Veterinary Medicines Directorate

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NATIONAL PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Tri-Solfen Cutaneous Solution for Pigs

Date Created: **March 2022**

MODULE 1

PRODUCT SUMMARY

Name, strength and pharmaceutical form	Tri-Solfen Cutaneous Solution for Pigs, Cutaneous solution
Applicant	Dechra Limited, Snaygill Industrial Estate, Keighley Road, Skipton, North Yorkshire, BD23 2RW
Active substance	Lidocaine Bupivacaine Adrenaline/Epinephrine Cetrimide
ATC Vetcode	QD04AB51
Target species	Pigs
Indication for use	Local anaesthesia during and following castration of piglets, and provision of castration wound antisepsis.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Product Information Database of the Veterinary Medicines Directorate.

(www.gov.uk/check-animal-medicine-licensed)

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	MA application submitted in accordance with Article 12.3 of Directive 2001/82/EC, as amended by 2004/28/EC
Date of conclusion of the procedure	25/01/2022

I. SCIENTIFIC OVERVIEW

The application is for an MA submitted in accordance with Article 12.3 of Directive 2001/82/EC, as amended by 2004/28/EC.

The proposed dosage is 1 or 2 ml, depending on the weight of the piglet, divided equally between the two spermatic cords and the cut skin edges.

The proposed indication is local anaesthesia during and following castration of piglets, and provision of castration wound antisepsis.

The product is produced and controlled using validated methods and tests which ensure the consistency of the product released on the market. It has been shown that the product can be safely used in the target species, any reactions observed are indicated in the SPC.[1] The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC. The efficacy [2] of the product was demonstrated according to the claims made in the SPC. The overall benefit/risk analysis is in favour of granting a marketing authorisation.

II. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTIUENTS

II.A. Composition

The product contains 40.6 mg Lidocaine (equivalent to 50 mg lidocaine hydrochloride monohydrate), 4.2 mg bupivacaine (equivalent to 5 mg bupivacaine hydrochloride monohydrate), 0.025 mg adrenaline (equivalent to 0.045 mg adrenaline tartrate) and 5.0 mg cetrimide. The product contains the excipients Sodium metabisulfite, Sodium hydroxide (for pH adjustment), Sulfuric acid (for pH adjustment), Sorbitol, liquid (crystallising), Hydroxyethylcellulose,

[1] SPC – Summary of product Characteristics.
[2] Efficacy – The production of a desired or intended result.

Citric acid (anhydrous), Disodium edetate, Brilliant Blue FCF (E133) and Purified water.

The container/closure system consists of 250 ml, 500 ml, 1 litre and 5 litre high-density polyethylene containers with a polypropylene cap, with an induction seal. Included with the product is a polypropylene spigot cap, with or without a low-density polyethylene dip tube, and a polypropylene/ polyoxymethylene dosing gun with brass nozzle and polyvinyl chloride tubing. The particulars of the containers and controls performed are provided and conform to the regulation.

The choice of the formulation and the absence of preservative are justified.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

II.B. Description of the Manufacturing Method

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site. The manufacturing method consists of a straightforward sequential addition and mixing process, with pH adjustment and inerting of the headspace with nitrogen.

The product is manufactured using conventional manufacturing techniques. Process validation for full-scale batches will be performed post-authorisation.

II.C. Control of Starting Materials

The active substances are Lidocaine, Bupivacaine, Adrenaline/Epinephrine and Cetrimide, they are established active substances described in the European Pharmacopoeia. The active substances are manufactured in accordance with the principles of good manufacturing practice.

The active substance specifications are considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification were provided.

Each of the active substances was supported with a CEP issued by the EDQM.

Apart from Brilliant Blue FCF (E133) and 30% sulfuric acid solution, reference was made to the current European Pharmacopoeia monographs as specifications for each excipient. The specifications for Brilliant Blue FCF (E133) and 30% sulfuric acid solution (E513) were in accordance with their JECFA monographs and were considered satisfactory.
The packaging for the final product was well described and supported by appropriate specifications, technical drawings, and regulatory compliance statements.

II.C.4. Substances of Biological Origin

There are no substances within the scope of the TSE Guideline present or used in the manufacture of this product.

II.D. Control Tests Carried Out at Intermediate Stages of the Manufacturing Process

Not applicable.

II.E. Control Tests on the Finished Product

The finished product specification controls the relevant parameters for the pharmaceutical form. The tests in the specification, and their limits, have been justified and are considered appropriate to adequately control the quality of the product. Satisfactory validation data for the analytical methods have been provided. Batch analytical data from the proposed production site have been provided demonstrating compliance with the specification. Control tests on the finished product are those for: appearance, weight, pH, viscosity, identification, assays and microbial limits.

II.F. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life when stored under the approved conditions.

G. Other Information

Shelf life of the veterinary medicinal product as packaged for sale: 2 years
Shelf life after first opening the immediate packaging: 3 months

III. SAFETY AND RESIDUES DOCUMENTATION (PHARMACO-TOXICOLOGICAL)

III.A Safety Documentation

Pharmacodynamics

The pharmacodynamic properties of the four active substances are summarised below:

Lidocaine is an amide local anaesthetic which works by blocking sodium channels. It is characterised as having a fast onset of action, within 30 seconds of application to mucosal tissues.

