CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205098Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	205098
Supporting document/s:	SDN 001
Applicant's letter date:	2/1/13
CDER stamp date:	2/4/13
Product:	VARITHENA™ Polidocanol Injectable Microfoam
Indication:	treatment of incompetent great saphenous veins, accessory saphenous veins and visible varicosities of the great saphenous vein (GSV) system
Applicant:	Provensis Ltd.
Review Division:	Div CardioRenal Products DCRP
Reviewer:	William T. Link, Ph.D.
Supervisor/Team Leader:	Albert DeFelice, Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Michael Monteleone

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of [NDA 205098] are owned by [Provensis Ltd.] or are data for which [Provensis Ltd.] has obtained a written right of reference.
Any information or data necessary for approval of [NDA 205098] that [name of applicant] does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of [NDA 205098].

Reference ID: 3367720

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 3

2 DRUG INFORMATION 12

2.1 DRUG 12
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS: IND 63420 12
2.3 DRUG FORMULATION 12
2.4 COMMENTS ON NOVEL EXCIPIENTS 13
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN 13
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN 13
2.7 REGULATORY BACKGROUND 13

3 STUDIES SUBMITTED 14

3.1 STUDIES REVIEWED 14
3.2 STUDIES NOT REVIEWED 14
3.3 PREVIOUS REVIEWS REFERENCED 14

4 PHARMACOLOGY 15

4.1 PRIMARY PHARMACOLOGY 15
4.2 SECONDARY PHARMACOLOGY 20
4.3 SAFETY PHARMACOLOGY 20

5 PHARMACOKINETICS/ADME/TOXICOKINETICS 56

5.1 PK/ADME 64
5.2 TOXICOKINETICS 117

6 GENERAL TOXICOLOGY 118

6.1 SINGLE-DOSE TOXICITY 119
6.2 REPEAT-DOSE TOXICITY 124

7 GENETIC TOXICOLOGY 142

7.1 *IN VITRO* REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES) 142
7.2 *IN VITRO* ASSAYS IN MAMMALIAN CELLS 145
7.3 *IN VIVO* CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY) 148
7.4 OTHER GENETIC TOXICITY STUDIES 150

8 CARCINOGENICITY 151

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY 151

9.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT 151
9.2 EMBRYONIC FETAL DEVELOPMENT 159
9.3 PRENATAL AND POSTNATAL DEVELOPMENT 171

10 SPECIAL TOXICOLOGY STUDIES 201

Reference ID: 3367720

1 Executive Summary

1.1 Introduction

Polidocanol endovenous microfoam (PEM) is a proprietary, engineered microfoam produced using an aqueous polidocanol solution and a gas mixture consisting of oxygen and carbon dioxide (65:35) with low nitrogen content. PEM is produced using a sterile bi-canister system designed to create a microfoam with stable physical characteristics, consistent density and bubble size (median diameter <100 µm and no bubble greater than 500 µm) at the time of use, and rapid bubble absorption following injection into the vein.

PEM was developed for the treatment of incompetent great saphenous veins (GSV), accessory saphenous veins, and visible varicosities of the GSV system above and below the knee. PEM is intended to improve the symptoms of superficial venous incompetence and the appearance of visible varicosities in the GSV system.

PEM remains coherent upon administration and displaces the blood in the vein, allowing the sclerosant, which is neither diluted nor de-activated, to act upon the endothelium. PEM empties even large veins of blood, allowing patients to be treated effectively with a small total dose of active sclerosant (i.e., 1.3 mg of polidocanol per 1 mL of PEM 1.0%).

PEM is also highly echogenic. With ultrasound, it is possible to observe PEM filling the targeted segments of incompetent vein. This allows for controlled treatment of the incompetent vein segment for which treatment is intended and limits unintended systemic exposure.

PEM is dispensed from a proprietary canister device which generates sufficient microfoam to treat several patients during the course of its in use shelf life. The Microfoam Transfer Unit (MTU), a component of the device used to dispense PEM to a syringe for injection into the target veins, must be replaced between patients

The dose-concentration and maximum volume of PEM proposed for licensure, up to 15 milliliters (mL) of PEM 1.0% per treatment session, provide the best balance of benefit and risk for all treated patients.

1.2 Brief Discussion of Nonclinical Findings

<u>Pharmacology</u>

The primary pharmacodynamics program consisted of four *in vivo* studies. These were conducted in non-rodent species with the aim of assessing and comparing the sclerosant action of polidocanol microfoam and solution and elucidating the most effective concentration. The non-rodent species selected were the sheep and the New Zealand White rabbit. The sheep was initially chosen due to the readily accessible

Reference ID: 3367720

jugular veins which are comparable to the size of human varicose veins. This latter study (study 256277) showed, however, that the sheep was not an appropriate model for assessing the pharmacodynamic activity of polidocanol as there were no signs of venospasm in the external jugular vein or saphenous vein following intravenous administration of the microfoam and histological evaluation of the vein sections indicated minimal endothelial damage. No further studies with the sheep were initiated. The rabbit was chosen as it was possible to model the clinical situation where the vein is occluded. It was also chosen as it is an established model for investigating the effect of sclerosants on vein endothelium in vivo.

The rabbit marginal ear vein studies which evaluated the pharmacodynamics of polidocanol as a solution versus the pharmacodynamics of polidocanol as a microfoam demonstrated that both formulations caused endothelial necrosis which elicited endothelial fibroplasia. The microfoam was shown to be more effective as a sclerosing reagent than polidocanol solution.

Safety Pharmacology

An extensive program of safety pharmacology studies was conducted with both polidocanol solution and microfoam. This comprised a series of safety pharmacology studies to investigate the effects of polidocanol solution on the central nervous system, the gastro-intestinal system, the renal system and the cardio-respiratory systems. In addition to this safety pharmacology program with polidocanol solution, a series of standard and specialized *in vitro* studies and *in vivo* cardio-respiratory studies in the dog and rat were conducted with polidocanol microfoam. This included a program to address the FDA concerns relating to the effects of microbubbles in the circulation arising from the administration of PEM.

Polidocanol solution at intravenous dosages up to 10 mg/kg was without significant effects on locomotor activity, intestinal motility or general behavior in the male NMRI mouse. This dose provides approximately 2.5 times that at the maximum human dose of polidocanol, 19.5 mg in the proposed clinical dose of 15 mL 1% PEM, based upon body surface area.

Polidocanol solution at intravenous dosages up to 3 mg/kg was without biologically relevant effects on renal function, in the male rat, up to 24 hours after dosing. At 10 mg/kg, blood in the urine was noted at 6 hours after dosing but this effect was no longer evident 24 hours after dosing. The 10 mg/kg dosage provides approximately 5 times that at the maximum human dose of polidocanol, 19.5 mg in the proposed clinical dose of 15 mL 1% PEM, based upon body surface area.

Sequential intravenous administration of 1, 3, 10, 15 and 20 mg/kg polidocanol solution was without effect on systolic blood pressure and heart rate in the anesthetized dog for up to 30 minutes after each dosing. No statistically significant effects on blood flow and respiratory parameters were measured following administration of polidocanol at dosages up to 20 mg/kg. At dosages up to 15 mg/kg, there were no effects on diastolic

Reference ID: 3367720

and mean blood pressure or electrocardiographic parameters. At 20 mg/kg, polidocanol slightly increased diastolic and mean blood pressure and increased P-Q and QRS intervals in the anesthetized dog. The 20 mg/kg dosage provides approximately 33 times that at the maximum human dose of polidocanol, 19.5 mg in the proposed clinical dose of 15 mL 1% PEM, based upon body surface area.

A series of experiments in the dog investigated the physiological response to the 'worst case' scenario with a rapid uncontained bolus injection of polidocanol microfoam into a peripheral leg vein. The 10 mL dose, twice the average human clinical dose (v/w) or equivalent based upon body surface area at the proposed clinical dose of 15 mL 1% PEM, resulted in no significant changes. Larger doses produced increasingly large changes in measurements suggesting pulmonary vascular occlusion, which might in part be reflex in origin, but all these changes rapidly returned to normal values without sustained deleterious effects.

Another study in the dog using microfoam showed that despite the presence of microbubbles and infusion of PEM up to a total volume of 150 mL (approximately 20 times a clinically relevant dose based upon (v/w), or 16 times based upon body surface area at the proposed clinical dose of 15 mL 1% PEM), the histopathology of lung, liver, heart and kidney was the same as observed in untreated control animals and, therefore, was not adversely affected by PEM. The hemodynamic and gas exchange responses produced by the higher doses (45 to 150 mL total dose) of PEM were consistent with transient occlusion of pulmonary arterioles with microbubbles and initiation of compensatory effects.

The following conclusions were made from the program of specialized safety pharmacology studies to address the FDA concerns relating to microbubbles in the circulation.

It was shown that residual gas in the blood was dependent on starting nitrogen content plus a small amount of residual oxygen. Carbon dioxide was completely absorbed at 2 minutes while only 5% of PEM NF1(<1% N2) gas remained in the blood compared to 24% of PEM OF ((b) (4) N2).

In a rat cremaster intravital microscopy study it was directly observed that PEM microbubbles did not lodge in the visualized arterioles, and blood flow remained normal indicating no evidence of microbubble trapping in smaller arterioles. The no-effect dose of 400 µL is equivalent to approximately 27 times the amount of microfoam estimated to cross a 20% patent foramen ovale (PFO) assuming the improbable migration of the entire clinical dose of 15 mL 1% PEM to the right atrium.

It was shown upon exposure to venous blood that the active agent polidocanol is diluted in the blood and does not remain associated with the residual gas bubbles. It was also shown that, despite the presence of microbubbles, an infusion of PEM in the dog up to a total volume of 150 mL (approximately 20 times a clinically relevant dose (v/w) or 16

Reference ID: 3367720

times based upon body surface area at the proposed clinical dose of 15 mL 1% PEM), resulted in no excess histopathology of lung, liver, heart and kidney.

A cardiovascular safety pharmacology study in the conscious beagle dog examined long term pulmonary effects following 4 weekly doses of PEM NF1 at dosage levels up to 8.0 mL/kg, 17 times the proposed maximum human dose of 15 mL 1% PEM based upon body surface area. There was a reduction in tidal volume and derived respiratory minute volume at the 15 minute assessment point which resolved within one hour. This same pattern occurred after each weekly dose but there was no indication of a cumulative effect. Blood gas concentrations were unaffected. Histopathology of the lung at the end of the 3 month follow-up period showed no abnormalities, and specifically no histological changes indicative of chronic pulmonary hypertension.

Injection of 2.5 µL microfoam into the carotid artery, at a dose eight times the maximal quantity of microfoam which might cross a right-to-left shunt and reach the human brain during administration of PEM NF1, did not produce clinical neurological deficits or induction of heat shock protein. At doses of 5 µL, sixteen times greater than the theoretical clinical dose to the brain, there was evidence of neuronal cell stress.

The acute cardiopulmonary effects in the dog of PEM NF1, administered in a fashion similar to clinical administration, demonstrated that no clinically relevant elevations of pulmonary artery pressure occur following a 20 mL dose (4 times the proposed maximal human clinical dose of 15 mL 1% PEM by weight or 2.8 times based upon body surface area). The injection of polidocanol microfoam made with 80% nitrogen produced greater and longer-lasting elevations, demonstrating improved safety of PEM NF1 over air-based microfoam.

This program of specialized safety pharmacology studies provided important reassurance of the safety of circulating microbubbles following intravascular administration of PEM.

Pharmacokinetics

No absorption studies were conducted as the route of administration is intravenous.

Distribution

Three single dose tissue distribution studies were carried out in the Sprague Dawley rat. One study measured radioactivity present in various organs and tissues by Quantitative Whole Body Autoradiography (QWBA) after injection of [^{14}C]-polidocanol in solution at a dosage of 3 mg/kg. This study also investigated tissue distribution in Lister Hooded rats to enable a comparison between pigmented and non-pigmented rats. Radioactivity was distributed widely to all organs and tissues in the rat with much of the dose reaching the organs of clearance rapidly. However, some radioactivity was retained in many tissues for the 168 hour duration of the study indicating an affinity of some polidocanol related material for cellular components. Lowest initial levels of radioactivity were seen in eye,

Reference ID: 3367720

testis and brain from the pigmented rats. There were no obvious differences between radioactivity retention in tissues from albino or pigmented rats.

A further study was performed with [^{14}C]-polidocanol in solution in the rat to provide additional information on the tissue distribution of total radioactivity. Levels of total radioactivity were measured by liquid scintillation counting (LSC) and the nature of radioactivity present in selected tissues investigated by Thin Layer Chromatography (TLC). Total radioactivity was well distributed throughout the tissues analyzed, with the highest concentrations in whole blood, plasma and the organs associated with elimination (liver and kidney) at 5 minutes post-dose.

The third study was a bridging study comparing the distribution of total radioactivity following administration of [^{14}C]-polidocanol microfoam or solution. Radioactivity was extensively distributed to the selected tissues analyzed. Radioactivity dispersed from the site of injection at a similar rate following each administration with only 1% remaining in the tail by 15 minutes post-dose. The amount of total radioactivity recovered in each tissue was generally comparable between the two formulations at each of the time-points analyzed. [^{14}C]-polidocanol was therefore shown to be distributed and cleared in a similar fashion following administration in either the microfoam or solution formulation to rats.

Two single dose tissue distribution studies were performed in the Beagle dog, one study using [^{14}C]-polidocanol solution and the other study using [^{14}C]-polidocanol microfoam. In the study with [^{14}C]-polidocanol solution, total radioactivity in selected tissues (except lungs) was highest in the animal killed at 168 hours post-dose (the first measurement time). Tissue concentrations of total radioactivity were significantly higher than the concentration present in whole blood and plasma. At 336 hours, the concentration in whole blood and plasma had decreased to close to the limit of reliable determination. Concentrations had also decreased in kidneys, renal fat and liver, but were slightly elevated in lung and similar in subcutaneous fat compared to 168 hours post-dose. By approximately 5 weeks post-dose, concentrations of radioactivity were much lower in all tissues measured, and by 15 weeks postdose, all tissues were at or below the limit of reliable determination.

In the study with [^{14}C]-polidocanol microfoam, radioactivity was well distributed in the tissues investigated. Total radioactivity levels were higher in organs associated with metabolism (liver and kidneys). Subcutaneous and renal fat retained some radioactivity. The terminal half-life values (168-672 hour interval) of approximately 650, 603, 582, 446, 359, 337 and 326 hours were estimated for liver, renal fat, subcutaneous fat, heart, spleen, kidneys, and lungs, respectively.

Metabolism

Metabolism was suggested to proceed via non-specific hydroxylation of the dodecyl chain with further hydroxylation and elimination to form the oxidized hydroxyl (keto/enol) metabolite series for polidocanol oligomers. The carboxylic acid metabolite series identified in study 169771 would be formed by oxidative cleavage of the double bond of

Reference ID: 3367720

the enol metabolite (oxidized hydroxyl metabolite). This carboxylic acid metabolite series was not observed in any of the *in vitro* samples analyzed. This was deemed to be due to the fact that metabolism had not proceeded to the same extent in microsomes as was observed in rat and dog *in vivo.*

The metabolism of [^{14}C]- polidocanol was qualitatively similar in each species with some quantitative differences observed between the 3 species. Due to the oligomeric nature of polidocanol and its metabolites and the analytical challenges this posed, it was not possible to determine if the quantitative differences were genuine species differences or were a consequence of differences in the metabolic competency of the microsomal preparations.

Excretion
The pharmacokinetic data package was completed with four studies investigating the excretion profile. Three of these studies were conducted in the Beagle dog and one in the Sprague Dawley rat with the aim of investigating the excretion profile of polidocanol following intravenous administration of [^{14}C]-polidocanol solution and microfoam (dog only). Radioactivity levels in urine, feces, plasma and carcass were determined using LSC. The nature of the radioactivity was analyzed by HPLC.

In the rat, the majority of the total radioactivity was eliminated within the first 24 hours post dose. The predominant route of excretion was via the urine followed by feces and then expired air. Low levels of radioactivity were detected in the gastrointestinal tract and residual carcass. The majority of radioactivity in urine and feces was postulated to be polar metabolites of [^{14}C]-polidocanol.

A similar pattern was observed for the dog and the excretion patterns were similar for the solution and the microfoam.

<u>Toxicology</u>

General toxicology
In a single dose toxicity study using polidocanol microfoam rats were shown to be physiologically inappropriate for safety evaluation of the preparation, since only a fraction of the required dose level could be achieved using this route of administration. The maximum tolerated dose (MTD) was established as two doses of 0.05 mL (at approximately 0.2 mg/kg dosage).

