposes.

Is the project related to other AWI-funded or other past/present research

The project is not related to other AWI- funded work, however AWI have co-funded other TRI-SOLFEN work for use in mulesing and tail docking in lambs, and de-horning and disbudding in calves.

Potential/real next steps in the research/project

TRI-SOLFEN has been shown to enhance wound healing and assist in the alleviation of pain at the treatment sites. The work from this project may provide data to that can expand the therapeutic claims and support further registration of TRI-SOLFEN to support other uses during husbandry procedures in livestock, both in Australia and internationally.

Names(s)/roles(s)/contact details of the potential spokesperson/people

Dr Brendan Sharpe as Study Director, Invetus Pty Ltd – 02 6770 3200 | bsharpe@invetus.com Dr Ruth Davis as Sponsor's Representative, Red Cap Solutions Pty Ltd – 02 9810 1104 | ruth@redcapsol.com.au
Names(s)/roles(s)/contact details of the key personnel in the project that can be contacted for information for communication purposes (if different from above)
As above.
Current images/video assets and potential opportunities
Nonapplicable.

12.5 Appendix 5 – List of Milestones & Dates Submitted

Milestone 4002261 – 0190 - Trial Protocol Finalised – 21 June 2017 (submitted)

Milestone 4002261 - 0200 – Commencement of Animal Phase – 21 June 2017 (submitted)

Milestone 4002261-0210 – Completion of Animal Phase – 31 July 2017 (submitted)

Milestone 4002261-0240 – Completion of Analytical Phase and Change of Study Director – 01 April 2019 (submitted)

Milestone 4002261-0220 – Final Report (herein).

12.6 Appendix 6 – Financial Summary

This project was fee for service (AWI Contract 4500009444) and the financial details of such are outlined by reference to the following documents:

Invetus Quotation Number 3220 $ 453,545.00 (excluding GST)

Contract Variation (24th April 2018) ON-00305

1st Invoice (Veterinary Health Research # 24059) $142,032.50 (excluding GST)

2nd Invoice (Invetus Pty Ltd #3219.2) $76,756.00 (excluding GST)

3rd Invoice (Invetus Pty Ltd #3219.3) $64,309.00 (excluding GST)

4th Invoice (Invetus Pty ltd #3219.4) $85,224.00 (excluding GST)

5th Invoice (awaiting acceptable of final report) $85,224.00 (excluding GST)

12.7 Appendix 7 – Any Project Intellectual Property

There was not any intellectual property arising out of this study. Australian Wool Innovation Limited retains ownership of all data generated and reported herein.

12.8 Appendix 8 – Storage of Primary Research Data (Paper based and Electronic)

All paper-based research data generated will be archived for a period of six years in the Invetus GLP archive (Invetus Pty Ltd, Armidale Research Centre, Trevenna Road, Armidale NSW) which is consistent with the Organisation for Economic Cooperation and Development (OECD) Guidelines for Good Laboratory Practice (GLP) and that of its Australian affiliate, the National Association of Testing Authorities (NATA).

All electronic data generated from this study will be archived indefinitely in line with Invetus Standard Operating Procedures (SOPs) – currently archived on secure server. Invetus SOPs are audited and approved by NATA based on compliance to the principles of GLP.

12.9 Appendix 9 – Animal Ethics Authority

une
University of New England

AUTHORITY No.: AEC17-053

ANIMAL ETHICS COMMITTEE

ANIMAL RESEARCH AUTHORITY
And Approval for Animal Experimentation

RESEARCH TEAM:	Dr Michael Chambers, Dr Bruce Chick, Dr Sarah Bailey, Dr Rad Nielsen, Dr Brendan Sharpe, Mr Henry Chambers, Mrs Jill Dawson, Mr Gavin Rogers, Mr Tim Dale, Ms Jane Lamb, Dr Tim Elliott, Mrs Jocelyn Baker, Mr James Radburn & Miss Lucy Pointing
EMERGENCY CONTACT:	Dr Michael Chambers 02 6770 3200, 02 6775 1221 or 0427 008 806

Are authorised to conduct the following research:

TITLE:	Metabolism study to determine the quantity and identify the nature of parent compounds and metabolites in ovine tissue and fluids following the topical administration of a lignocaine,

bupivacaine, adrenaline and cetrimide formulation). AETO3219AW

LOCATION(S): UNE Animal House, Ring Road, UNE NSW 2351
"Waitahuna" 658 Cluny Road Armidale NSW 2350

ANIMALS:

Species	Strain	No's Required	Procedure Details
08 - Sheep	Merino	46	3,4 & 6

This authority remains in force from **04/07/2017** to **04/07/2018** unless suspended, cancelled or surrendered.

This statement must be read in conjunction with the Conditions for Animal Experimentation at UNE as stated on the reverse.

Jo Sozou.

Jo-Ann Sozou
AEC Secretary and UNE Research