Bupivacaine is also an amide agent that, like lidocaine, works by blocking ion channels to prevent nerve conduction. Bupivacaine has a slower onset of action when compared to lidocaine, but a longer duration of action (1 hour). Lidocaine in combination with bupivacaine results in an additive effect of both, with the rapid onset of action of lidocaine and the prolonged effect of bupivacaine.
Adrenaline (also known as epinephrine) is a hormone produced naturally by the adrenal glands. It is a neurotransmitter and is an agonist of alpha and beta-adrenergic receptors. Alpha-adrenergic stimulation by adrenaline leads to vasoconstriction. Topical adrenaline produces vasoconstriction at the treated site, counteracting the vasodilatory properties of the local anaesthetics. By reducing the rate of systemic absorption of the active substances, this results in prolonging the anaesthetic effect, minimising the risk of systemic toxicity, and helping produce haemostasis.
Cetrimide is an antiseptic that works by binding strongly to skin and mucous membranes. Cetrimide delivers its antiseptic effect by reducing surface tension between bacterial cell membranes, causing cellular disintegration and leakage of cell contents.

Pharmacokinetics

Lidocaine and bupivacaine distribution is extensive with the ability to cross the blood-brain and placental barriers, and can be found in milk. There is initial widespread distribution, particularly to highly perfused tissues including kidney, liver, lung and heart, followed by slower redistribution to muscle and fat. These local anaesthetics are eliminated more slowly from fat. Metabolism, through hydroxylation and alkylation via the P450 pathway, and (for lidocaine), amide hydrolysis via hepatic carboxylesterase enzymes, occurs rapidly in the liver with elimination via urine.
Adrenaline is poorly absorbed following topical administration. It is very rapidly metabolised by the liver with a plasma half-life of 2.5 minutes. It is metabolised by catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) to inactive metabolites which are then excreted in the urine after conjugation with glucuronic acid or sulphates.
Cetrimide is poorly absorbed due to its cationic nature and binds strongly to the skin surface, mucosae and tissues. It is rapidly excreted in bile and faeces, mostly unmetabolized.

Toxicological Studies

The applicant has provided bibliographical data on the below aspects:

- Single Dose Toxicity

Several literature references were used to assess the acute toxicity of lidocaine. Acute toxicity of lidocaine was determined in rats, mice, dogs, and monkeys. From the available data, it was concluded that acute toxicity is dependent on the route of administration, since acute toxicity of lidocaine is of a similar order of magnitude following oral, SC, or IM administration, but somewhat higher when given IP and even more so after IV administration. Demonstrated in one study in

rats where IV administration gave an LD_{50} value of 27.8 mg/kg bw compared to IM administration in another giving an LD_{50} value of 260 mg/kg bw. Similar trends were shown in studies with mice where an LD_{50} value of 133 mg/kg bw was obtained via the IP route compared to an LD_{50} value range of 200 – 400 mg/kg bw, after oral and SC administration. The adverse effects after single exposure are linked to the intended pharmacodynamic action of lidocaine, i.e. initially there is CNS depression and convulsions, followed by cardiopulmonary toxicity.

A number of adverse effects following a single administration of bupivacaine, via several exposure routes and in various species (IV, SC, IP, intranasal, corneal, and intratracheal), are reported in the published literature. Like with lidocaine, it was concluded that acute toxicity of bupivacaine is dependent on the route of administration. For instance in one of the reviewed studies in mice, LD_{50} values range from 7.8 mg/kg bw (IV) to 82 mg/kg (SC). Severe systemic toxicity, such as seizure, ventricular arrhythmia, respiratory depression, or cardiovascular collapse, were observed at bupivacaine plasma levels from 4 µg/ml upwards in dogs, monkeys, piglets, and pigs. It was concluded that adverse effects after single exposure cause CNS, cardiac and circulatory toxic changes that are caused by the intended pharmacodynamic action of bupivacaine.

A sufficiently comprehensive overview of published data available on adrenaline, regarding its administration or exposure in humans via various routes (topical, oral, parenteral), including exposure via intact and broken skin and across mucosal membranes was provided and did not raise concerns.

Cetrimide has a long history of safe use in human medicine and is considered to have a low toxic potential.

- Repeated Dose Toxicity

Lidocaine: No repeat dose studies with lidocaine were submitted by the applicant but plenty of published literature and other studies have been referenced. A lower bound NO(A)EL value of 63.4mg/day (approximately 1md/kg/day in a 60kg adult) for repeat dose toxic effects of lidocaine in humans was established from a dermally applied dose of 35mg.kg,

Bupivacaine: As well as citing a number of repeat-dose toxicity studies with bupivacaine from the published literature, two older, proprietary sub-acute studies in rats and rabbits administered levobupivacaine by the SC route were submitted. In rats, a of NOAEL 20 mg/kg bw/day was derived based on convulsive episode at the next higher dose level of 30 mg/kg bw/day. In rabbits a LOAEL of 20 mg/kg bw/day was derived based on slightly impaired mobility.