In a single dose toxicity study of 1% polidocanol microfoam in the dog, the maximum tolerated dosage was established as 6 mL/kg.

In the pivotal repeat dose toxicity study in rats, daily intravenous administration of polidocanol solution for 28 consecutive days at dosage levels of 9 and 27 mg/kg/day was associated with moderate clinical signs which included mastication, salivation and changes in respiration rate (labored and irregular). A dosage level of 3 mg/kg/day was associated with the same clinical signs, however, the incidences were infrequently

Reference ID: 3367720

observed and this was considered to be the No Observed Adverse Effect Level (NOAEL).

In the first of the three pivotal repeat dose toxicity studies in dogs, daily intravenous administration of polidocanol solution at dosage levels of 6 and 15 mg/kg/day for 28 consecutive days was associated with moderate clinical signs which included: body tremors, licking of lips, salivation, retching and emesis. There were no histological changes noted which were considered to be related to treatment with polidocanol. A dosage level of 2 mg/kg/day was considered to be the No Observed Effect Level (NOEL).

In the second pivotal repeat dose toxicity study in the dog, four once weekly intravenous administrations of polidocanol as microfoam or solution at both dose levels of 2.25 and 6.75 mg/kg/dose were associated with clinical signs, which included emesis, salivation, lip licking and retching. A dose level of 6.75 mg/kg/dose with polidocanol microfoam was also associated with ataxia and changes in respiration and (for the majority of female animals) an inability to support their own body weight. Both the microfoam and solution resulted in intimal proliferation in the vena cava at a dose of 6.75 mg/kg/dose. Intravenous administration of both polidocanol microfoam and polidocanol solution was associated with enlarged or prominent injection sites at necropsy and with venous thrombosis, vasculitis and intimal proliferation histologically, which was attributed to the sclerosing nature of the test item.

In the final pivotal repeat dose toxicity study in dogs, polidocanol microfoam was administered intravenously to an unoccluded (i.e. open) vein to investigate immediate systemic exposure. In addition, doses were "trapped" in a closed vein (occluded by direct pressure or pneumatic tourniquet proximal to the site of injection) to more accurately simulate the intended clinical use. In the open vein design, animals were dosed with polidocanol microfoam at 1.44 mg/kg and 12.8 mg/kg once weekly on four occasions. In the closed vein design, animals were dosed with 6 mg and 12 mg polidocanol microfoam once weekly on four occasions.

Toxicokinetic data indicated that overall systemic exposure was greater following administration of the test article to an open vein as compared to a closed vein. In some dogs, severe clinical observations followed the systemic administration of 12.8 mg/kg polidocanol. Abnormal administration site observations (swelling and/or erythema usually of mild severity) were often present after dosing in dogs in all of the test article-treated groups. Although these conditions reversed with time, under the conditions of this study, an overall NOAEL could not be determined for either the open or closed vein routes of administration.

Genetic toxicology

The genotoxicity of polidocanol has been investigated using the standard battery of tests as recommended in ICH guidance S2 (R1): *in vitro* bacterial reverse mutation assay; *in vitro* mouse lymphoma tk assay and an *in vivo* test for chromosomal damage using rodent hematopoietic cells. Polidocanol solution was used rather than the

Reference ID: 3367720

microfoam as the nature of the formulation was not considered to impact on the outcome of the studies.

In the bacterial reverse mutation assay, it was shown that polidocanol was not mutagenic to *Salmonella typhimurium* or *Escherichia coli*, in either the presence or absence of S9 mix, when tested at concentrations extending into the toxic range.

Two mouse lymphoma tk assays were conducted. In the first assay a significant mutagenic response was observed in the presence of S9 mix. However the responses were very weak (approximately 1.7-fold increase in mutant fraction over control values), and occurred close to the limit of acceptance for the accompanying level of toxicity (10-17% survival). A mutagenic response was not observed in the absence of S9 mix.

In the second mouse lymphoma assay, polidocanol was also shown to be very weakly mutagenic when tested in the presence of S9 mix, only when tested at toxic concentrations. A mutagenic response was not observed in the absence of S9 mix.

In the mouse micronucleus study, animals were dosed by the intravenous route with polidocanol solution at concentrations of 20, 40 and 80 mg/kg. It was shown that polidocanol did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dosage of 80 mg/kg.

Carcinogenicity
No carcinogenicity studies have been performed using either polidocanol solution or microfoam formulations as the product is intended for acute short-term administration. This approach is in accordance with the recommendations presented in ICH guidance S1A.

Reproductive toxicology
A program of reproductive toxicity studies has been conducted with polidocanol to investigate fertility, embryo-fetal development and peri/post natal development. Polidocanol solution was used rather than the microfoam as the nature of the formulation was not considered to impact on the outcome of the studies.

In the rat male and female fertility studies, dose levels up to and including the highest dosage of 27 mg/kg/day were not associated with any effects on male or female fertility. Although a no-effect level was not established for clinical signs of reaction to treatment in males, the effects at the lowest dosage of 3 mg/kg/day were confined to mastication in 13 out of 25 animals, and occasional animals with a fixed stare and abnormal respiration. Similarly in females, although a no-effect level was not established for clinical signs of reaction to treatment, the effects at the lowest dosage of 3 mg/kg/day were confined to mastication in 14 out of 24 animals, and occasional animals with a fixed stare.

Reference ID: 3367720

In the pivotal rat embryo-fetal development study, dose levels of polidocanol up to and including the highest dosage of 27 mg/kg/day were not associated with any effects of treatment on pregnancy performance, fetal weight, or the incidences of fetal abnormalities and variants. Maternal toxicity was indicated at 9 and 27 mg/kg/day by clinical signs including subdued behavior and mastication; at 27 mg/kg/day abnormal respiration and red staining of the cage were also observed. Although a maternal no-effect level was not established, effects at the lowest dosage of 3 mg/kg/day were confined to mastication in occasional animals.

In the pivotal rabbit embryo-fetal development study, dose levels of polidocanol up to and including the highest dosage of 10 mg/kg/day did not produce any indication of maternal toxicity or any adverse effects on embryo-fetal mortality, fetal weight or the incidences of fetal abnormalities and variants. The NOEL for both maternal and fetal effects was 10 mg/kg/day.

In the peri and post natal study in rats, dosage levels of polidocanol up to and including 9 mg/kg/day were without effects on the development of the conceptus and offspring to sexual maturity. At a dosage level of 27 mg/kg/day, effects were confined to an equivocal reduction in body weights of F1 males and associated equivocal delay in the age of preputial separation. Although a maternal no-effect level was not established, effects at 3 mg/kg/day were confined to clinical signs of reaction in 3 animals.

Local tolerance
In a local tolerance study, paravenous injection of polidocanol solution and polidocanol foam around the marginal ear vein of rabbits produced evidence of a local irritant effect which was slightly less marked with polidocanol foam.

In an antigenicity study in guinea pigs, polidocanol was shown not to be antigenic.

1.3 Recommendations

No recommendations.

1.3.1 Approvability

In the opinion of this reviewer, the product is approvable.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

A separate labeling review will be filed.

Reference ID: 3367720

2 Drug Information

2.1 Drug

CAS Registry Number: 9002-92-0 or 3055-99-0

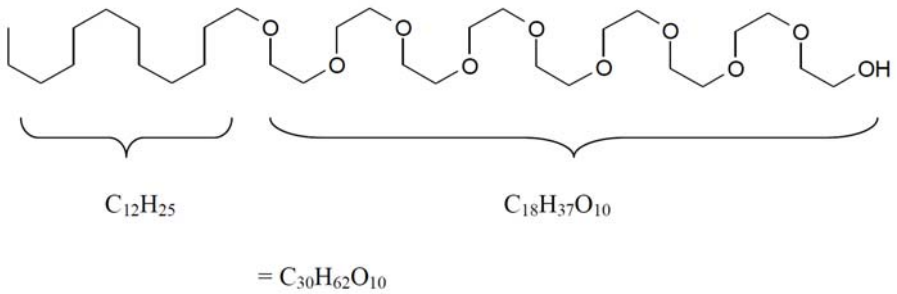
Generic Name: Polidocanol

Code Name

Chemical Name(s): α-dodecyl-ω-hydroxypoly(oxyethylene), oxypolyethoxydodecane, macrogol lauryl ether, polyoxyl lauryl ether, polyethylene glycol monolauryl ether

Molecular Formula/Molecular Weight: The molecular formula is $CH_3(CH_2)_{11}(OCH_2CH_2)_nOH$ where n = an average of nine. The molecular mass corresponds to an average 582.9g based on an average of 9 ethylene glycol moieties.

Structure or Biochemical Description:



Pharmacologic Class: endovenous sclerosant

2.2 Relevant INDs, NDAs, BLAs and DMFs: IND 63420

2.3 Drug Formulation

Polidocanol injectable microfoam is a unique intravenous pharmaceutical form. It is a microfoam product containing polidocanol solution for intravenous administration.

The product utilizes a drug-device combination to generate injectable microfoam which is the dosage form. The product is a bi-canister unit format consisting of the following components:

> A “Polidocanol Canister” containing a 1.0 % w/w polidocanol solution held under an atmosphere (approx. 1.2 bar absolute) of carbon dioxide.
>
> An “Oxygen Canister” containing oxygen at approximately 5.4 bar absolute which is used to pressurize the polidocanol canister immediately prior to use so as to permit generation of the microfoam.

Reference ID: 3367720

[redacted] (b) (4)

The product is activated by charging the Polidocanol Canister with oxygen from the Oxygen Canister, resulting in a gas mixture of oxygen and carbon dioxide at an approximate ratio of 65:35, respectively, at approximately [redacted] (b) (4) absolute pressure in the Polidocanol Canister. The dosage form, injectable microfoam, is only generated when polidocanol solution and the oxygen/carbon dioxide gas mixture are fed into the polidocanol canister valve assembly to create foam of a specified density. The resultant microfoam [redacted] (b) (4) is extracted by the user through the microfoam transfer unit (MTU). The density of the polidocanol solution in the microfoam is [redacted] (b) (4)

2.4 Comments on Novel Excipients

none

2.5 Comments on Impurities/Degradants of Concern

none

2.6 Proposed Clinical Population and Dosing Regimen

Varithena™ is intended for single use in treatment of incompetent great saphenous veins (GSV), accessory saphenous veins, and visible varicosities of the GSV system above and below the knee.

2.7 Regulatory Background

On 11-Oct-2001, an Investigational New Drug application for PEM was submitted to the FDA. Following a series of discussions with FDA and protocol amendments, in September, 2002 the Division of Dermatology and Dental Products granted approval to begin Study 003 under Investigational New Drug (IND) Application 63,420 under the conditions of a partial clinical hold. Study 003 was a Phase 2 study in which patients were treated with the original formulation of PEM. The specific concern prompting this partial clinical hold was the lack of information on whether the microbubbles in PEM dissipated in the leg or entered the systemic circulation before being cleared.

On 14-Nov-2003, IND 63,420 was placed on full clinical hold following FDA review of echocardiographic data from Study 003, which indicated that bubbles were detectable in the right heart chambers of all 10 patients providing data and also in the left heart chambers of one patient subsequently diagnosed with a PFO. The Agency noted that it was unclear to what extent there might be systemic exposure to the polidocanol drug substance in the heart and lungs. Provensis was required to provide sufficient information from non-clinical studies regarding the fate of the microbubbles, and relate these findings to clinical safety. Additionally, Provensis was asked to provide evidence that exposures to microbubbles do not have subclinical effects such as microinfarctions.

Reference ID: 3367720

The Agency asked Provensis to consider modifying the polidocanol microfoam to minimize the risk of gas embolism. To address FDA's concerns, during 2004, PEM was altered (b) (4), resulting in the current gas composition of 65:35 oxygen and carbon dioxide. At the time of this change, (b) (4) was also introduced, which resulted in changes to (b) (4) the canister. The development and clinical trial material manufacturing was performed by (b) (4)

On 16-Jun-2005, a complete response to the clinical hold was submitted to the FDA. This response included data from 9 non-clinical studies and 1 clinical study (Study 011). On 12-Jul-2005, the sponsor was given permission to resume clinical studies in the US and responsibility for oversight of IND 63,420 was transferred from the Division of Dermatology and Dental Products to the Cardiovascular and Renal Drug Products Division of the Center for Drug Evaluation and Research (CDER).

In 2007, clinical trials of PEM in the US resumed with the initiation of Study 012, in which the current, low-nitrogen formulation of PEM was administered. The clinical trial material for this study was manufactured by (b) (4) All subsequent studies have been conducted using the current, low-nitrogen formulation of PEM (range of dose-concentrations of the active pharmaceutical ingredient [API]: 0.125% to 2.0%) produced at (b) (4)

In 2009, the Microfoam Transfer Unit (MTU) was incorporated into the product and was used throughout the remainder of the PEM clinical development program. This unit was attached to the pressurized polidocanol canister to facilitate filling syringes with PEM. In 2012, API manufacture was transferred to (b) (4) and manufacture of the final product was transferred to Biocompatibles Ltd. (Farnham, UK; an affiliate of Provensis Ltd.).

3 Studies Submitted

3.1 Studies Reviewed

GLP safety pharmacology, general toxicology, genotoxicity and reproductive toxicology studies are reviewed herein.

3.2 Studies Not Reviewed

Pharmacology and pharmacokinetics studies are summarized as presented but were not formally reviewed.

3.3 Previous Reviews Referenced

none

Reference ID: 3367720

4 Pharmacology

4.1 Primary Pharmacology

A program of *in vitro* and *in vivo* studies has been conducted to evaluate the pharmacodynamics of polidocanol solution and polidocanol endovenous microfoam (PEM). All *in vivo* studies were carried out by intravenous administration as this is the proposed route of clinical administration. Polidocanol solution, rather than microfoam, was used in a number of studies because of limitations on how much polidocanol could be injected using the foam and because of effects of the foam on the vein that are independent of polidocanol.

The primary pharmacodynamics program consisted of four *in vivo* studies (study 256146, study 256696, study KMWW-0001, study 256277). These were conducted in non-rodent species with the aim of assessing and comparing the sclerosant action of polidocanol microfoam and solution and elucidating the most effective concentration. The non-rodent species selected were the sheep and the New Zealand White rabbit. The sheep was initially chosen due to the readily accessible jugular veins which are comparable to the size of human varicose veins. This latter study (study 256277) showed, however, that the sheep was not an appropriate model for assessing the pharmacodynamic activity of polidocanol as there were no signs of venospasm in the external jugular vein or saphenous vein following intravenous administration of the microfoam and histological evaluation of the vein sections indicated minimal endothelial damage. No further studies with the sheep were initiated. The rabbit was chosen as it was possible to model the clinical situation where the vein is occluded.

The rabbit marginal ear vein studies (study 256146, study 256696, study KMWW-0001) which evaluated the pharmacodynamics of polidocanol as a solution versus the pharmacodynamics of polidocanol as a microfoam demonstrated that both formulations caused endothelial necrosis which elicited endothelial fibroplasia. However, the microfoam was shown to be more effective as a sclerosing reagent than polidocanol solution. Polidocanol microfoam at concentrations of 0.25%, 0.5%, 1% and 2% was shown to cause sclerosis in a dose-responsive manner with comparable effectiveness observed at concentrations of 1% and 2%.

Evaluation of two formulations of polidocanol using the rabbit marginal ear vein (Study 256146) (GLP)

<u>Objective</u>
The objective of the study was to evaluate the sclerosant action of polidocanol solution and polidocanol microfoam in the marginal ear vein of the rabbit.

<u>Methods</u>
Concentrations of 0.5% or 2.0% of both solution and microfoam were administered intravenously into the left marginal ear vein of female New Zealand White Rabbits.

Reference ID: 3367720

Physiological saline (0.9%) was administered intravenously via the marginal vein in the corresponding right ear.

Histological examination of the ears was carried out 2 hours, 24 hours, 7 days, 14 days or 21 days after treatment. Two sites, 10 mm and 30 mm proximal to the injection site were evaluated.

Results

Polidocanol microfoam 0.5% caused venous thrombosis and endothelial degeneration after 7 days. Complete venous occlusion was noted at the 10 mm site and partial occlusion at the 30 mm site after 14 days. Recanalization was observed after 21 days.

Polidocanol microfoam 2% caused endothelial degeneration to progress from minimal and focal after 2 hours to moderate and diffuse after 24 hours and after 7 days. There was partial venous occlusion by thrombus formation and fibroplasia at the 10 mm site and complete venous occlusion at the 30 mm site after 21 days. Histological samples from the 14 day treatment were unsatisfactory for evaluation.