Cetrimide has a long history of safe use in human medicine and is considered to have a low toxic potential.

- Reproductive Toxicity, including Teratogenicity:

Concerning reproductive and developmental toxicity, for lidocaine, NO(A)ELs were generally the highest doses tested. From the data provided, which included two FDA reports for new drug applications (NDAs) comprising several GLP-compliant studies, and published literature, it could be concluded that adverse reproductive and teratogenic effects are not expected at subcutaneous doses up to 60 mg/kg/day. Studies in rats looked at the effects of exposure to lidocaine at various reproductive stages (implantation, embryogenesis, organogenesis, foetal development and growth) and also post-natal growth. Data from three embryofoetal studies in rabbits were also provided. The data presented indicated no reproductive or developmental safety risks from lidocaine exposure. Data from human therapeutic use of lidocaine was discussed and exposure to lidocaine in pregnancy in humans was also investigated. Overall the findings in humans were consistent with results from studies in animals.

Reproductive/developmental toxicity study data for bupivacaine was also submitted, including subcutaneous studies of fertility and embryo-foetal development as well as pre- and post-natal development in rats and rabbits. For the purpose of risk assessment, a LO(A)EL for developmental toxicity of 5 mg/kg bw/day is considered appropriate (a maternal NO(A)EL of 5 mg/kg bw/day was also established).

Cetrimide has a long history of safe use in human medicine and is considered to have a low toxic potential.

- Mutagenicity

In order to evaluate the genotoxic potential of lidocaine, bupivacaine and its metabolites (i.e., 2,6-xylidine), extensive data from the published literature, together with two proprietary GLP-compliant studies for bupivacaine (an *in vitro* bacterial gene mutation assay and an *in vitro* chromosomal aberration test) were submitted. Also submitted was a combined acute bone marrow micronucleus/comet study performed with the metabolite 2,6-xylidine. Regarding the parent compounds themselves, these data were sufficient to conclude that there is little concern regarding genotoxicity in humans; however, concerning 2,6-xylidine, its detoxification is saturated above a certain dose *in vivo*, resulting in metabolites that potentially may drive genotoxicity through ROS (reactive oxygen species) formation. As a consequence, a threshold mechanism of action can be assumed and risk assessment of systemic exposure to 2,6-xylidine should be based on carcinogenicity data.

- Carcinogenicity:

No carcinogenicity studies using lidocaine are available, instead a review of available literature about lidocaine carcinogenicity was submitted. A metabolite of lidocaine, 2,6-xylidine, has been associated with carcinogenicity in rats. A two-year carcinogenicity study in which rats were given 2,6-xylidine, in feed, at doses of up to 3000 ppm/day (219 mg/kg bw/day) was provided. This study established a TD25 value of 25.9 mg/kg bw/day and this was considered a satisfactory toxicological value for use in risk characterisation. A NO(A)EL was not

established in the study. No carcinogenicity studies using bupivacaine are available. Bupivacaine is not known as an animal or human carcinogen and there is no concern for genotoxicity of bupivacaine in humans.

Observations in Humans

Lidocaine is authorised as a human medicine for local anaesthesia in a variety of indications. Therefore, data from human exposure are available. The routes of administration are numerous: Topical, oral, epidural, etc. Bibliographical data were provided which show that based on data on the local pharmacological effects of an oromucosal gel containing lidocaine, a topical pharmacological LO(A)EL of 10 mg/person/day is defined for local effects.

In studies with bupivacaine lozenges, an oral dose of 5 mg per person resulted in local analgesia and may be considered as the local pharmacological LO(A)EL. Systemically, the absence of cardiovascular or CNS side effects in humans up to an oral dose of 100 mg bupivacaine per person for up to 7 days, with plasma levels remaining below the threshold for systemic pharmacological effects (<1 µg/ml), has been used to establish a pharmacological NO(A)EL of 100 mg/day.

Microbiological Studies

Additional studies/ bibliographical data were provided which show that following ingestion, both lidocaine and bupivacaine are highly and rapidly absorbed. This greatly reduces the fraction of the administered dose that may reach the hindgut/colon so very little is available to the microorganisms. In humans, lidocaine and bupivacaine are principally excreted in the urine (as metabolites) and faecal excretion is low. For lidocaine, based on the available data, it was considered that 16% of an orally administered dose (based on urinary recovery of 84%) is a conservative estimate of the fraction of lidocaine excreted in the faeces in humans and which may be bioavailable for gut microflora. Based upon similar data available for bupivacaine in humans, the same estimate is also considered to be a conservative estimate for bupivacaine.

- Studies on Metabolites, Impurities, Other Substances and Formulation.

The applicant has provided data from the published literature on each of the active substances. The available data from the published literature suggest that the active substances (with the exception of adrenaline) may elicit hypersensitivity type reactions in sensitised individuals and that the formulation may cause skin and eye irritation (in particular due to its low pH). The proposed user warnings mitigate these risks.