Treatment with polidocanol solution 0.5% indicated that no changes to the ear vein endothelium occurred in any of the biopsies from 2 hours to 21 days. Treatment with polidocanol solution 2.0% showed that after 24 hours the ear vein of one animal (Animal 17) revealed an intraluminal fibrinous thrombus with focal endothelial degeneration. After 7 days, the ear vein of another animal (Animal 18) revealed a well-established thrombus with endothelial degeneration and fibroplasia within the thrombus. However, the veins examined from Animals 19 and 20, examined after 14 and 21 days were patent, without evidence of thrombosis.

The right ears which were treated with 0.9% physiological saline in general indicated no abnormalities present in the tissue.

Evaluation of polidocanol microfoam using the rabbit marginal ear vein (main study) (Study 256696) (GLP)

Objective

Polidocanol microfoam and polidocanol solution were evaluated for sclerosant action in the rabbit marginal ear vein model. Clinical pathology parameters pre and post treatment were evaluated and histological evaluation was determined up to 21 days post treatment.

Methods

Concentrations of 0.25%, 0.5%, 1.0%, 2.0%, 3.0% polidocanol microfoam and 0.5%, 1.0%, 2.0% polidocanol solution were administered intravenously into the left marginal ear vein of male New Zealand White rabbits. Physiological saline (0.9%) was administered intravenously via the marginal ear vein in the corresponding ear.

Reference ID: 3367720

Histological examination of the ears was carried out 4 hours, 24 hours, 7 days, 14 days or 21 days after treatment. Two sites, 10 mm and 30 mm proximal to the injection site were evaluated. In addition, blood samples were obtained before treatment and prior to necropsy for the measurement of hematology, clinical chemistry and coagulation parameters.

Results

Following treatment redness was noted at the sites of the sutures on both the left and right ears for all animals. In some cases the coloration was present at the dose site and also extended to the auricular artery. Animals 18, 19, 20, 22, 23, 24 and 25 which were treated with 2% or 3% polidocanol foam indicated scab tissue at the injection site. Necropsy evaluation indicated that most rabbits showed some reddening of the ears around the injection site, consistent with the intravenous technique.

Clinical pathology data indicated increased levels of urea and creatinine in blood samples 4 hours post treatment from animals treated with polidocanol foam or polidocanol solution. The increased levels were transient and returned to normal values 24 hours post treatment. There were also mild increases in the aspartate aminotransferase and alanine aminotransferase levels, 4 hours after treatment with polidocanol foam or polidocanol solution. A protocol extension involving four animals treated with phosphate buffer confirmed that the elevated levels of urea and creatinine were due to the effects of anesthetic and not the test items.

There were no other significant changes in clinical pathology data to indicate a systemic effect following treatment with polidocanol foam or polidocanol solution.

Histology findings indicated that treatment with polidocanol microfoam (0.25%, 0.5%, 1.0%, 2.0%, 3.0%) and polidocanol solution (0.5%, 1.0%, 2.0%) caused endothelial necrosis which elicited endothelial fibroplasia. Where the necrosis was sufficiently severe, the fibroplasia progressed to cause luminal occlusion, which persisted for 21 days. The control ear veins treated with physiological saline from the right ears showed no histological abnormalities.

The concentration of polidocanol considered to be the most effective in relation to the desired clinical effect is that which caused the most rapid onset of total venous occlusion along the length of the vein. From the histological findings, polidocanol microfoam was considered to be the most effective at 1% concentration as this caused total venous occlusion at both sites in the shortest time (14 days, animal 14) and was more effective than the solution at the lower concentrations of 0.25% and 0.5%. For the polidocanol solution the most effective concentration was 1%.

Reference ID: 3367720

A dose range finding study of polidocanol in Varisolve® (PEM) PD Microfoam in a rabbit model of sclerosis (Study KMWW-0001) (non- GLP)

Objective

The objective of this study was to determine the range of PEM containing varied dose concentrations of the active ingredient polidocanol, which is able to initiate the sclerosing process by causing endothelial denudation, acute thrombosis and resolution without recanalization.

Methods

Female New Zealand White rabbits were dosed with vehicle (Group 1) or PEM containing 0.25, 0.5, 1, or 2% polidocanol (Groups 2, 3, 4 and 5, respectively) on Day 1 via intravenous injection in the marginal ear vein (usually the right ear). Four animals each from Groups 1 and 2 and 3 animals each from Groups 3-5 were euthanized on Day 1 at 1 hour. Three animals per group from Groups 1 through 5 were euthanized at each of the following time points: on Day 1 at 12 hours, Day 8, Day 22, and Day 43. Five additional groups, comprised of three animals each, were treated with vehicle or PEM containing 0.25, 0.5, 1, or 2% polidocanol via intravenous injection on Day 1 in the marginal ear vein using a modified clamp to improve the model. These animals were euthanized on Day 8.

Effectiveness of the PEM to cause sclerosis was assessed by administration site observations, Doppler ultrasound of the treated ear, and macroscopic and microscopic pathological examination of the ears. General health of the animals was assessed by clinical observations and body weights.

Results

There were two unscheduled deaths in the study. A Group 4 animal (1% polidocanol microfoam) died shortly prior to scheduled euthanasia at 1 hour post treatment, possibly due to anesthesia. A Group 5 animal (2% polidocanol microfoam) was found dead on Day 2. Gross necropsy on Day 2 did not reveal any potential cause of death, though the tissues were very autolyzed. Neither death was attributed to administration of PEM.

There was no effect on body weights or clinical observations related to administration of PEM. Administration of vehicle or PEM resulted in edema at the administration site and/or erythema for approximately one week following treatment. Edema and particularly erythema persisted up to 25 days post treatment in PEM-treated animals in a dose-responsive manner.

PEM was able to reduce blood flow in the treated ear in a dose-responsive manner as assessed by Doppler ultrasound. Several animals in Group 2 had diminished blood flow, and more animals in Group 3 had diminished blood flow. By Day 31, all Group 4 (1% polidocanol microfoam treated) animals had no detectable blood flow (absent) and all Group 5 animals (2% polidocanol microfoam treated) had diminished or absent blood flow.

Reference ID: 3367720

Macroscopic alterations related to PEM administration included discoloration of the treated veins at 1 hour from vascular congestion with or without perivascular hemorrhage. At Day 8, thickening was observed of the treated veins in a dose-responsive incidence, which corresponded microscopically to thrombosis. By Day 43, the veins of the animals in Groups 4 and 5 were devoid of blood and difficult to visualize.

Microscopic lesions in PEM-treated veins progressed from edema at 1 hour post treatment to pronounced necrosis and organizing thrombi at 12 hours post treatment. Hemorrhage was present in a dose-responsive manner at 1 and 12 hours post treatment in Groups 3, 4, and 5. By Day 8, thrombi, fibroplasia, and sub-acute inflammation were observed in polidocanol-treated veins. Complete sclerosis of the polidocanol veins was noted in several veins of Groups 3, 4, and 5 at Day 22. Recanalization was an infrequent finding. Complete sclerosis of the polidocanol-treated veins was apparent in most sections from Group 4 and 5 animals. Microscopic lesions were generally dose responsive in incidence and severity.

Results in animals treated using a modified clamp were generally similar to animals treated using a normal clamp.

In vivo evaluation of the effect of polidocanol microfoam on the jugular vein of the sheep (Study 256277) (GLP)

Objective

A study was conducted to assess venospasm in the external jugular vein of the sheep following intravenous administration of various concentrations of polidocanol microfoam. The sheep was the selected model as it possesses readily assessable external jugular veins comparable to the size of human varicose veins.

The original intention was to dose 2 sheep per group with polidocanol microfoam at concentrations of 0.25%, 0.5%, 1.0%, 2.0% or 3.0% polidocanol. However as animal 1 indicated no signs of venospasm following intravenous administration of 3% polidocanol microfoam the treatment schedule was abandoned and animal 2 was dosed with 10% polidocanol microfoam.

Methods

Two anesthetized sheep were treated with polidocanol microfoam and the extent of venospasm was assessed. For animal 1 both the right and left external jugular veins were treated with 3% polidocanol microfoam followed by 15 minutes observation period of the vein segment.

Animal 2 was injected with 10% polidocanol foam into the right jugular vein followed by injection of 10% polidocanol into the right saphenous vein. The vein segments were observed for signs of venospasm. Histological sections were prepared from the vein

Reference ID: 3367720

tissues from both animals and stained with hematoxylin-eosin. In addition, blood samples were taken for measurement of hematology and coagulation parameters.

Results
Administration of 3% polidocanol microfoam via both external jugular veins indicated no signs of venospasm. Histological data indicated minimal endothelium damage. Similarly, administration of 10% polidocanol microfoam via the right external jugular vein or the right saphenous vein indicated no signs of venospasm. Histological data indicated minimal or no abnormalities present. Hematology and coagulation parameters for both animals were similar pre and post administration of the microfoam.

The data from the two animals indicated that the sheep was not a useful model to illustrate the mechanism of action of the polidocanol microfoam. The texture of the external jugular veins did not appear to possess sufficient muscular tissue to cause it to go into venospasm. Similarly the saphenous vein which is considered to have more muscle than jugular vein did not go into venospasm.

Conclusion
On the basis of these findings it was concluded that the sheep was not a suitable model for the evaluation of the mechanism of action of polidocanol microfoam. The study was formally cancelled and no further studies were undertaken in sheep.

4.2 Secondary Pharmacology

No secondary pharmacodynamic studies were conducted.

4.3 Safety Pharmacology

An extensive program of safety pharmacology studies has been conducted with polidocanol solution and polidocanol microfoam. These studies were standard safety pharmacology studies designed to evaluate possible pharmacological effects of polidocanol on the cardio-respiratory system of the dog (study RCC 767676), the renal function of the rat (study RCC 767687) and the gastrointestinal system (study RCC 767698), general behavior (study RCC 767654) and locomotor activity (study RCC 767665) of the mouse. The rodent species and strains used were the Wistar rat and the NMRI mouse which are both standard rodent models. The canine species were the Beagle and the Hound which were selected because of the ready availability of high quality animals, their large size in the case of the Hound dogs and their acceptability as suitable models by regulatory authorities. In addition to the completion of the standard safety pharmacology program with polidocanol solution, a series of standard and specialized cardio respiratory studies in the dog and the rat together with a series of *in vitro* studies were conducted. This program included studies specifically designed to assess the effect of microbubbles in the circulation from the administration of PEM in order to address FDA concerns in this regard.

Reference ID: 3367720

In Vitro Safety Pharmacology

Three *in vitro* safety pharmacology studies were conducted. One of these studies investigated the hemolytic potential of polidocanol in human blood (study 256188). The other two studies (study RD198/23925, study RD198/23926) were part of the program to address the FDA concerns relating to the effects of microbubbles in the circulation arising from the administration of PEM.

Polidocanol: evaluation of the hemolytic potential of polidocanol in human blood *in vitro* (Study 256188) (GLP)

Objective
This study was conducted to evaluate the hemolytic potential of polidocanol solution and polidocanol microfoam using human blood. Methods Blood samples were taken from 3 healthy human male volunteers, transferred to tubes containing polidocanol solution, polidocanol microfoam, saponin (4.0 g/L) or saline and monitored at 37°C for 1 hour. The concentrations evaluated were 0.05%, 0.2%, 0.5% and 2.0% for both polidocanol solution and polidocanol microfoam.

Results
The values obtained for experiments that used 0.05% and 0.2% polidocanol concentrations and the results obtained for 0.5% and 2.0% polidocanol concentrations are summarized in the following two Tables.

The hemolytic effect of 0.05% and 0.2% polidocanol formulations on human blood *in vitro* (study 256188)

Sample	Hemolysis (%)			
	Subject A	Subject B	Subject C	Mean
0.05% microfoam	0.2	0.1	0.0	0.1
0.2% microfoam	0.1	0.2	0.6	0.3
0.05% liquid	0.2	0.0	0.0	0.1
0.2% liquid	0.3	0.1	0.0	0.1
Saponin (4.0 g/L)	64.8	36.6	46.9	49.4
Saline (154 mM NaCl)	0.2	0.0	0.0	0.1

Reference ID: 3367720

The hemolytic effect of 0.5% and 2.0% polidocanol formulations on human blood *in vitro* (study 256188)

Sample	Hemolysis (%)			
	Subject A	Subject B	Subject C	Mean
0.5% microfoam	0.0	0.0	0.2	0.1
2.0% microfoam	0.1	0.0	0.2	0.1
0.5% liquid	0.0	0.0	0.1	0.0
2.0% liquid	0.0	0.1	0.1	0.1
Saponin (4.0 g/L)	65.9	80.0	83.6	76.5
Saline (154 mM NaCl)	0.0	0.1	0.1	0.1

Conclusion
The data indicated that polidocanol solution and polidocanol microfoam were non–hemolytic at the concentrations evaluated in the study. This was compared with physiological saline which was also non-hemolytic and was in total contrast to the positive reference compound saponin which was found to be severely hemolytic.

***In vitro* experiments to assess microfoam gas absorption into human venous blood (Study RD198/23925) (non-GLP)**

This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective
The objectives of this *in vitro* study were to determine the rates of gas absorption from microfoam into blood and to investigate (b) (4)

Methods This experiment measures the change in volume of a syringe full of fresh human venous blood as polidocanol microfoam was added. The principle behind the experiment was as follows: The blood is contained in a 20 mL syringe connected to a manometer tube. The other end of the manometer tube is open to the atmosphere in an otherwise closed system. As microfoam is added to the syringe, the total volume increases and blood is displaced into the manometer tube. As the gas is absorbed it ceases to have any effective volume and the total volume of blood and microfoam reduces. As the volume reduces the column of blood displaced into the manometer tubing is drawn back into the syringe, the rate is dependent on the rate of gas absorption. After complete absorption, there will be an increase in the total volume, from the liquid component of the microfoam and also from any gas that remains unabsorbed. The volume of liquid added is determined by measuring the reduction in weight of the dosing syringe before and after administration of the microfoam (assuming the gas has no weight and the liquid a density of 1). In this way, both the rates and completeness of the gas absorption may be determined by measuring the movement of the column of blood in the manometer tube. The narrow bore of the manometer tube amplifies the

Reference ID: 3367720

volume change so that relatively small changes can be easily measured (1 µL equals approximately 3 mm).

All microfoam formulations used in the experiment contained 1% polidocanol aqueous solution. The formulations tested were (b) (4) PEM OF (b) (4) nitrogen) and PEM NF1(<1% nitrogen) as described below. The Sponsor provided the microfoam formulations to the testing facility as pre-charged aerosol canisters. Each set of experiments consisted of 6 runs.

(b) (4)

1 Page has been Withheld in Full as b4 (CCI/TS) immediately following this page

Summary statistics for each experiment for percentage residual gas volume at 120 seconds (study RD198/23925)

(b) (4)

[redacted]

Conclusion

This study clearly demonstrated the important elements of microfoam gas absorption. (b) (4) [redacted] PEM NF1 resulted in faster and more complete gas absorption when compared with PEM OF.

***In vitro* experiments for assessing whether there is a potential for relatively high concentrations of polidocanol to remain in the venous system following the Varisolve® (PEM) clinical procedure (Study RD198/23926) (non-GLP)**

Note: This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective

The primary objectives were to determine whether there is the potential for polidocanol to remain associated with gas bubbles after mixing with blood; and to investigate the magnitude of any such effect in terms of localized concentrations of polidocanol that could result. The secondary objective was to investigate the effect of mixing the blood/polidocanol/gas mixture.

Methods

The study introduced polidocanol microfoam into a sample of fresh human venous blood and following a short period of mixing the sample was held vertically, permitting the bubbles to float to the top. Without disturbance the blood was then separated into 5 equal aliquots, each of which was centrifuged and plasma portion was removed and assayed for polidocanol concentration. It was hypothesized that if there was any

Reference ID: 3367720

association between the polidocanol and gas bubbles in blood, then there would be excess polidocanol concentration in the upper (first) aliquot.

The formulations tested were [redacted] (b) (4) microfoam (1%, 0.1% and 0.01% polidocanol), PEM NF1 (<1% nitrogen) and air (70 µL of 1% polidocanol + 500 µL of air). The Sponsor provided the [redacted] (b) (4) microfoam test products and the PEM NF1 as pre-charged aerosol canisters. For the air/polidocanol formulation 70 µL of 1% polidocanol solution was introduced into the test syringe and mixed until fully dispersed. Then 0.5 mL of air was added and the syringe shaken vigorously for one minute. Each set of experiments consisted of 6 runs. The polidocanol concentrations were measured by liquid chromatography and tandem mass spectroscopy (LC-MS-MS).