Regarding the excipients. Sorbitol is commonly used in human medicines/ cosmetics /foodstuffs and is seen as unlikely to cause skin/eye irritation or hypersensitivity type reactions. Hydroxyethylcellulose is not a skin irritant but is a slight ocular irritant. Citric acid and disodium EDTA are not expected to be irritants at the levels present in the formulation. Brilliant Blue (E133) is also not

considered an eye/skin irritant nor expected to cause hypersensitivity reactions. Sodium metabisulfite is thought to possibly cause hypersensitivity type reactions with topical exposure. The user warnings have been revised to reflect these findings.

User Safety

A user risk assessment was provided in compliance with the relevant guideline.

Warnings and precautions as listed on the product literature are adequate to ensure safety to users of the product. Therefore the following applicant's user recommendations are appropriate:

- Contact with skin or eyes can cause irritation and repetitive exposure can lead to allergic reactions. Pharmacological effects (i.e. local anaesthesia) are likely to occur in case of contact with the product.
- Lidocaine and bupivacaine can form a metabolite (2,6-xylidine) in humans, which can induce carcinogenic effects at high doses in long-term toxicology studies in rats.
- Avoid skin, eye or oral contact with the product. Wear disposable impermeable gloves when handling the product and treating animals.
- In case of accidental spillage onto skin, wash off immediately with soap and water.
- Avoid ingestion of and do not smoke or eat while handling the veterinary medicinal product.
- In case of accidental ingestion, seek medical advice and show the package insert to the physician.
- People with known hypersensitivity to any of the active substances or to any of the excipients (e.g., sodium metabisulfite) should administer the product with caution. Exposure to this product whilst using another medicinal product which also contains a locally acting amide anaesthetic may cause cross sensitivity.
- Wash hands thoroughly after use.

Environmental Safety

The Environmental Risk Assessment (ERA) was carried out in accordance with VICH and CVMP guidelines.

Phase I:
In accordance with VICH guidance, as this is a combination product, the applicant has summed the amount of active substances (lidocaine, bupivacaine and cetrimide) in their calculation of the PEC_{soil}. The applicant did not include adrenaline in their calculations on the basis that it is present in very small amounts in the product (0.025 mg/ml) and would also be exempt from an environmental assessment as it is a naturally occurring substance (i.e., an endogenous hormone) which is rapidly metabolised/degraded.

Since the product is only indicated for use in piglets up to 7 days of age, the applicant adapted several of the default values provided in the CVMP guidance for other categories of pig (e.g., bodyweight, animal turnover, nitrogen production, and fraction of herd treated) in order to calculate the $PEC_{soil.}$ (34.6 µg/kg). In most part, the applicant has provided sufficient justification for the revised input values and it was accepted that they could represent the reasonable worst-case exposure.

The ERA concluded in Phase I at Question 17 of the decision tree, on the basis that the PEC_{soil} value calculated for the target species category (piglets under 7 days of age) was below the threshold value (100 µg/kg) for progressing to a Phase II risk assessment. The product is not expected to pose a risk for the environment when used in accordance with the recommendations included in the proposed SPC.

III.B.2 Residues documentation

Residue Studies

The applicant provided four proprietary studies with information on the pharmacokinetic and residue depletion patterns of the active substances contained in the proposed formulation of the product. Of these studies, one was considered to be the pivotal study, as it is stated to be conducted with the final formulation. It was also performed using the target population and following the recommended use of the product. Samples of tissues were taken from animals at several time points. Sufficient information regarding residues of lidocaine, bupivacaine and cetrimide in tissues at different timepoints were available from this study to establish a withdrawal period for the product. The applicant also provided clear arguments as to why no consumer risk should be expected from the presence of adrenaline in the formulation.
The applicant proposed a zero-day withdrawal period for this product, based on the 'No MRL required' status of the active substances, the low residue concentrations found in the residue studies, and the fact that the target animal is the male piglet up to 7 days of age, which, according to the applicant, will not enter the food chain for some months after treatment.

MRLs

All of the active substances of the formulation are listed in Table 1 of Regulation 37/2010 with a 'No MRL required' status for the target species.

The excipients all have a 'No MRL required' status or are considered to be not pharmacologically active at the doses given.

Withdrawal Periods

Based on the data provided, a withdrawal period of zero days for meat in male piglets up to 7 days is justified.

IV. CLINICAL DOCUMENTATION

IV.I. Pre-Clinical Studies

Pharmacology

The applicant provided bibliographical information describing the pharmacodynamic properties of the active substances.

The applicant provided three pharmacokinetic studies, two in piglets slightly older than the target population and one in the target population. These studies established the basic absorption, distribution, metabolism and excretion of the active substances lidocaine, bupivacaine and cetrimide.

Tolerance in the Target Species

The applicant conducted a target animal tolerance study using multiples of the recommended dose in the target species (0x, 1x, 3x and 5x). Multiples of duration were not investigated owing to rapid clotting/sealing of castration wounds. The study animals consisted of 48 entire male commercial hybrid piglets, aged 3-6 days and weighing between 1.51kg and 3.14kg on Day 1. Parameters evaluated included clinical observations, haematology/ biochemistry, gross pathology and histopathology. The blood and pathology results were unremarkable. Minimal adverse effects (transient application site inflammation) were seen following doses up to 5 times the recommended dose, but this was similar to that seen at the normal dose. Most of the piglets had healed well within seven days of castration.

IV.II. Clinical Documentation

Laboratory Trials

The applicant provided two studies from published literature in which Tri-Solfen was used. The applicant also conducted three proprietary studies with Tri-Solfen: a dose titration and methodology evaluation for efficacy study, a dose confirmation study; and a field efficacy and safety clinical trial.

Dose confirmation studies:

Study title	Pivotal study for the determination of the analgesic efficacy of TRISOLFEN, when applied topically to piglets undergoing surgical castration. Sponsor reference: AETP18176F
Objectives	This study was designed to determine, under clinical field use conditions the efficacy of TRISOLFEN topical anaesthetic formulation to alleviate pain during and following castration in piglets, when applied topically to the wound during surgical castration. Evaluation was via established pain measurement methodologies in

	piglets including vocalisation assessment, motor responses and assessments of direct sensory testing including a 300 g von Frey filament and a needle.
Test site(s)	Animal Phase: BettaPork Pty Ltd, 2242 Valentine Plains Road, Valentine Plains Queensland 4715 Australia.
Compliance with Regulatory guidelines	Good Clinical Practice (GCP) compliant
Test Product	Tri-Solfen, composition: 50.5 g/l Lignocaine hydrochloride monohydrate, 5.03 g/l Bupivacaine hydrochloride monohydrate, 0.0475 g/l Adrenaline acid tartrate and 4.87 g/l Cetrimide. Treatment dose: 1 – 2 kg BW, 1.0 ml 2.1 – 4 kg BW, 2.0 ml
Control product/placebo	0.9% saline solution (Baxter, Batch: W47P5, Expiry: April 2020) with blue food dye added at a rate of 2.5 ml per 250 ml saline. 2.0 ml dose given to animals in control group
Animals	40, porcine, male, Commercial hybrid, 3 – 7 days, 1.58 – 3.01 kg bw at allocation. Eligibility: Normal health Inclusion criteria: Entire males, 3-7 days old, no treatment with products containing the same active constituents as the investigatory veterinary product (IVP) within the animal's lifetime. Exclusion criteria: Animals that are debilitated, suffering from disease or injury, fractious or otherwise unsuitable for inclusion in the study, in the opinion of the Investigator, will be excluded.
Randomisation	Animals randomly allocated to two groups
Blinding	Animals in Group 1 were treated with blue saline solution (placebo product) which was of similar colour to the IVP. Piglet's motor response to castration assessed by blinded observer using video footage. Blinding arrangements for wound sensitivity measurements not clear from protocol and FSR.
Method	**Study Day -1; 04 Dec 2018** Selected litters and confirmed suitability of selected animals. Identified (using uniquely numbered ear tag) and weighed individual piglets. Allocated piglets to treatment groups. **Study Day 0; 5 Dec 2018** <u>Surgical and treatment procedures</u> Piglets restrained in a piglet cradle, castrated and treatment applied as described above.

	Intra-operative video and audio recording Sound and video recording during castration as detailed below. Post-operative wound sensitivity testing and clinical observations Assessed sensory threshold at each surgical site at ~ 1 minute, and then ~1, 2, 4, 8 and 12 hours post-treatment, as detailed below. Clinically observe animals at each of the pain assessment time points. **Study Day 1; 06 Dec 18** Post-operative pain assessments & clinical observations Assessed sensory threshold at each surgical site and on each animal using the methodology as detailed below at ~24 hours post-treatment. Clinically observed animals. **Efficacy measurements:** a) Behavioural motor response during castration A video camera recording device was used to record from time of application of the skin surface treatment to approximately 5 seconds following the severance of the second spermatic cord for each piglet, with piglets clearly identified throughout the recording. The behavioural response to castration was assessed using an intensity scale of 0 to 2 at each of four time points: traction of first testis; cutting first spermatic cord; traction of second testis and cutting second spermatic cord. Therefore, a total maximum score of eight was possible for each piglet. b) Audio responses during castration Sound was recorded during castration by holding a recorder approximately 60 cm from the snout. A verbal cue was given immediately preceding spermatic cord severance. The generated soundwaves were downloaded and supplied to a Sound Consultant who was blinded to piglet treatment. c) Wound sensitivity testing during and after castration The sensitivity threshold of each piglet to a 300 g von Frey filament (vFF) and then pin-prick (18G, 1.5 inch needle) at the castration site was assessed at ~1 minute, and then ~1, 2, 4, 8 and 12 hours post-