Results
There were no statistically significant differences in polidocanol concentration between any of the aliquots in any of the experiments.

Conclusion
There was no measurable or clinically important excess polidocanol concentration associated with gas bubbles following mixing microfoam *in vitro* with fresh human venous blood.

In Vivo Safety Pharmacology

An extensive program of 13 *in vivo* safety pharmacology studies was conducted. This comprised a series of safety pharmacology studies to investigate the effects of polidocanol solution on the central nervous system (study RCC 767665, study RCC 767654), the gastrointestinal system (study RCC 767698), the renal system (study RCC 767687) and the cardio-respiratory systems (study RCC 767676). However, in addition to this safety pharmacology program with solution, a series of standard (study Butler 1, study TMC002) and specialized cardio-respiratory studies in the dog and rat were conducted with polidocanol microfoam. These studies included a program to address the FDA concerns relating to the effects of microbubbles in the circulation arising from the administration of PEM (study TMC003, study TMC004, study UP001, study DVASF001, study DVASF002, and study CTBR 690666).

Central Nervous System

Polidocanol: Modified Irwin screen test in the mouse (Study RCC 767654) (GLP)

Objective
A study was conducted to evaluate possible pharmacological effects of polidocanol, when administered intravenously, on the general behavior of the NMRI mouse using a modified Irwin screen test for evaluation.

Reference ID: 3367720

Methods
Three male NMRI mice per group received vehicle (Water for Injection), 1 mg/kg, 3 mg/kg or 10 mg/kg polidocanol solution by intravenous bolus administration. Animals were assessed for behavioral changes before, shortly after and at 30 minutes, 1 hour and 2 hours after treatment. The observations included home cage assessments as well as the response to a new environment (arena) and handling as follows:

Home cage: – Increased locomotor activity, abnormal behavior, alertness, startle response.

In the arena: – Pilo-erection, tremor, twitches, respiration, spasms, abnormal behavior, abnormal body carriage, abnormal gait, exploratory activity, sedation. In the hand – Fearfulness, passivity, exophthalmos, pupil diameter, response of pupil to light, pinna reflex, corneal reflex, hypothermia, cutaneous blood flow, cyanosis, ptosis, lacrimation, salivation, body tone, pain response, aggressiveness, vocalization, diarrhea.

On return to the home cage: – Grooming.

Results
Following intravenous administration of vehicle or polidocanol at 1, 3 or 10 mg/kg, an increase in alertness in 1 or 2 or 3 animals per group was observed for up to 2 hours after dosing. In addition, an increased exploratory activity (1 of 3 animals) was noted at isolated time points for up to 2 hours, in all groups. However, as these behavioral effects were not dose related, were also seen in vehicle-treated animals and/or were present before dosing, they were not thought to be related to treatment with test article. In addition, in 1 of 3 animals dosed with 1 mg/kg polidocanol, a decrease in exploratory activity, immediately after dosing, was evident. As this effect was seen in only 1 of 3 animals, it was not considered to be of pharmacological relevance.

Conclusion
Intravenous administration of polidocanol at doses up to 10 mg/kg was without significant effects on general behavior in the male NMR1 mouse.

Polidocanol: Effect on locomotor activity in the mouse (Study RCC 767665) (GLP)

Objective
A study was conducted to evaluate possible effects of polidocanol on the locomotor activity (static, mobile and rearing) in NMRI mice following intravenous administration.

Methods
Six male mice per group received vehicle (Water for Injection), polidocanol in solution at 1 mg/kg, 3 mg/kg or 10 mg/kg by intravenous bolus administration. Locomotor activity was measured for 2 periods of 10 minutes prior to dosing and continuously for 2 hours after dosing. Values after dosing were registered and reported at 10 minute intervals. Activity was measured by interruptions in the infrared light beams caused by movement

Reference ID: 3367720

of the animals. The total activity during each measurement period was calculated by the monitoring system. Animals were kept individually in the activity monitoring cages.

Results
Following administration of 1, 3 and 10 mg/kg polidocanol, no significant effects on static activity (slow, fast and total), mobile activity (slow, fast and total) and mobile times were measured in the mouse when compared with vehicle treatment, for up to 2 hours after dosing. Administration of 1 mg/kg of polidocanol resulted in isolated statistically significant increases in rearing activity (slow, fast and total) and active time at 30 minutes after dosing ($p<0.05$). However, when data were presented as percent change from before dosing values, effects on rearing activity were no longer significant. Effects on active time at the same time point are not thought to be treatment-related as they were not seen following administration of polidocanol at 3 and 10 mg/kg..

Conclusion
Polidocanol at intravenous dosages up to 10 mg/kg was without pharmacologically relevant effects on locomotor activity for up to 2 hours after dosing in the male NMRI mouse.

Gastrointestinal System

Polidocanol: Effect on the intestinal motility (charcoal propulsion) in the mouse (Study RCC 767698) (GLP)

Objective
A study was conducted to evaluate possible effects of polidocanol on the motility of the gastrointestinal tract in NMRI mice following intravenous administration.

Methods
Six male mice per group received vehicle (Water for Injection), polidocanol in solution at 1 mg/kg, 3 mg/kg or 10 mg/kg by intravenous bolus administration. Animals were fasted overnight with access to water *ad libitum*. On the test day animals were weighed and test or control article administered. Charcoal was administered immediately after dosing. Animals were sacrificed 30 minutes after the administration of charcoal. The gastrointestinal tract was removed immediately after sacrifice. The total length of the small intestine and the distance traversed by the charcoal was measured and recorded.

Results
The mean distance traversed by the charcoal meal in control animals treated with vehicle was 33 ± 3 cm. This represented 75% of the total intestinal length. Following intravenous administration of polidocanol at 1, 3 or 10 mg/kg, the distance traversed by the charcoal meal was 30 ± 5 cm, 33 ± 5 cm and 27 ± 5 cm, respectively. The percentage of the intestine through which the charcoal passed was 68%, 74% and 62%, respectively. These results were not statistically different to those seen in the controls.

Reference ID: 3367720

Conclusion Intravenous administration of polidocanol at dosages up to 10 mg/kg was without significant effect on intestinal motility in the male NMRI mouse.

Renal/Urinary System

Polidocanol: Effect on renal function in the rat (Study RCC 767687) (GLP)

Objective
A study was conducted to evaluate possible effects of polidocanol on renal function in Han Wistar rats following intravenous administration.

Methods
Six male rats per group received vehicle (Water for Injection) or polidocanol in solution at 1 mg/kg, 3 mg/kg or 10 mg/kg by intravenous bolus administration. Immediately after dosing animals were transferred to metabolism cages where they were housed individually. Animals were deprived of food but allowed access to water *ad libitum* for the collection period of 24 hours. Urine was collected in specimen vials at 6 (0 to 6) and 24 (6 to 24) hours after dosing. At the end of the 24 hour collection period animals were lightly anesthetized with isoflurane and approximately 0.6 mL blood was sampled from the retro-orbital plexus into tubes containing lithium heparin as anticoagulant. The volume, specific gravity, osmolality and pH of urine collected at 0 to 6 and 6 to 24 hours after dosing were measured and the contents of sodium, potassium, chloride, calcium, phosphorous, protein (total), urea, bilirubin, urobilinogen and creatinine were analyzed. At 24 hours after dosing the plasma creatinine was measured and creatinine clearance calculated.

Results
Administration of 1 mg/kg polidocanol was without effect on urine volume, specific gravity, osmolality, electrolyte excretion, total protein, urea, bilirubin, urobilinogen or creatinine, for up to 24 hours after dosing. Six hours after dosing, a significant reduction in urine volume was seen following administration of 3 mg/kg ($p<0.05$). In these animals specific gravity (not significant) and osmolality ($p<0.05$) were correspondingly increased but no marked effects on pH or excretion of electrolytes or other parameters were seen. These effects were no longer evident at 24 hours after dosing. Following administration of 10 mg/kg, polidocanol induced a slight but not statistically significant increase in phosphorous and total protein excretion at 6 hours after dosing. However, in contrast to 3 mg/kg polidocanol where a reduction in urine volume was measured, no effects on urine volume were seen. Between dosing and 6 hours after dosing, it was noted that 4 of the 6 rats had blood in the urine. At 24 hours after dosing, slight increases in the excretion of potassium ($p<0.05$), calcium (not significant) and total protein (not significant) were observed but no blood in the urine was noticed at this later time point. Polidocanol at 1, 3 and 10 mg/kg produced no effects on the plasma levels of creatinine or creatinine clearance when measured 24 hours after dosing.

Reference ID: 3367720

Conclusion
Intravenous administration of polidocanol at doses up to 3 mg/kg was without biologically relevant effects on renal function in the male rat for up to 24 hours after dosing. At 10 mg/kg, blood in the urine was noted at 6 hours after dosing but this effect was no longer evident at 24 hours after dosing.

Cardiovascular and respiratory systems

Polidocanol: Effect on the cardiovascular and respiratory systems in the anaesthetized dog (Study RCC 767676) (GLP)

Objective
A study was conducted to evaluate possible pharmacological effects of polidocanol on the cardiovascular and respiratory systems of the anesthetized beagle dog when administered intravenously.

Methods
Four anesthetized male beagle dogs (fasted for 18 hours prior to surgery) received sequential doses of vehicle (Water for Injection) and polidocanol solution at 1, 3, 10, 15 and 20 mg/kg by intravenous infusion. Arterial blood pressure (systolic, diastolic and mean), heart rate and respiratory parameters (respiratory rate, tidal volume, minute volume) were recorded before dosing, and at 5 minute intervals for 30 minutes after each administration. Electrocardiographic tracings were recorded and arterial blood flow was measured at intervals before, during and following each administration of vehicle or polidocanol.

Results
Polidocanol at all doses was without effect on systolic blood pressure and heart rate for up to 30 minutes after each dosing. Dosages up to 15 mg/kg were also without effect on diastolic and mean blood pressure. Small but significant increases in diastolic blood pressure were noted before dosing of polidocanol at 20 mg/kg and at 5 to 15 and 25 to 30 minutes after dosing ($p<0.05$ to 0.01). Consequently mean blood pressure was also slightly increased at 10, 15 and 30 minutes after dosing ($p<0.05$).

No effects on electrocardiographic parameters were noted at dosages of polidocanol up to 15 mg/kg. At 20 mg/kg, no effects on P-wave amplitude or duration or on Q-T interval were evident. Statistically significantly higher values for P-Q interval were measured before dosing at 20 mg/kg, during dosing, after dosing and at all time points up to 30 minutes after dosing ($p<0.05$ to 0.01). An increase in QRS interval was also measured after dosing of 20 mg/kg and at 5 and 10 minutes after dosing ($p<0.01$, maximally 12.5%). This effect was short lasting and was no longer seen at 15 minutes after dosing.

No statistically significant effects on blood flow were measured at any dose of polidocanol tested, although a slight reduction in blood flow was seen following

Reference ID: 3367720

administration of 20 mg/kg. No significant effects on respiratory parameters were measured following administration of polidocanol at dosages up to 20 mg/kg.

Conclusion
Sequential intravenous administration of 1, 3, 10, 15 and 20 mg/kg polidocanol was without effect on systolic blood pressure and heart rate in the anesthetized dog for up to 30 minutes after each dosing. No statistically significant effects on blood flow and respiratory parameters were measured following administration of polidocanol at dosages up to 20 mg/kg. Dosing at up to 15 mg/kg was also without effect on diastolic or mean blood pressure or electrocardiographic parameters. At 20 mg/kg, polidocanol slightly increased diastolic and mean blood pressure and increased P-Q and QRS interval in the anesthetized dog.

A physiological study of venous injection of Varisolve® (PEM) PD Microfoam: Investigating the effect of bolus infusions of polidocanol microfoam in dogs (Study Butler 1) (non-GLP)

Objective
This study examined the cardiovascular and pulmonary effects in a canine model of a worst-case scenario of a clinician injecting the maximum dose of polidocanol microfoam directly into the venous circulation, with no trapping of the foam within the target veins.

Methods
Polidocanol microfoam was bolus infused into anesthetized male and female hound breed dogs. Infusion doses were 10 mL, 20 mL, 40 mL or 60 mL of 1% polidocanol microfoam. The study tested four dogs per treatment group. The microfoam was prepared by syringe delivery of polidocanol solution (1%) to the microfoam aerosol device supplied by the Sponsor. A new aerosol nozzle apparatus was attached to the microfoam preparation device and the device pressurized with test gas to 40 pounds per square inch. The composition of the test gas was (b) (4)

For microfoam infusion, a glass syringe was filled by plunger displacement of foam directly from the aerosol canister connector. The size of the glass syringes was selected according to the dose injected, using 10 mL, 20 mL or 30 mL syringes. The syringe port was then diverted to the peripheral venous injection cannula and the test material infused into the animal at 5 mL/ sec (as fast as practicable).

Trans-esophageal echocardiographic imaging was used to monitor microbubbles in the pulmonary artery or vena cava. The following hemodynamic, blood gas, and blood cell parameters were assessed: mean arterial blood pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, central venous pressure, cardiac output, heart rate, stroke volume, pulmonary vascular resistance, total peripheral resistance, oxygen saturation, end-tidal CO_2, arterial O_2, arterial CO_2, arterial pH, arterial white cell counts, arterial neutrophil counts. Electrocardiograms were recorded continuously throughout

Reference ID: 3367720

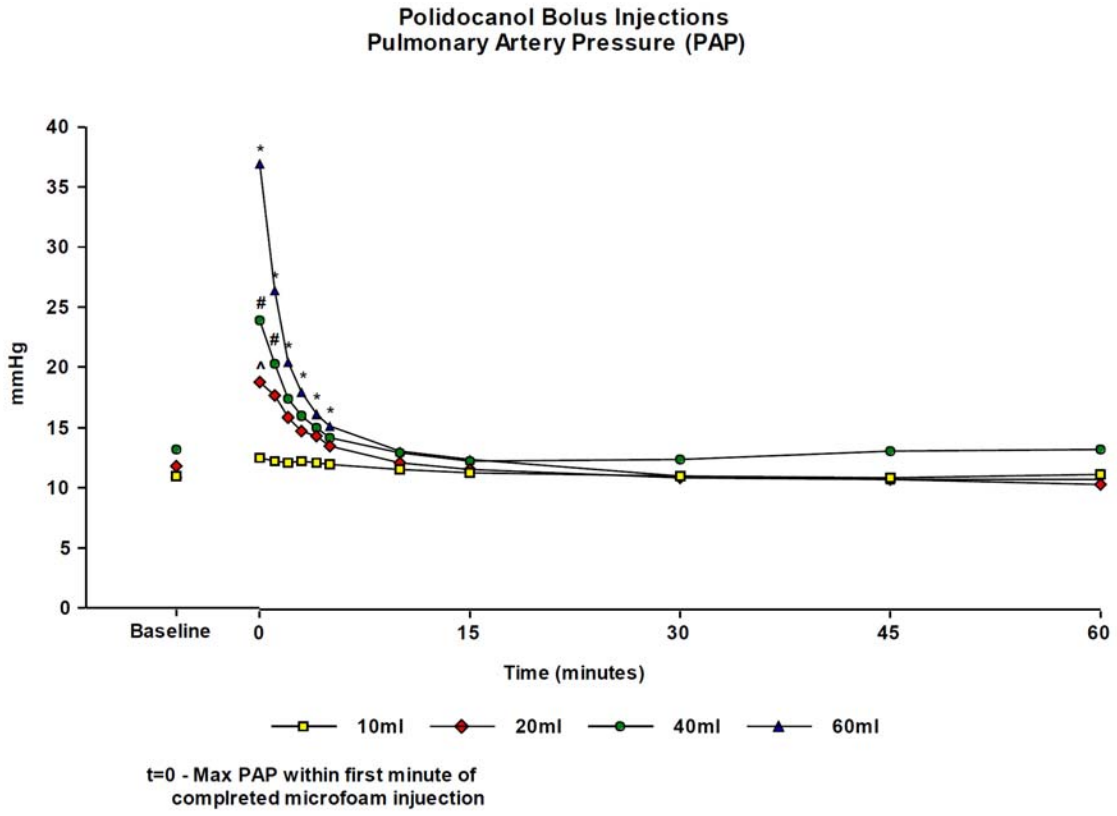
the experiments. The animals were euthanized at the conclusion of each experiment and a gross examination performed. Tissue samples from the heart, liver, spleen, lung and kidneys were collected for histopathological evaluation (*note: no histopathological data were reported in the study report).*

Results

Bolus infusions of polidocanol microfoam in doses ranging from 10 mL to 60 mL resulted in transient dose-related hemodynamic and blood gas effects. The duration of the significant events usually lasted for no longer than 5 minutes post infusion. There were some indications of dose response relationships, especially with pulmonary artery pressures.

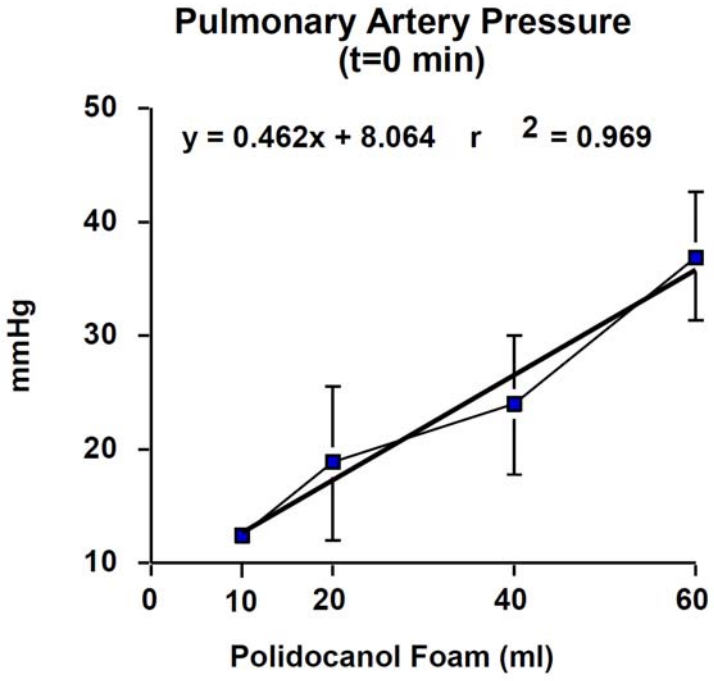
The important changes were seen most clearly in pulmonary artery pressure (PAP), which rose significantly ($p<0.05$) above baseline after each dose, except after the 10 mL dose (Figure below).

Mean pulmonary artery pressure following bolus intravenous infusion of polidocanol microfoam 10mL, 20mL, 40mL and 60mL (study Butler 1)



The response was directly related to volume injected with a good straight-line correlation (Figure below).

Correlation between dose volume and PAP (study Butler 1)

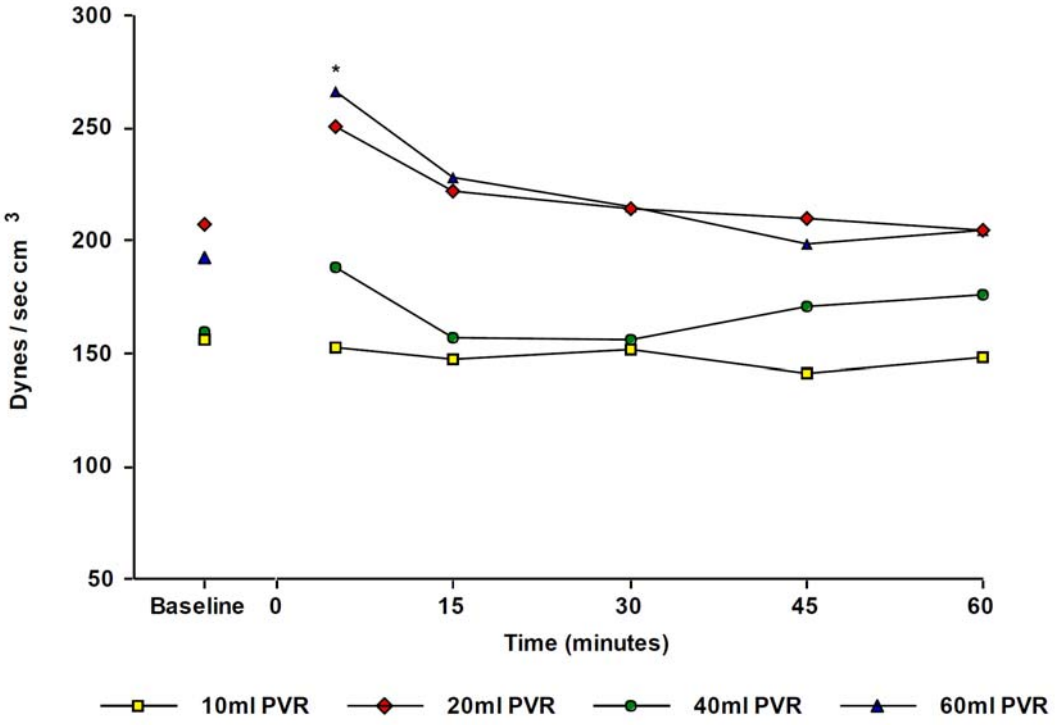


There was a corresponding fall in mean arterial pressure (MAP) although this was less dramatic; the 40 mL dose produced the greatest change. These changes reached their peak by minute 1 in the case of PAP, and MAP lagged reaching its peak at 3 minutes. These rapid changes were matched by the rapid visualization and clearance of bubbles in the pulmonary artery seen on trans-esophageal ultrasound. Both changes decayed rapidly with no significant difference from baseline at 5 minutes with the exception of the PAP, 60 mL dose.

Compensatory changes were seen in increased heart rate, significant only at 0 to 3 minutes and central venous pressure that failed to reach significance.

Cardiac output (CO) was measured by thermal dilution and was first measured at 5 minutes, it showed no deviation from base line, earlier changes which could have been expected to influence CO had already returned to normal. The derived measurements of vascular resistance similarly showed no change at 5 minutes, except for a statistically significant ($p<0.05$) increase in pulmonary vascular resistance (PVR) at the 60 mL dose (Figure below).

Pulmonary vascular resistance following bolus intravenous infusion of polidocanol microfoam 10mL, 20mL, 40mL and 60mL (study Butler 1)



End tidal CO_2, a measure of ventilation perfusion imbalance, showed no consistent variation. There were no significant changes attributable to the polidocanol foam beyond 5 minutes with the exception of the arterial O_2 in the 60 mL group, and may have been caused by pulmonary edema formation following the maximal but transient rise in PAP.

Conclusion

These series of experiments investigated the physiological response to the 'worst case' scenario with a rapid uncontained bolus injection of polidocanol microfoam into a peripheral leg vein of dogs. Even at the largest dose, equivalent to 180 mL in man w/v, this was well tolerated in comparison to previous experience with air injections (Butler, 1989). The 10 mL dose, equivalent to the average human clinical dose of 30 mL 1% foam, resulted in no significant changes. (*Note: the current proposed maximum clinical dose is now 15 mL of 1% PEM so the 10 mL dose is twice the clinical dose (w/v) or equivalent based upon body surface area*). Larger doses produced increasingly large changes in measurements suggesting pulmonary vascular occlusion, which might in part be reflex in origin, but all these changes rapidly returned to normal values without sustained deleterious effects.

Reference ID: 3367720

Effect of Varisolve® (PEM) PD Microfoam (prototype formulation) on cardiopulmonary function in the anesthetized dog: dose response with repeat rapid dosing, variation of dosing interval and delayed microfoam administration (Study TMC002) (non-GLP)

Objective

In this study, 3 separate experiments were conducted to investigate the cardiopulmonary effects of PEM given by rapid intravenous infusion in the anesthetized hound breed dog.

Methods

The PEM formulations were comprised of the sclerosant, polidocanol at a concentration of 1% and a mix of gases: oxygen, carbon dioxide and nitrogen. Further information on the microfoams is provided below. The anesthetized dog model was the same in each experiment and involved bolus infusion of PEM into the saphenous vein of the leg via infusion catheter inserted by a cut down. Measurement of cardiopulmonary variables and echocardiographic ultrasound recordings were performed at baseline, throughout infusion and continuously for one hour after the last dose. At the completion of data collection, the dog was euthanized. Each experiment tested 4 male and female dogs per treatment group. In Experiment 1 only, selected organs were examined for gross and histological pathology.

The study design was varied to determine the cardiopulmonary, blood gas and blood cell effects of: (1) repeat rapid infusions of five escalating volume doses of PEM (Experiment 1), (2) variation in the dosing interval between repeat infusions of a single volume dose of PEM (Experiment 2) and (3) a single infusion of PEM whose administration was delayed by a predetermined amount of time (Experiment 3).

Experiment 1

Infusion doses were 5 mL x 3 (total 15 mL), 10 mL x 3 (total 30 mL), 15 mL x 3 (total 45 mL), 25 mL x 3 (total 75 mL) and 50 mL x 3 (total 150 mL) of PEM injected at 5 mL/second (as fast as practicable). The interval between each dose was 5 minutes.

Experiment 2

Infusion doses were 20 mL x 3 (total 60 mL) with 5, 15 and 30 minute intervals of PEM injected at 5 mL/second (as fast as practicable).

Experiment 3

The infusion dose consisted of 20 mL of PEM injected 0, 90 and 150 seconds following preparation at 5 mL/second (as fast as practicable).

PEM used in Experiments 1 and 2 was prepared in a pressurized device with a final concentration of (b) (4) nitrogen. Forty (40) mL of polidocanol solution (1%) were removed from a sterile 50 mL vial (single use only) and delivered by clean syringe to the microfoam aerosol device supplied by the Sponsor. A new aerosol nozzle apparatus was attached to the microfoam preparation device and the device pressurized with test gas from a cylinder to 40 pounds per square inch pressure. The gas mixture was

Reference ID: 3367720

comprised of oxygen (b) (4), nitrogen (b) (4) and carbon dioxide (b) (4) This resulted in a final nitrogen concentration (b) (4) Experiment 3, in contrast used PEM manufactured in special canisters by the Sponsor designated as PEM OF and certified to contain (b) (4) nitrogen.

Results

All animals survived to scheduled sacrifice. Findings showed that repeat rapid infusions of PEM at doses 5 to 50 mL (total dose 15 to 150 mL) had no effect on heart rate (HR), cardiac output (CO), systemic vascular resistance (SVR), stroke volume (SV), arterial carbon dioxide partial pressure ($PaCO_2$), arterial total concentration of carbon dioxide, oxygen saturation (SpO_2, SaO_2) and percent neutrophils. Doses of 5 mL and 10 mL (total dose of 15 and 30 mL) of PEM also had no effect on the remaining variables of mean pulmonary artery pressure (MPAP), peripheral vascular resistance (PVR), pulmonary capillary wedge pressure (PCWP), mean arterial pressure (MAP), central venous pressure (CVP), end-tidal CO2 (ET-CO_2), arterial oxygen partial pressure (PaO_2) and white blood cell (WBC) count. Infusions of PEM at higher doses of 25 mL and 50 mL (total dose of 75 and 150 mL), produced transient increases in mean and maximal MPAP and PVR, transient decreases in MAP, ET-CO_2, PaO_2 and arterial pH. Additionally, repeat infusions of 15 mL (total dose of 45 mL) of PEM increased MPAP, PCWP and decreased white blood cell count. The latter variable was also depressed with repeat infusions of 25 mL of PEM. Transient changes produced by repeat infusions of PEM lasted no longer than 5 to 6 minutes with the exception of ET-CO_2 and WBC which returned to baseline over a longer period. The no-effect dose in this dose-response experiment was 10 mL per injection, or total of 30 mL 1% foam, given to a dog with average weight of 30 kg, which is approximately two times the anticipated maximum human exposure based on a volume/weight ratio and a clinical dose of 30 mL. *(Note: this was the maximum anticipated dose at the time of writing the report; the current proposed maximum clinical dose is 15 mL 1% foam and, therefore, the ratio is now approximately four times (v/w) or three times based upon body surface area).*

With a 5 minute interval between repeat infusions of higher doses (25 mL and 50 mL) of PEM, an enhanced increase in MPAP and decrease in ET-CO_2 occurred following the third infusion compared to the first infusion. In experiment 2, lengthening the dosing interval by 3 and 6 fold (15 and 30 minutes, respectively) to allow for sufficient clearance of microbubbles between the three repeat infusions of PEM given at a supra clinical dose of 20 mL, showed an ameliorating effect on some parameters such as MPAP, PVR and PaO_2 but no effect at all on others such as MAP, arterial pH, and WBC. Infusion of PEM at a 30 minute interval exacerbated changes in CVP and ET-CO_2. Therefore, the 15 minute interval between repeat dosing of PEM is sufficient to minimize cardiopulmonary and blood gas variables under the conditions tested.

Variation in the time (0, 90 seconds, 150 seconds) between preparation of PEM and infusion (Experiment 3) had no effect on some variables of MPAP, MAP and ET-CO_2, while others such as arterial pH and PVR were minimized with PEM used immediately or within 90 seconds of preparation compared to microfoam prepared 150 seconds prior to administration. These results support the use of PEM within 90 seconds of formation.

Reference ID: 3367720

The cardiopulmonary changes observed following PEM infusion in Experiment 3 were diminished compared to responses observed in Experiment 2 following the first dose. The reduction of nitrogen content of PEM (b) (4) used in Experiment 3 may account, in part, for these differences.

Conclusion

As expected, microbubbles were observed on trans-esophageal echocardiography following infusion of all doses of PEM in all experiments. The transient appearance of microbubbles following infusions in the three experiments was similar. In Experiment 1 opacification time was dose dependent. The hemodynamic and gas exchange responses produced by the higher doses (45 to 150 mL total dose) of PEM are consistent with transient occlusion of pulmonary arterioles with microbubbles and initiation of compensatory effects. Nevertheless, despite the presence of microbubbles and infusion of PEM up to a total volume of 150 mL (approximately 10 times a clinically relevant dose), the histopathology of lung, liver, heart and kidney was the same as observed in untreated control animals and therefore, under acute conditions, was not adversely affected by PEM. *(Note: the dose volumes were selected to span a 10 fold range of volumes. The minimum dose (3x = 15 mL total) was equivalent to a clinically anticipated dose of 30 mL (v/w basis). The maximum dose of 50 mL (3x = 150 mL total) was a survivable volume for the size of the test animals (20 to 35 kg), which thus exceeded the maximum clinical dose by approximately 10 times on a dose volume equivalency. The current proposed maximum clinical dose is 15 mL 1% PEM and, therefore, 150 mL is approximately 20 times the clinically relevant dose or 16 times based upon body surface area).*

Assessment of Varisolve® (PEM) NF1 on cardiopulmonary parameters in the anesthetized dog (Study TMC003) (non-GLP)

Note: This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective

This study evaluated the acute cardiopulmonary hemodynamic effects of the PEM NF1 formulation, administered intravenously as two rapid bolus injections separated by a 5 minute interval, in the anesthetized canine model (hound breed). PEM NF1 comprised 1% polidocanol and a gas mix of oxygen, carbon dioxide and low nitrogen (<1%). The study also evaluated the cardiopulmonary hemodynamic effects of polidocanol solution and polidocanol foamed with room air administered in the same manner as PEM NF1. Further information on the microfoam formulations is provided below.

Methods

For evaluation of PEM NF1 and polidocanol 1% solution, sixteen dogs were randomized into one of four groups with n=4 dogs per group (Table 8). The cardiopulmonary parameters of three doses of PEM NF1 and one dose of liquid polidocanol were assessed. For evaluation of air-based polidocanol microfoam (air-based PD microfoam),

Reference ID: 3367720

six dogs (2 groups of n=3 dogs) received one of two doses, equal to the two lower doses of PEM NF1. Each dog received one dose of test material given intravenously as two rapid bolus infusions separated with a 5 minute interval.

Study design (study TMC003)

Group number	Number of animals	Test article	Dose, mL*
1	4	1% polidocanol solution	12.8 (2 x 6.4)
2	4	PEM NF1	10 (2 x 5)
3	4	PEM NF1	20 (2 x 10)
4	4	PEM NF1	80 (2 x 40)
5	3	Air-based polidocanol microfoam	10 (2 x 5)
6	3	Air-based polidocanol microfoam	20 (2 x 10)
*All doses administered at 5 minute intervals at 5 mL/sec through an indwelling saphenous vein catheter.			

PEM NF1 was supplied by the Sponsor as a bi-canister, consisting of one canister containing carbon dioxide (1.2 bar absolute) and 1% aqueous polidocanol solution, and the other canister containing oxygen pressurized to (b) (4) bar absolute. Microfoam was generated through a (b) (4) immediately before use. PEM NF1, contained nitrogen only as a trace contaminant of less than 1% and typically below the level of analytical detection of 0.4%.

For preparation of air-based PD microfoam, 40 mL of polidocanol solution (1%) were delivered by syringe to an aerosol device supplied by the Sponsor. A (b) (4) was attached to the aerosol valve and pressurized with Medical Air USP (b) (4) to 40 pounds per square inch.