	treatment at four sites. These four sites comprised the two lateral aspects of the cut edge of the castration wound and the intact skin at the dorsal and ventral aspects of the castration wound ~3 mm from the cut edge. Each assessment was scored on a scale of 0-3.
Statistical method	The protocol stated that 'key pain measurement parameters (video and von Frey/needle scores and audio data) will be compared as appropriate using suitable software (Spotfire 5+, Version 8.2 for Windows, TIBCO Software Inc 2010 or equivalent). Data for efficacy categores "a" and "b" were compared using both parametric t-tests and equivalent non-parametric test (Wilcoxon rank-sum tests) as the underlying data distribution was not fully known. Statistical comparisons were performed using S+.
RESULTS	
Outcomes for endpoints	a) Behavioural motor response during castration Tri-Solfen treated piglets demonstrated numerically lower group mean motor response scores associated with traction of each testis and cutting of each spermatic cord. The group mean total motor response score of Tri-Solfen treated piglets (3.7/8) was significantly different to that of placebo treated counterparts (6.9/8) ($p<0.0001$). b) Audio responses during castration The applicant reported the audio AUC (decibels x time) prior to cutting the spermatic cord i.e. during traction on the first testis ('pre-cut'), after cutting the spermatic cord ('post-cut') and in 'total' (i.e. pre-cut plus post-cut data combined). Treatment with Tri-Solfen resulted in a significant reduction in audio AUC 'pre-cut' ($P<0.0001$). No significant differences were observed in 'post-cut' ($P=0.968$) or 'total' audio AUC ($P=0.495$). c) Wound sensitivity testing during and after castration Wound sensitivity data were analysed in multiple different ways using different statistical methods. Data were collated by treatment group, location (cut edge or intact skin), time point and method of stimulation (vFF or needle stimulation). Total scores for cut edge, intact skin and overall were calculated. Overall the data suggest a possible effect of Tri-Solfen on castrated piglets under the conditions of the study. Total pain scores were significantly lower in Tri-Solfen treated than

	placebo treated piglets. The duration of the treatment effect was up to 1 hour by most methods.
DISCUSSION	It was concluded that the application of Tri-Solfen to the castration wound at the time of surgery and prior to testes removal afforded significant analgesic efficacy in comparison to placebo treated piglets from the time of teste removal (based on motor response scores from video assessments and vocalisations prior to severance of the spermatic cord) and through to and including ~1 hour post-treatment (based on wound sensitivity assessments).

Field Trials

Study title	Multi-site safety and efficacy field study in pigs – study to confirm the safety and efficacy of Tri-Solfen a topical anaesthetic and antiseptic solution for pain relief in piglets during and following castration when applied at the recommended dose level. Sponsor reference: PN2182
Objectives	This study is designed to confirm the safety and efficacy of Tri-Solfen, a topical anaesthetic and antiseptic solution for pain relief in piglets during and following castration when applied at the recommended dose level to piglets under commercial production conditions.
Test site(s)	Am Bartelsbusch 2, 23911 Pogeez, Klein Disnack, Germany. Bompieri Marco/Natural Healthy Pig, Via Rossa Baselle, Lombardy, Italy. Multicentred, negatively controlled, randomised and blinded field study performed on two farm sites; one in Germany (DE) and one in Italy (IT).
Compliance with Regulatory guidelines	Good Clinical Practice (GCP) compliant
Test Product	Tri-Solfen, composition: 40.37 g/l Lidocaine (as hydrochloride), 4.16 g/l Bupivacaine (as hydrochloride), 24.89 mg/l Adrenaline (as acid tartrate). 4.84 g/l Cetrimide. Treatment dose: 0.5-1 ml/kg BW (<2 kg BW pigs received 1.0 ml; ≥2 kg BW piglets received 2.0 ml).
Control product/placebo	None. Castrated piglets which did not receive treatment acted as the control and were physically handled and castrated in the same way as the piglets which received treatment/IVP.
Animals	173 piglets: Commercial hybrids, Danish and Landrace x Large White (DE site) and Duroc x Landrace x Large

	White (IT site). Entire males, 3-7 days old on Study Day 0, bodyweight 1.2--4.1 kg on Study Day -1. Healthy with normal conformation with no birth defects. *Inclusion criteria:* Entire males; 3-7 days of age on Study Day 0; weighed ≥1 kg on Study Day -1; from a litter of piglets which had at least 6 and a maximum of 12 male piglets; had confirmation that blue spray marker had been applied to the scrotum on Study Days -3 and -1. Routine husbandry procedures (e.g. iron injection, teeth clipping, ear tagging) were permitted provided they were performed at or before Study Day -3 or else postponed until after the collection of efficacy data (on Study Day 1). *Exclusion criteria:* Birth defects; had received antibiotic treatment since birth; had received any other treatment which may have affected the response to pain or the wound healing process since birth; had other wounds or obvious sources of pain inflammation or infection present; piglets (or corresponding sows) which had received any of the actives in the IVP or pain relief medication (e.g. Xylazine) within the previous 48 hours (prior to Study Day 0) which potentially may confound clinical responses; were tail docked; recent (within the previous 48 hours prior to study inclusion) or current treatment which may have had an effect on analgesia or wound healing.
Randomisation	Included piglets were randomly allocated to treatment according to the Random Treatment Allocation Plan (RTAP) produced by Triveritas. Each site was provided with a site specific RTAP. The study protocol had planned that the piglets would then be assigned to treatment based on the order of “catch” (from the selected piglets included on Study Day -1, as detailed above). However, litters 1-4 in Germany and all litters from Italy were assigned to treatment in numerical ear tag order (e.g. piglet DE0101 assigned to the first treatment group on the randomisation, DE0102 assigned to the second treatment group and so on). This deviation was not considered to have an impact on the validity of the study as the approach was still random.
Blinding	Personnel who made clinical observations were blinded to the treatment groups because treatments were administered by a separate member of the study site personnel (the treatment administrator). Study personnel conducting safety and clinical observations did not have access to the treatment records, inventory