A plastic, sterile, non-pyrogenic syringe was filled by plunger displacement with foam directly from a microfoam transfer device attached to either the PEM NF1 canister or the aerosol device. The size of the syringe was selected according to the dose injected, using 10 mL or 20 mL syringes. The syringe port was diverted to the peripheral venous injection cannula and the test material infused into the animal at 5 mL/second followed by a saline flush (10 mL). The microfoam was infused within 90 seconds of preparation.

Dogs were anesthetized with pentobarbital sodium, mechanically ventilated and instrumented with catheters for measurement of pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), mean arterial pressure (MAP), central venous pressure (CVP), and cardiac output (CO). Heart rate (HR), end-tidal carbon dioxide (ET-CO_2) and oxygen saturation (SaO_2) were recorded with capnography and oximetry, respectively. Peripheral vascular resistance (PVR), stroke volume (SV) and systemic vascular resistance (SVR) were calculated from measured parameters. Measurements were made in triplicate prior to the first infusion of test agent (baseline),

Reference ID: 3367720

during infusions and up to 60 minutes following the second infusion. The presence of microbubbles was determined with transesophageal echocardiography (TEE).

Results

All animals survived to scheduled sacrifice. PEM NF1 at doses of 10 mL and 20 mL did not change cardiopulmonary parameters of MPAP, PVR, HR, CVP, CO, SV, SVR and SaO_2. Minor changes in MAP, ET-CO_2 and PCWP occurred following infusion of 20 mL of PEM NF1, but were small and considered clinically irrelevant.

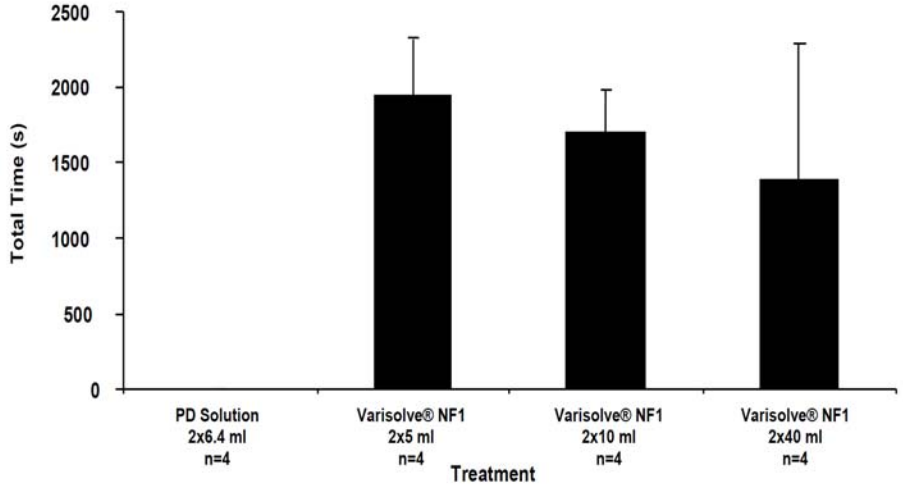
PEM NF1 at the highest dose of 80 mL had no effect on CVP, SVR, CO, SV, and SaO_2 but significantly and transiently increased MPAP, PVR, and HR and decreased ET-CO_2. The maximum increase in MPAP was approximately 5 mm Hg. Minor but statistically significant changes in PCWP and MAP were observed following the second infusion which were considered to include baseline drift and too small to be clinically relevant. Repeat infusions of PD solution, at a dose equal to that contained in 80 mL of PEM NF1, produced a small increase in MPAP but no change in PVR. Infusions of PD solution changed other variables to the same (PCWP, HR, ET-CO_2) or greater extent (MAP, CVP) compared to PEM NF1.

Infusion of air-based PD microfoam elevated MPAP and PVR at doses of 10 mL and 20 mL. The same doses of PEM NF1 had no effect on these parameters. Since the concentration of PD sclerosant was comparable for the two formulations, and the foaming technique (effecting bubble size and stability) was similar, the difference is attributed to the high nitrogen content in air.

Total persistence time and disappearance rate of microbubbles (Figures below) as observed with TEE were independent of the test agent.

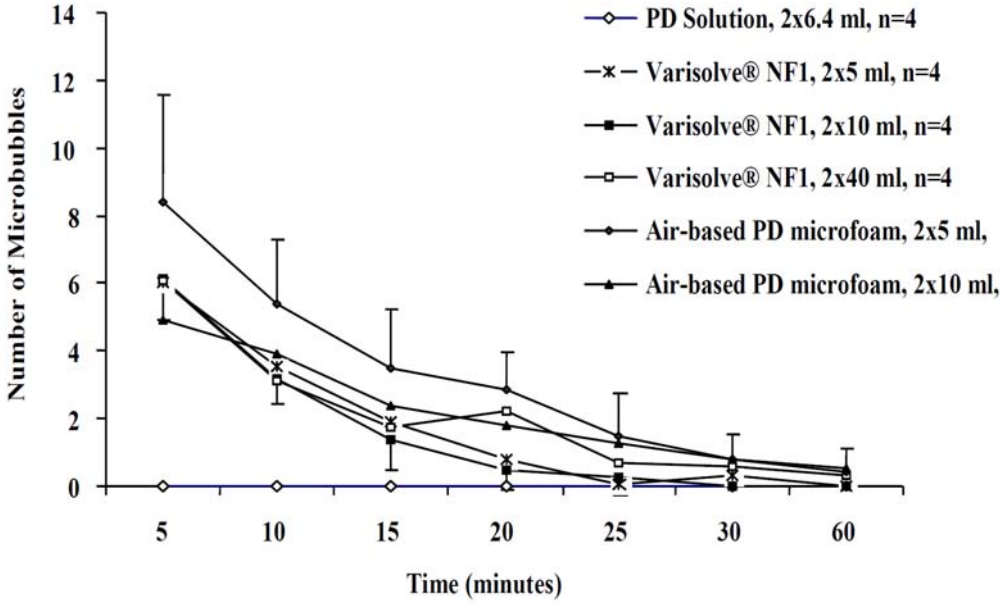
Reference ID: 3367720

Total time of TEE detection of bubbles following treatment with Varisolve® (PEM) NF1 in the anesthetized dog (study TMC003)



Values given as mean ± SD. Total TEE time for Air-based PD microfoam at 2x5 ml (n=2) were 2356s and 1733s and at 2x10 ml (n=2) were 3767s and 1797s.
PD signifies 1% polidocanol solution.

Number of microbubbles versus time following infusion of Varisolve® (PEM) NF1 and air-based PD microfoam (study TMC003)

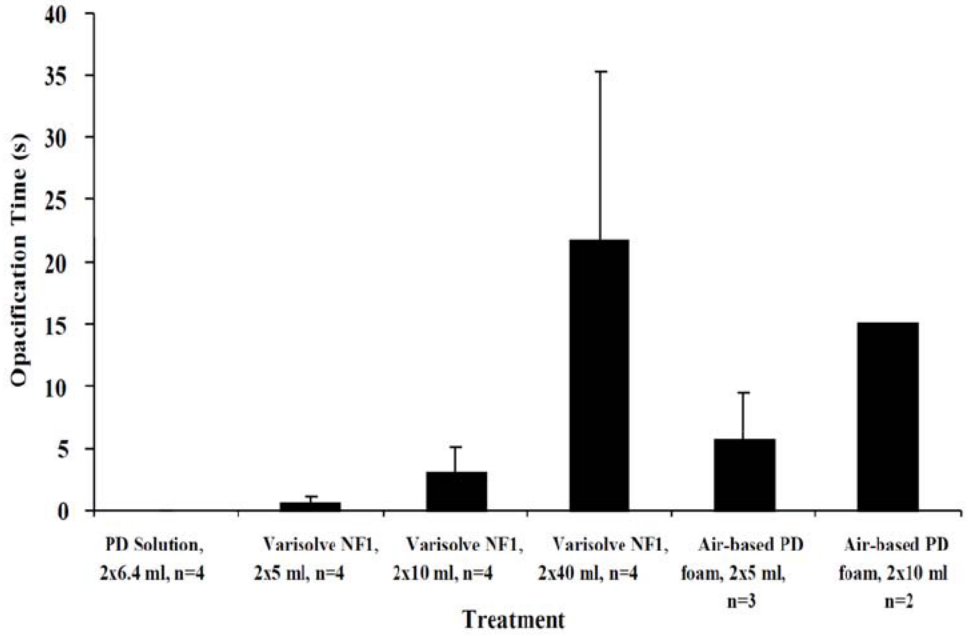


Values given as mean±SD. For clarity, SD shown only for Air-based PD microfoam (2x5 ml) and Varisolve® NF1(2x10 ml). PD signified 1% polidocanol solution.

As expected, PD solution produced no evidence of bubbles and opacification time following rapid infusion of PEM NF1 was dose-related (Figure below). Infusions of 5 mL and 10 mL of PEM NF1 (Group 2 and 3) that produced no change in MPAP,

opacification time was 1 and 3 seconds, respectively. The highest dose of PEM NF1 (Group 4) produced a longer opacification of 22 seconds. The opacification time following infusion of air-based PD microfoam (Group 5) was 6 seconds longer than observed with the same dose of PEM NF1 (Group 2).

Opacification time following infusion of Varisolve® (PEM) NF1 and air based PD microfoam (study TMC003)



Values given as mean±SD. Opacification time for air-based PD microfoam at 2x10ml for n=2 dog were 24 and 5 s. PD signifies 1% polidocanol solution.

Conclusion

PEM NF1 had no effect on cardiopulmonary hemodynamics at total doses of 10 mL and 20 mL, administered intravenously. Evidence of transient and minor pulmonary obstruction was observed with infusion of PEM NF1 at a total dose of 80 mL. This is equivalent (v/w) to 12 times the usual therapeutic dose. (*Note:* (b) (4) *The current proposed maximum clinical dose is 15 mL of 1% PEM and therefore this ratio is now 16 times (v/w) and 11 times based upon body surface area*).

***In vivo* canine model to evaluate association of the active drug substance, polidocanol, with the gaseous component of Varisolve® (PEM) PD microfoam (prototype formulations) (Study TMC004) (non-GLP)**

Note: This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective

This study was conducted to measure the concentration of the drug substance, polidocanol, in the pulmonary artery (PA) of hound dogs following administration of two formulations of 1% polidocanol (PD) microfoam: PD microfoam (<1% N_2, equivalent to PEM NF1) and PD microfoam (approximately (b) (4), equivalent to PEM OF).

The study also determined the extent of association of the sclerosant, polidocanol, with microbubbles of PD microfoam immediately following release of PD microfoam from a venous depot site in the anesthetized canine model.

Note: A pilot study was conducted before the main study with air based polidocanol microfoam (approximately 80% N2), polidocanol microfoam (<1% N_2) and polidocanol microfoam (approximately (b) (4) There were no differences in polidocanol concentration in the bubble-rich and the bubble-depleted fractions for the three formulations tested.

Methods

Microfoam mixed with systemic blood entering the pulmonary artery (PA) prior to passage into the pulmonary vascular bed was sampled and analyzed to determine the concentration of polidocanol in the bubble-rich and bubble-depleted portions of blood. Plasma levels of polidocanol following administration of polidocanol-spiked saline administered similarly to PD microfoam but without foaming were also measured in this model as a non-gas control.

An anesthetized dog model was used for bolus infusion of PD microfoam into the saphenous vein. The saphenous vein was cannulated and a ligature was placed around the femoral vein and tightened to occlude the vein immediately prior to administration of test agent. The occlusion was maintained for a 5 minute period following dosing and then released to allow the vein content to be released systemically. This was to simulate the clinical situation for varicose vein treatment where microfoam is maintained between the point of injection and the saphenofemoral junction for up to 5 minutes until venospasm occurs.

In order to prepare the PD microfoam (<1% N_2, equivalent to PEM NF1), the microfoam generating aerosol device provided by the Sponsor was purged with gas mix for 1 minute (b) (4)). Twenty (20) mL of 1% polidocanol solution was withdrawn from a vial using a syringe. The gas purge continued as the 20 mL PD solution was added to the aerosol device. A valve was placed into position on the aerosol device and the gas bleed tap opened. (b) (4)

The PD microfoam (b) (4), equivalent to PEM OF) was prepared by the same method as the PD microfoam (<1% N_2) with the following exceptions.

The aerosol device was allowed to equilibrate with air (valve and solution removed). Twenty (20) mL of 1% polidocanol solution was added to the device chamber and the valve sealed into place. The gas mix was generated by serial dilution, with the intent of achieving a gas mix containing (b) (4) To achieve this, the device (b) (4)

To evaluate the degree of association of polidocanol with the gas bubbles entering the PA, a total of four female dogs were treated with PD microfoam (<1% N_2) and PD microfoam (approximately (b) (4)). To maximize the number of administrations both the right and left saphenous veins were treated.

Each dog received a total of 6 treatments of 10 mL or 20 mL volumes per hind limb. The first 2 treatments in each hind limb were saline containing 1.3 mg, then 2.6 mg polidocanol, respectively. The next four injections included two injections each of PD microfoam (<1% N_2) and PD microfoam (approximately (b) (4) administered in randomized order. Test agents and polidocanol spiked saline controls were injected at 1 mL/second into the occluded treatment vein.

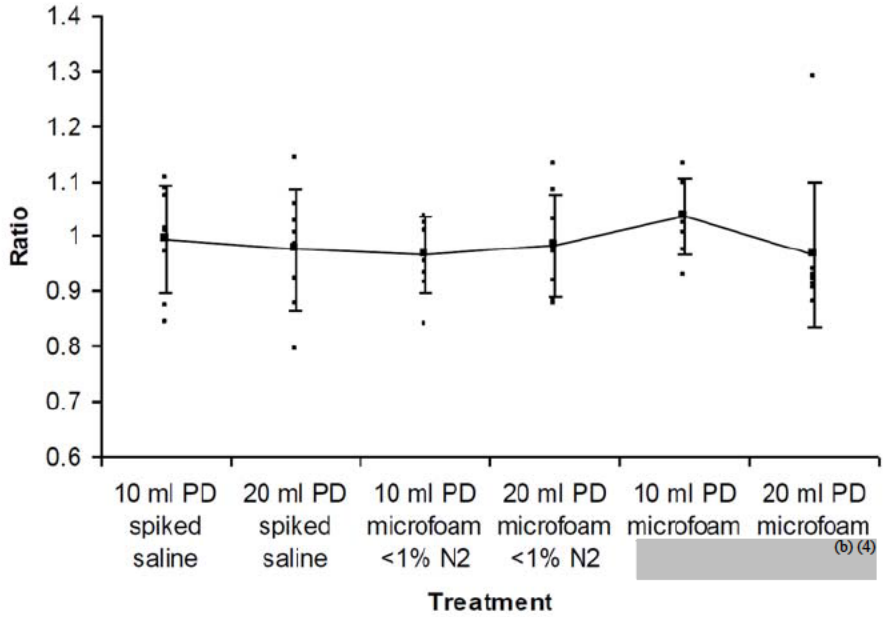
Each treatment dose was followed by a 30 minute clearance period. Control doses were followed by a 10 minute clearance period.

Measurement of cardiopulmonary variables and pulmonary artery ultrasound imaging were performed at baseline, throughout infusion and continuously throughout the study until the last dose. The PA was monitored continuously by trans-esophageal echocardiography (TEE) for the presence of circulating systemic gas bubbles. A PA blood sample was collected at the time of maximum PA opacification following the release of microfoam from the leg. The blood sample containing bubbles was separated into two portions, a bubble-rich portion and a bubble-depleted portion. The polidocanol concentration in each of these two portions was then measured by LCMS- MS and compared statistically for any significant differences in polidocanol concentration.

Results

Following treatment with either PD microfoam (<1% N_2) or PD microfoam (approximately (b) (4)) there were no significant differences in the mean ratio of PD concentration between the bubble-rich portion of the plasma sample and the bubble-depleted portion of the plasma sample (Figure below). Therefore, there was no evidence to suggest that systemically circulating gas bubbles were associated with more PD than the surrounding plasma.

Mean (+/- 1 SD) ratio (% top/bottom) of PD concentration by treatment (dog numbers 05-02 to 05-05) (study TMC004)



Conclusion
These results suggested that residual bubbles that circulate to the lung following microfoam sclerotherapy would not cause an increase in polidocanol toxicity to the lung vasculature.

Assessment of the behavior of Varisolve® (PEM) NF1 in the microcirculation of rat cremaster using intravital microscopy (Study UP001) (non-GLP)

This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective
A study was conducted to evaluate the behavior of PEM NF1 (<1% nitrogen) at five escalating doses in the arterial microcirculation of the rat cremaster using intravital microscopy and hence to gain insight into the potential risk of occlusive cerebral gas embolism in patients with a right to- left shunt e.g. patent foramen ovale (PFO). By way of comparison, the behavior of two other sclerosant microfoam preparations, PEM OF (approximately (b) (4)) and liquid polidocanol foamed with room air (approximately 79% nitrogen), were similarly evaluated in the rat cremaster microcirculation. The study also determined the effect of the sclerosant, liquid polidocanol on agonist-induced vascular reactivity of the cremaster vasculature.

Methods
Intravital microscopy of the rat cremaster is a long-standing reliable model used by others to study microvascular changes to inflammatory mediators, vasoactive agents,

sympathetic nerve stimulation and microvascular embolization with air bubbles. The visual presence of microbubbles, their behavior and dimensions were assessed following infusion of PEM into the rat femoral artery. To approximate the exposure of microfoam to venous blood during the migration of microfoam from a treated varicose vein to the heart, the test article was an aerobically mixed *ex vivo* with venous blood from the rat prior to infusion into the arterial microcirculation.

Eighteen rats were randomized into one of 3 groups with n=6 rats per group. Additionally, 4 rats were assigned to a fourth group and the effect of a single dose of polidocanol on vasoreactivity was determined by intravital microscopy with use of a video micrometer to measure change in vessel diameters.

Study design (study UP001)

Group number	Number of animals	Treatment	
		Test article	Dose (µL)*
1	6	PEM NF1 (<1% nitrogen)	50, 100, 200, 300, 400
2	6	PEM OF (b) (4)	50, 100, 200, 300, 400
3	6	Air-foamed polidocanol (b) (4)	50, 100, 200, 300, 400
			Dose
4	4	Polidocanol solution (3%)	1 mL/kg (30 mg/kg)

* 50 µL mixed in 200 µL venous blood; remaining doses mixed with 400 µL venous blood

PEM NF1 contained nitrogen only at trace levels of less than 1%, and typically less than the minimum detectable 0.4%.

PEM OF contained approximately (b) (4), which was deliberately added as part of the inert atmosphere in the polidocanol canister.

PEM NF1 and PEM OF were supplied as bi-canisters, consisting of one canister containing 1% polidocanol solution in a gas atmosphere and a pressurized oxygen canister. Microfoam was generated (b) (4) immediately before use in both PEM formulations. In order to prepare the air-based polidocanol microfoam (~79% nitrogen), (b) (4)

(b) (4)

. One part sclerosant was mixed with 4 parts of air.

Each rat received an intra-arterial (IA) infusion of test agent mixed with venous blood in an escalating dose manner at dose levels of 50 µL in 200 µL of venous blood, and 100 µL, 200 µL, 300 µL and 400 µL test agent in 400 µL of venous blood. Dose escalation continued until embolization occurred. Embolism was defined as the sudden blocking of the arteriole by a foreign substance (in this case, microbubble) brought to the site by the blood flow. This was assessed by video microscopy. After administration of each dose, if no embolization occurred, the appropriate position of the arterial catheter was confirmed by a saline flush. In some cases, if no embolization occurred at the highest dose tested, the test agent was infused without dilution in venous blood.

On the day of the experiment, the rat was anesthetized and a catheter was placed in the right jugular vein (RJV) for intravenous fluid administration and aspiration of venous blood; a second catheter was placed in the right femoral artery (RFA), positioned to deliver the test agent into the cremaster artery. Each test agent was administered following a 30 second period of mixing with freshly drawn venous blood. The microbubbles of the test agent-venous mixture were detected with an 8 MHz Doppler ultrasound probe present on the RFA catheter. Dosing of the microfoam began at 50 µL in 200 µL of venous blood and continued to the highest dose (400 µL in 400 µL of venous blood) or until embolization occurred. Embolism was defined as the sudden blocking of the arteriole by a foreign substance (in this case, microbubble) brought to the site by the blood flow.

Microbubble behavior was microscopically visualized in each experiment in arterioles in the range of 10 to 200 µm in diameter. Measurement of vessel and bubble dimensions with a video micrometer, observation of erythrocyte motion, and a videotaped recording of the experiment were made. Microscopic observations continued until clearance of microbubbles or up to 2 hours, whichever occurred first.

Results

PEM NF1 up to the highest dose tested (400 µL in 400 µL venous blood) produced no obstructive embolic events within the cremaster microvasculature. Despite convincing evidence of microbubble infusion and correct catheter placement, no bubbles were visualized in 5 out of 6 experiments. In one experiment, two bubbles visible only on viewing individual video frames (approximately 0.20 nL volume) rapidly transited the microcirculation without evidence of peripheral lodgment or reduced blood flow. Several very small bubbles were on a black and white reproduction of a single still video frame. Direct IA infusion of PEM NF1 without mixing with venous blood exhibited continuous downstream movement of the microbubbles and clearance from the microcirculation in less than 3 minutes. These observations are consistent with predicted gas reabsorption rates for microbubbles with PEM NF1 composition.

Microbubbles generated from PEM OF produced no evidence of microvascular occlusion up to the highest dose tested (400 µL in 400 µL blood) although bubbles were

larger and more frequently seen than with PEM NF1. The bubbles rapidly transited the microcirculation without peripheral lodgment. Microbubbles (~ 2.72 nL volume) generated from air-based polidocanol foam extensively embolized and occluded the cremaster arteriolar network with a continuous chain of bubbles, even at the lowest dose tested (50 µL). A no-effect dose level could not be determined suggesting that polidocanol microfoam prepared with room air might seriously impair arterial microcirculation.

Systemic injection of a 3% polidocanol solution, at a dose 56 times greater than the amount of polidocanol used in a therapeutic dose of 20 mL of PEM had no effect on endothelium and smooth muscle-dependent vasoreactivity. (*Note:* (b) (4) *. The current proposed maximum clinical dose is 15 mL 1% PEM and therefore the ratio becomes 75 times (v/w)*).

Conclusion

PEM NF1 was without effect on the arteriolar microcirculation as typified by the cremaster muscle, at doses up to 400 µL. This no-effect dose of 400 µL is equivalent to approximately 20 times the amount of microfoam estimated to cross a 20% PFO assuming the improbable migration of the entire clinical dose of 20 mL to the right atrium. (*Note:* (b) (4) *The current proposed maximum clinical dose is 15 mL 1% PEM and therefore the no-effect dose is now approximately 27 times (v/w)*).

PEM OF was also without effect on the arteriolar microcirculation of the rat cremaster at doses up to 400 µL whereas microfoam generated by foaming liquid polidocanol with room air occluded microvascular arterioles at a dose of 50 µL, the lowest dose tested. The sclerosant alone also had no effect on classic agonist-induced arteriolar responses.

Assessment of the effect of Varisolve® (PEM) NF1 on focal cerebral ischemia and infarction in the rat (Study DVASF001) (non-GLP)

Not : This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective

In the clinic, microbubbles generated from the use of PEM NF1 formulation are predicted to absorb rapidly in venous blood. In patients with a right-to-left shunt condition such as a patent foramen ovale (PFO), the migration of microbubbles from the venous depot site across the PFO into the arterial system was considered to pose a potential risk. This study was conducted to obtain information relevant to assessment of this potential risk.

Reference ID: 3367720

Methods

The effect of intra-arterial (IA) administration of PEM NF1 on subclinical cerebral effects was evaluated in a rat model previously shown to display cerebral ischemia and infarction in response to intracarotid injection of atheroembolic particles (Rapp, 2003). The rat model was adapted to simulate an extreme clinical scenario in which the entire dose of microfoam escaped from a treated vein, mixed within the venous system and 20% traversed a right-to-left shunt to enter the arterial and ultimately the cerebral circulation. The adaptation allowed anaerobic mixing of foamed sclerosant with venous blood prior to IA infusion directly into the right cerebral hemisphere of the rat.

Using immunohistochemical methods, cerebral ischemia was detected by the expression of the neuronal stress marker, inducible heat shock protein72 (iHSP72); infarction was assessed by histological evaluation of neuronal degeneration with the standard hematoxylin stain. Inducible HSP72 is a member of a large family of protein chaperones that assist with protein folding and refolding. HSP72 does not exist in the normal rat brain but is rapidly (within hours) induced in response to a variety of stresses including ischemia, hypoxia and trauma. Once induced, iHSP72 initiates multiple cytoprotective mechanisms suppressing apoptosis and necrosis. It is a very sensitive early marker of stress and its pleiotropic functions are essential for reversal of harmful effects of ischemia.

The initial study design included seven groups with n=10 rats per group except for the microspheres treatment group (n=6), randomized with regard to treatment (Table 11). Signs of unexpected neurological deficits following treatment of rats with the high dose (25 µL) of PEM NF1 prompted the decision to discontinue treatment with this dose. This occurred approximately at the midpoint of the study. Two additional treatment groups (8 and 9) of 8 rats each (Table 11) were created. The remaining treatments were re-randomized.

Reference ID: 3367720

Study design (study DVASF001)

Group number	Number of animals	Treatment			
		Test article[a]	Dose level (µL)	Time of euthanasia	
				Hours	Days
1	10	Saline	25	24	
2	10	PEM NF1	2.5[b]	24	
3	10	PEM NF1	10	24	
4	10	PEM NF1	25	24	
5	10	PEM NF1	25		7
6	10	Air-foamed polidocanol (1%)	10	24	
7	6	Microspheres	100 @ 98 micron	24	
Amended study design					
8	8	PEM NF1	5[b]	24	
9	8	PEM NF1	5[b]		7

[a] All rats received a single intra-arterial infusion of test article into the right internal carotid artery (ICA).

[b] 10 µL PEM NF1 added to 200 µL venous blood and one-fourth (2.5 µL) and one half (5 µL) of foam-blood mixture was administered; other doses were mixed with 400 µL venous blood and entire amount was infused.

PEM NF1 was supplied as a bi-canister, consisting of one canister containing 1% polidocanol solution in a gas atmosphere and a pressurized oxygen canister. PEM NF1 contained nitrogen only as a trace contaminant, less than 1% and typically less than the minimum detectable 0.4%. Microfoam was generated [redacted] (b) (4) immediately before use.

In order to prepare the air-based polidocanol microfoam, [redacted] (b) (4) One part sclerosant was mixed with 4 parts of air.

Each rat received a single IA injection of one of four doses (2.5, 5, 10 and 25 µL) of PEM NF1, one dose (10 µL) of air-foamed polidocanol, one dose of microspheres (100 at 98 micron) or saline (equivalent to 25 µL of microfoam). On the day of the experiment, the rat was anesthetized with an isoflurane and a nitrous oxide/oxygen oxide (N_2O/O_2) mixture (rat was fasted for 12 hours prior to anesthesia). An arterial catheter was placed in the right external carotid artery (ECA) and advanced to the origin of the internal carotid artery (ICA). A second catheter was placed in the right external jugular vein (EJV). The catheters were connected to a Y-connector attached to a series of three syringes. Following a stabilization period, the experiment was initiated with

withdrawal of venous blood (200 µL or 400 µL depending on dose) into the dosing/mixing syringe (syringe left side). The test agent was prepared and injected (middle syringe) and flushed into the venous blood with saline (syringe right side). Since 10 µL was the lowest quantity of microfoam that could be accurately delivered, administration of 2.5 µL and 5 µL doses was achieved by addition of 10 µL of microfoam to 200 µL venous blood and infusion of one fourth and one half of the venous foam mix, respectively. Microbubbles of the foam/blood mixture were detected with an 8 MHz Doppler ultrasound probe positioned on the ICA catheter.

Following the infusion, the ECA and EJV were ligated and the neck incision was closed. The animal was returned to its cage and observed for one hour. Twenty-four hours or 7 days following the ICA infusion, the rat was euthanized and prepared for perfusion fixation. Rats, euthanized 7 days following surgery, were observed daily until euthanasia. The brain was removed, post-fixed and slides of coronal views were immunohistochemically and histologically evaluated according to pre-set criteria. Upper and lower limits were defined by observations in rats infused intra-arterially with microspheres (100 at 98 microns) or saline (equivalent to 25 µL microfoam).

Results

Two rats received 1 to 3 air bubbles during infusion of test agent and were terminated immediately as directed by the protocol. One rat, receiving 5 µL PEM NF1, died during surgery and was replaced with a naïve rat. One rat was found dead several hours following infusion of microspheres. Due to possible autolysis, the brain was not obtained from this animal. All other rats survived to scheduled sacrifice.

There was no evidence of cerebral neuronal degeneration or infarction in any rat infused with PEM NF1 up to the highest tested dose of 25 µL. There was no evidence of ischemia in rats receiving either 2.5 µL or 25 µL of PEM NF1, assessed 24 hours following treatment or in rats infused with 5 µL or 25 µL of PEM NF1 measured 7 days post treatment. Inducible HSP72 expression was observed 24 hours following infusion of 5 µL and 10 µL of PEM NF1 in 1 out of 8 rats and in 2 out of 9 rats, respectively.

Neurological deficits were absent in rats receiving 2.5 µL and 5 µL of PEM NF1. At least one rat in the treatment groups receiving the higher doses of 10 µL and 25 µL of PEM NF1 exhibited neurological dysfunction.

There was no difference in the incidence of neurological dysfunction following infusion of air-based polidocanol foam and the same dose of PEM NF1 measured 24 hours post.

The subclinical cerebral and neurological effects of IA administration of PEM NF1 were attributed in large part to the inhalation of N_2O gas, a widely used gas supplement in rodent surgery. It was proposed that in blood saturated with N_2O, the initial entry of N_2O into microbubbles impedes size reduction and bubble extinction and promotes bubble stabilization.

Reference ID: 3367720

Conclusion
A 2.5 µL no-effect dose of PEM NF1 for ischemia and infarction was determined. This dose is equivalent to 5.8 times the amount of PEM NF1 expected to cross a PFO, with a documented maximal size of 20% of cardiac flow, assuming the entire clinical dose of 20 mL reached the right atrium. (*Note:* (b) (4) *The current proposed maximum clinical dose is 15 mL of 1% PEM and the no-effect dose is therefore 8 times the amount of PEM expected to cross the PFO under these conditions*).

There was no morphological evidence of cerebral infarction at a 5 µL dose of PEM NF1 at both 1 and 7 days following treatment. The minimal ischemia observed at 24 hours in 1 out of 8 rats but completely absent in all rats at 7 days suggests that the no adverse effect dose of PEM NF1 is 5 µL. This dose is equivalent to 11.6 times the amount of PEM NF1 expected to enter the arterial circulation, given the entire clinical dose traversed an estimated 20% PFO. (*Note:* (b) (4) *The current proposed maximum clinical dose is 15 mL of 1% PEM and the no adverse effect dose is therefore 16 times the amount of PEM expected to cross the PFO under these conditions*).

A 5 µL no-effect dose of PEM NF1 for gross neurological deficits was established.

It was proposed that the inability of this study to demonstrate a higher no-effect dose was attributed to the use of N_2O inhalation which produced an adverse and artificial *in vivo* environment that favored the stabilization of PEM NF1 microbubbles and increased the probability of an ischemic event. Since N_2O inhalation is not used clinically during PEM NF1 therapy, a second study (DVASF002) to ascertain the subclinical effects of PEM NF1 in rats supplemented with air compared to rats supplemented with N_2O was initiated.

Re-assessment of the effect of Varisolve® (PEM) NF1 on focal cerebral ischemia and infarction in the rat (Study DVASF002) (non-GLP)

Note: This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective
The primary objective was to test the hypothesis that intra-arterial infusion of PEM NF1 at doses of 10 µL and 25 µL has no effect on cerebral ischemia/infarction in rats breathing an anesthetic inhalation gas mixture containing air in place of nitrous oxide (N_2O) used previously in DVASF001.

A secondary objective was to implement procedural changes that would reduce random cerebral ischemic and infarction events in the rat.

Reference ID: 3367720

Background:
Results of the DVASF001 study showed that intra-arterial (IA) infusion of PEM NF1 into the ipsilateral cerebral hemisphere of the rat produced neurological deficits at a dose of 10 µL. The DVASF001 experimental protocol specified the use of N_2O in the isoflurane anesthetic inhalation mixture throughout the surgical procedures and administration of PEM NF1. DVASF001 findings were unexpected and conflicted with results from a parallel study (UP001) assessing the behavior of PEM NF1 microbubbles in the rat cremaster arteriolar microcirculation by intravital microscopy. It was expected that there would be rapid dissolution of the microbubbles in venous blood and the results of UP001 showed that in rats breathing air/O_2, microbubbles derived from PEM that embolize the microcirculation up to doses 8 times that used in DVASF001 did not cause microvascular occlusion or cessation of blood flow. It was concluded that the discrepancy between the result of DVASF001 and UP001 and supporting gas theories was in large part due to the presence of N_2O that would stabilize and retard reabsorption of PEM NF1 microbubbles.