	records or the randomisation and were not present during the treatment administration. To assist in the blinding of the clinical observations all study piglets had standard blue spray-on piglet marker dye applied to the scrotum on Study Day -3 and Study Day -1, and product run-off from treated piglets was physically wiped off.
Method	**Administration of test product:** The IVP was administered once on Study Day 0. IVP brought to (approximately) room temperature prior to application to minimise reaction to the application. IVP was delivered via a study specific applicator which delivered 1.0 ml in 0.1 ml graduations. The applicator tip was bulb-shaped to prevent tissue trauma during treatment. Each piglet was gently restrained to expose the ano-genital region of the piglet by use of a cradle. The following procedure was then performed: • With the piglet restrained and with time allowed to permit the piglet to settle/become calm within the cradle, the handler pinched the scrotal skin and underlying tunica vaginalis compressing both testicles towards the anus. • The pinched scrotal skin and underlying tunica vaginalis area was then incised in a single transverse cut at the level of the upper 1/3 of the testis to expose and exteriorise the testis. • Tri-Solfen was then applied (to the IVP piglets) into the scrotal sac, following the incision of the scrotal skin, tunica vaginalis and exposure of the testicles. Half the total dose was applied to each side. Care was taken to ensure that the IVP coated the spermatic cord as well as the cut edges of the scrotal sac. • A period of 30 seconds was then allowed to elapse. • The testis was then removed by severing the cord as per routine procedure, taking care to minimise traction on the cord during severance. **Observations/measurements:** <u>Motor responses</u> Measured by the assessment of motor response during the procedure at four different time points: a) traction on first testis, b) first spermatic cord severance, c) traction on second testis, and d) the cutting of the second spermatic cord. Motor response grading scale: 0 = No motor response; 1 = Mild motor response such as short lived leg extension or front leg paddling or kicking but no major body resistance movement in the cradle); 2 =

	Marked motor response such as prolonged leg movements or marked body resistance movement in the cradle. Scores were added to form a total motor response score out of 8. The behavioural response during the castration procedure was determined from video recording. To ensure consistency, all scoring was performed by the same person. <u>Scan sampling</u> Observations were conducted during an approximate three-hour period in the mornings and an approximate two-hour period in the afternoons on Study Day -1, 0 and in the morning only on Study Day 1. On Study Day 0, morning scan samples were performed as soon as possible post castration/treatment with the afternoon assessments being performed later that same day. During the scan sampling, behaviours were recorded approximately every 10 minutes. <u>Focal assessments</u> Focal assessments were conducted twice on Study Day -1 (morning and afternoon), and post castration/treatment on Study Day 0/1 at approximately 1 (+1 min), 15 (+/- 2 mins), 30 (+/- 5 mins) minutes and then at approximately 60 and 90 minutes, and at 2, 3, 6, 8, 24 and 30 hours. Pain related behaviour of individual piglets was recorded at each assessment time point. Piglets were observed continuously for approximately 1 minute and all activities were recorded. Piglets were scored as 1= behaviour present or 0= behaviour absent for each of the behaviours. <u>Audio recording</u> Sound response of piglets during the castration procedure was recorded using a validated Digital Sound Level Meter (DSLM) measuring and logging peak audio values each second. The DSLM recorded was mounted ca. 60cm away from the snout of the piglet. Recording started approximately 20 seconds into the 30 second wait following the treatment (or the period of wait to mimic treatment for control piglets), i.e. approximately 10 seconds prior to castration. This is except for piglets from Italy where the video was started approximately 10 seconds into the 30 second wait. <u>*Safety-related*</u> Safety observations and measurements were made on Days -1, 1, 6 and 12 and comprised clinical examinations, including measurements of rectal temperature, body weight, wound healing (days 6 and 12 only) and adverse events. At each timepoint, wounds were scored as follows: 1 completely healed

	(no scab); 2 slight scab present at site of incision; 3 fully formed scab over wound (thick and bumpy in appearance); 4 fully formed scab over wound (thin in appearance); 5 wound still open and signs of fresh blood; 6 wound still open and looks raw.
Statistical method	*Differences between treatment groups and study sites* Any differences in weight between treatment groups and between study sites, along with any evidence of an interaction between treatment group and study site were tested at the 5% level using a generalised linear model (GLM) with normally distributed outcome and linear link. *Primary efficacy parameters* The motor response data collected during the castration procedure was analysed using an ordinal regression on the ordinal behavioural response scale. Efficacy was determined by demonstrating statistically significant differences at the 5% level. Analysis of pain control following castration was performed using a binomial model, including treatment, time, and if it was significant at the 5% level an interaction between the two. Effects significant at the 5% level were kept in the final model and those not significant at the 5% level were removed by backwards elimination. Efficacy was determined by demonstrating a statistically significant difference between treatments or a statistically significant difference in reduction in pain-associated behaviour over time at the 5% level. *Secondary efficacy parameters* The peak volume of vocalisation and volume of vocalisation (area under the curve per second) during spermatic cord cutting were analysed using a mixed effects linear model to estimate the effect of treatment, site, and the interaction between treatment and site. A cubic transformation of the volume of vocalisation and the natural logarithm of area under the curve per second were taken to ensure normality in the model residuals. Effects significant at the 5% level were kept in the final model, others were removed by backwards elimination. Litter was fitted as a random intercept. Following castration, assessment of efficacy was achieved by comparing the proportion of animals demonstrating pain-associated behaviour in the treatment and negative control groups. *Safety* Safety observations and measurements comprised of

	clinical examinations, including measurements of rectal temperature, body weight, wound healing and adverse events. Clinical examination data from all piglets both prior to treatment and following treatment was tabulated by treatment group for interpretation. Basic statistics (means, standard deviations, number in calculation) was produced for quantitative data (rectal temperature). Data on adverse events were tabulated by treatment group for the purpose of interpretation. Proportions of piglets in the treatment and negative control groups dying or demonstrating adverse effects were compared using Fisher's exact test. Lack of toxicity was demonstrated by absence of increased rates statistically significant at the 5% level. Wound healing data were analysed using an ordinal mixed effects regression model.
RESULTS	
Participant flow	86 piglets (42 DE and 44 IT) received IVP. 87 piglets (43 DE and 44 IT) were in the negative control group. 7 piglets (4 IVP, 3 Control group) were removed from the per protocol group for various reasons (Intestinal prolapse, castration cut too long, 1ml overdose of IVP, treated but didn't meet inclusion criteria, not castrated following correct procedure). Of the 7 animals removed, all were included in the intention to treat (ITT) population, with the exception of the one piglet which was given an overdose of IVP, which was removed from all efficacy populations.
Outcomes for endpoints	The mean total motor response score was greater in the negative control group compared to the IVP treated piglets (4.37 and 2.86 respectively). The nociceptive motor response was statistically significantly greater (at the 5% level) in the negative control ($p < 0.001$). The results indicate that if a response score of 2 or above was applied then the odds ratio of a nociceptive motor response score being in an equal or greater category in the negative control group as opposed to the IVP group was 2.9 ($e^{1.07}$). The piglets exhibited behaviours associated with pain prior to castration (Study Day -1) with comparable frequencies in the IVP and negative control groups (number of piglets showing at least one pain related behaviour (by Focal Assessment or Scan Sampling) was 57 and 55, respectively). The corresponding figures in the 30 minutes post-castration were 60 and 82, respectively. A greater number of IVP-treated piglets showed no pain response (N= 23) in the same period, compared to their negative control group counterparts (N=4). By >30 minutes there was little

	difference noted between the pain response reported in both the IVP group and negative control groups (N=80 and N=83, respectively). The Generalised Linear Mixed Model fitted to the pain associated behaviour during the first 30 minutes following castration found a difference in the odds of showing pain that was statistically significant at the 5% level (p-value <0.0001) in favour of the Tri-Solfen treated group. A piglet in the negative control group had the odds of showing a pain-related behaviour which was 2.39 ($e^{0.87}$) times that of a piglet in the Tri-Solfen treated group, across all observations. The focal observations were higher than the scan observations, and this effect was statistically significant at the 5% level (p-value <0.0001).
Adverse events (AE)	AE's were identified in the observations the study and ABON coding was given to the Veddra symptoms identified in the Tri-Solfen group. At least one AE was reported in 14 IVP treated compared to 9 negative control animals out of 86 and 87 piglets, respectively, in each group. There was one Serious AE (anaphylactic shock) reported in the IVP group and assessed as probably IVP-related. There were five other AEs considered reported in the IVP group only. Four involved application site inflammation and one, scratching. They were assessed as possibly IVP-related. The frequencies for anaphylactic shock and for application site inflammation (1.16 in 100 and 4.65 in 100 animals treated, respectively).
DISCUSSION	The results from the study show that Tri-Solfen provides significant reduction in pain-associated responses in piglets during and for the 30 minutes following castration when applied at the recommended dose level to piglets under commercial production conditions, and that it is safe when administered at the recommended dose level to piglets.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics the benefit/risk profile of the product is favourable.

MODULE 4

POST- AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)

The post-authorisation assessment (PAA) contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)