DVASF002 was, therefore, designed to test the hypothesis that the effect of N_2O inhalation was responsible for the cerebral ischemia/infarction following infusion of PEM NF1 observed at lower than expected doses.

Methods
The experimental procedure was similar to that described in DVASF001 with the exception that only two doses (10 µL and 25 µL) of PEM NF1 were tested and half of the animals at each dose received an anesthetic inhalation mixture containing air/O_2 and the other half received an anesthetic inhalation mixture of N_2O/O_2, as before. Therefore, N=10 rats received a single IA infusion of 25 µL of PEM NF1 during which N=5 rats inhaled N_2O/O_2 (Group 1) and N=5 inhaled air/O_2 (Group 2). Twenty rats received an IA infusion of 10 µL of PEM NF1 with N=10 inhaling N_2O/O_2 (Group 3) and N=10 inhaling air/O_2 (Group 4). A saline treatment group (N=6, Group 5) was added to provide a baseline control for immunohistochemical and histological evaluation (Table 12). Other procedural changes included control of infusion rate, reduction of common carotid artery (CCA) occlusion time, use of femoral venous blood for mixing with test agent and omission of dilution of foam-blood mixture with heparinized saline.

Study design (study DVASF002)

Group	Number of Animals	Treatment		
		Test agent[a]	Dose level (µL)	Inhalation mixture[b]
1	5	PEM NF1	25	N_2O/O_2 (70/30)
2	5	PEM NF1	25	Air/O_2 (70/30)

Reference ID: 3367720

3	10	PEM NF1	10	N_2O/O_2 (70/30)
4	10	PEM NF1	10	Air/O_2 (70/30)
5	6	Saline	25	Air/O_2 (70/30)

[a] The test substance was mixed anaerobically with venous blood and administered as a single IA infusion into the right internal carotid artery. The rate of infusion was 5 µL/second. The rat was euthanized 24 hours following administration of the test agent.

[b] Administered with isoflurane anesthetic

PEM NF1 was supplied as a bi-canister, consisting of one canister containing 1% polidocanol solution in a gas atmosphere and a pressurized oxygen canister. PEM NF1 contained nitrogen only as a trace contaminant, less than 1% and typically less than the minimum detectable 0.4%. Microfoam was generated [redacted] (b) (4) immediately before use.

On the day of the experiment, the rat was anesthetized with 5% isoflurane and a mixture of either N_2O/O_2 (70%/30%) or air/O_2 (70%/30%). Saline treated rats were anesthetized with isoflurane containing the air/O_2 (70%/30%) mixture. An arterial catheter was placed in the right external carotid artery (ECA) and advanced to the origin of the internal carotid artery (ICA). A second catheter was placed in the ipsilateral femoral vein (FV). The catheters were connected to a series of syringes to permit flushing of the catheter system with heparinized saline and to allow withdrawal of venous blood and subsequent mixing with microfoam prior to infusion. The audio output from a Doppler ultrasound probe on the arterial infusion line confirmed injection of microbubbles.

Following the infusion, the ECA and FV were ligated and the incisions were closed. The animal was returned to its cage and observed for one hour. All rats were assessed prior to euthanasia for gross neurological deficits. Twenty four hours following the ICA infusion, the rat was euthanized and immediately underwent perfusion fixation. Coronal brain slices were prepared and stained immunohistochemically with the antibody to the iHSP72 and histochemically with H & E and cresyl violet.

Results

Regardless of the inhalation mixture, IA infusion of PEM NF1 or saline did not produce any neurological deficits at the tested doses. There was no evidence of cerebral ischemia or infarction following infusion of the 25 µL dose of PEM NF1 into rats receiving either an inhalation gas mixture of air/O_2 or N_2O/O_2. Infusion of the 10 µL dose of PEM NF1 produced ischemia in 1 out of 7 rats inhaling the N_2O/O_2 mixture and neuronal degeneration in 1 out of 6 rats inhaling the air/O_2 mixture. Mild ischemia and neuronal degeneration occurred in two of the 5 rats infused with saline. The subclinical lesions produced by IA infusion of 10 µL dose of PEM NF1 were one grade level above

Reference ID: 3367720

that observed in saline controls. A no-effect dose of PEM NF1 on subclinical ischemia or infarction could not be determined in rats supplemented with N_2O/O_2 or air/O_2.

Conclusion
This study did not confirm the previous findings of DVASF001 where neurological signs were seen in 10 µL and 25 µL doses (5 out of 16) compared with the same doses (0 out of 15) in this study with nitrous oxide inhalation and therefore suggests that implementation of procedural changes may have eliminated some cerebral events unrelated to treatment. Consequently, the contribution of N_2O inhalation to adverse cerebral effects of PEM NF1 in this model cannot be determined. Aspects of the model such as *ex vivo* microfoam-venous blood mixing and presence of anesthesia and inhalation gases that are absent in the clinical use of PEM NF1 in man, yet are likely to independently influence cerebral ischemia and infarction in the rat, raised concerns about the clinical relevance of the model.

A cardiovascular and respiratory safety pharmacology study of Varisolve® (PEM) NF1 in the beagle dog (Study CTBR 690666) (GLP)

Note: This was part of the program of studies conducted to address the FDA concerns relating to microbubbles in the circulation following the use of PEM.

Objective
A study was conducted to evaluate the pharmacological effects of PEM NF1 on the cardiovascular and respiratory systems following intravenous bolus injection in the conscious beagle dog, in particular to determine if repeated high dose administration of the test article could cause chronic pulmonary hypertension.

Methods
Four male beagle dogs per group were administered a once-weekly intravenous dose of saline or test article at dose levels of 1.04 mL/kg (saline), 1.0 or 8.0 mL/kg (test article) over a three-week period (4 doses total) (Table 13) followed by a 12 week observation period.

Study design (study CTBR 690666)

Group	Treatment	Dose level mL/kg	Number of animals (male)
1	Saline	1.04	4
2	Low dose PEM NF1	1.0	4
3	High dose PEM NF1	8.0	4

Following dosing, clinical signs, respiratory parameters, blood gas concentrations and hemodynamic measurements were recorded in all animals for a period of four hours. Body weight was also recorded for all animals at least once weekly for calculation of dose volumes. Systemic blood pressures (systolic, diastolic, mean) and heart rate were

Reference ID: 3367720

measured for all dogs using Microrenethane® catheters which had been surgically placed in the abdominal aorta via a femoral artery approximately five days prior to dosing on the study. Blood samples for gas concentrations were also taken as required from this catheter. For collection of pulmonary artery pressure data, a Swan-Ganz® catheter was passed through a jugular venous access port in each animal to the pulmonary artery. On completion of the in-life phase of the study, all animals were euthanized and subjected to a gross necropsy. Histopathological examination of the lungs was performed to determine any pathological effect of possible chronic pulmonary hypertension.

Technical problems were encountered during the study with respect to placement of the catheter for pulmonary artery pressure (PAP) data collection. Initially, exact quantitative measurements for PAP were not obtained as the transducer position relative to the catheter tip was not standardized for each individual animal. An attempt was made to correct this prior to the final dose administration for each group, however physical placement of the catheters at this time was difficult due to jugular vein thrombosis. In light of these issues, the pulmonary pressure data collected in this study was not considered valid and was therefore not analyzed. An assessment of histopathological change in the lungs was concluded to be the final determinant of potential effects due to pulmonary hypertension.

Results

There we no treatment-related mortalities. Animal 303 (high dose- Group 3) was euthanized approximately 48 hours following the completion of Dose 1. Clinical signs observed in this animal prior to euthanasia were indicative of problems related to the experimental procedure or resulting from infection, such as blood leaking from the jugular access site, signs of hemorrhage (black skin), increased body temperature and dehydration, therefore this dog was replaced by Animal 305. Occasional vomiting and decreased activity was noted for some animals administered PEM NF1 at the high dose. No other treatment related clinical signs were noted for any animal.

There was no apparent dose-related effect of PEM NF1 on systemic hemodynamic parameters measured in this study (systolic, diastolic, mean pressure and heart rate). Variations in these measurements were considered related to the experimental and/or dosing procedures.

There was an apparent reduction in tidal volume and derived respiratory minute volume (RMV) observed for animals dosed at 8 mL/kg at the 15 minute post dose time point, this effect being seen following all four administrations of test article. A similar change was also observed for animals dosed at 1 mL/kg following the third administration of test article; this effect was not noted at any other week. In all cases, the decrease was transient and tidal volume and RMV appeared to be normal by the one hour post dose assessment.

Blood gas concentrations appeared unaffected by dosing with test article throughout the study, as assessed by measurements of pCO_2 and pO_2. Blood pH was slightly reduced

Reference ID: 3367720

for animals administered 8 mL/kg at the 15 minute post dose assessment following all four test article doses. On all occasions this had recovered to predose levels by 4 hours post dose.

Gross examination of animals at study termination revealed pale areas in the endocardium of the right ventricle in one animal of Group 2 and two animals of Group 3. These were compatible with catheter-inflicted trauma, however, as they were only observed in animals given PEM NF1 at doses of 1.0 mL/kg and up, a test-article effect cannot be ruled out.

Histopathological examination of the lungs revealed only miscellaneous findings in animals from all groups which were considered to be incidental and not related to the administration of the test article. Changes compatible with chronic pulmonary hypertension (i.e. arterial medial hypertrophy) were not observed in the samples of lung examined.

Conclusion
A once weekly for four weeks intravenous bolus injection of PEM NF1 at dose levels of 1.0 mL/kg and 8.0 mL/kg to beagle dogs caused only a transient effect on respiration, evidenced by a decrease in tidal volume and RMV at 15 minutes post dose, resolving by one hour post dose. There was no effect on blood gas concentrations and no effect on the lungs, assessed by histopathological examination. No changes compatible with chronic pulmonary hypertension were observed and accordingly, the no observed adverse effect level (NOAEL) for this study was considered to be 8.0 mL/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

Summary

The pharmacokinetic data presented in this summary were derived from single dose studies performed at dosages within the range of those used in the repeat dose toxicity studies (rat 3.0 to 30.0 mg/kg, dog 1.0 to 20.0 mg/kg). All studies were carried out by intravenous administration as this is the proposed route of clinical application. Unlike the clinical setting however, where there will be retention of the gas from the microfoam within the target vein, these studies examined systemic exposure since no trapping of the microfoam in the vein occurred. Polidocanol solution, rather than microfoam, was used in a number of studies because of limitations on how much polidocanol could be injected using the foam and because of effects of the foam on the vein independent of the polidocanol.

Several *in vivo* studies have been performed to characterize the distribution, metabolism and excretion of polidocanol in the Lister Hooded and Sprague Dawley rat and the Beagle dog. These species were selected because of the ready availability of high quality animals and their acceptability as suitable models by regulatory authorities. In addition, both the Sprague Dawley rat and the Beagle dog were employed in toxicity

Reference ID: 3367720

testing and enable meaningful assessment of the systemic exposure levels associated with No Adverse Effect doses, thus allowing a confident prediction for the safety of similar exposures in humans.

No absorption studies were conducted as the route of administration is intravenous.

Three single dose tissue distribution studies were carried out in the Sprague Dawley rat. One study measured radioactivity present in various organs and tissues by Quantitative Whole Body Autoradiography (QWBA) after a 3 mg/kg dose of [^{14}C]-polidocanol solution (study 167690). This study also investigated tissue distribution in Lister Hooded rats to enable a comparison between pigmented and non-pigmented rats. Radioactivity was distributed widely to all organs and tissues in the rat with much of the dose reaching the organs of clearance rapidly. However, some radioactivity was retained in many tissues for the 168 hour duration of the study indicating an affinity of some polidocanol related material to cellular components. Lowest initial levels of radioactivity were seen in eye, testis and brain from the pigmented rats. There were no obvious differences between radioactivity retention in tissues from albino or pigmented rats. A further study was performed with [^{14}C]-polidocanol solution in the rat to provide additional information on the tissue distribution of total radioactivity (study 169609). Levels of total radioactivity were measured by liquid scintillation counting (LSC) and the nature of radioactivity present in selected tissues investigated by Thin Layer Chromatography (TLC). Total radioactivity was well distributed throughout the tissues analyzed, with the highest concentrations in whole blood, plasma and the organs associated with elimination (liver and kidney) at 5 minutes post-dose.

The third study was a bridging study comparing the distribution of total radioactivity following administration of [^{14}C]-polidocanol microfoam or solution (study 171995). Radioactivity was extensively distributed to the selected tissues analyzed. Radioactivity dispersed from the site of injection at a similar rate following each administration with only 1% remaining in the tail by 15 minutes post-dose. The amount of total radioactivity recovered in each tissue was generally comparable between the two formulations at each of the time-points analyzed. [^{14}C]-polidocanol was therefore shown to be distributed and cleared in a similar fashion following administration in either the microfoam or solution formulation to rats.

Two single dose tissue distribution studies were performed in the Beagle dog, one study using [^{14}C]-polidocanol solution (study 169572) and the other study using [^{14}C]-polidocanol microfoam (study 175188). In the study with [^{14}C]-polidocanol solution, total radioactivity in selected tissues (except lungs) was highest in the animal killed at 168 hours post-dose (the first measurement time) (study 169572). Tissue concentrations of total radioactivity were significantly higher than the concentration present in whole blood and plasma. At 336 hours, the concentration in whole blood and plasma had decreased to close to the limit of reliable determination. Concentrations had also decreased in kidneys, renal fat and liver, but were slightly elevated in lung and similar in subcutaneous fat compared to 168 hours post-dose. By approximately 5 weeks post-

Reference ID: 3367720

dose, concentrations of radioactivity were much lower in all tissues measured, and by 15 weeks postdose, all tissues were at or below the limit of reliable determination.

In the study with [^{14}C]-polidocanol microfoam, radioactivity was well distributed in the tissues investigated (study 175188). Total radioactivity levels were higher in organs associated with metabolism (liver and kidneys). Subcutaneous and renal fat retained some radioactivity. The terminal half-life values (168-672 hour interval) of approximately 650, 603, 582, 446, 359, 337 and 326 hours were estimated for liver, renal fat, subcutaneous fat, heart, spleen, kidneys, and lungs, respectively.

Two studies were carried out to investigate the plasma kinetics of total radioactivity following intravenous administration of [^{14}C]-polidocanol solution to Sprague Dawley rats (study 167706) and Beagle dogs (study 167711). Whole blood kinetics was also investigated in the dog study. Levels of radioactivity were measured by LSC and the nature of the radioactivity was examined by High Performance Liquid Chromatography (HPLC). In the rat, the peak mean plasma concentration was observed at 5 minutes post-dose with a value of 6.51 µg equivalent/mL. Thereafter, the mean concentration of total radioactivity declined rapidly to 3.87 µg equivalent/mL at 0.25 hours post-dose and further to 1.43 µg equivalent/mL at 2 hours post-dose. The mean concentration of total radioactivity continued to decline and at the final time-point, 48 hours post-dose, the mean concentration of total radioactivity was 0.06 µg equivalent/mL. In the dog, the highest mean plasma concentration of total radioactivity was 3.91 µg equivalent/mL and was observed immediately after the end of the infusion. The mean concentration of total radioactivity in plasma then decreased to 1.57 µg equivalent/mL at 2 hours and further to 0.10 µg equivalent/mL at 24 hours post-dose. The mean concentration of total radioactivity continued to decline steadily and by 168 hours post-dose, the last sampling time, the mean concentration of total radioactivity in plasma was 0.02 µg equivalent/mL. The mean concentration of total radioactivity in whole blood showed a similar kinetic pattern to that found in plasma, although at lower levels.

To complete the distribution studies, an *in vitro* study was performed to determine the binding of polidocanol solution to plasma proteins from Sprague Dawley rats, Beagle dogs and humans (study 167727). Protein binding was determined by ultracentrifugation followed by LSC. The degree of plasma protein binding decreased with increasing concentration of [^{14}C]-polidocanol. The extent of protein binding varied between species and was higher in the dog than in the other two species. Protein binding was ranked; rat < man < dog at the lower concentration and man < rat < dog at the higher concentration.

Several of the *in vivo* studies already described included limited metabolite profiling. However, one study investigated the biotransformation of polidocanol in the rat and dog in greater detail using samples retained during these previous studies (study 169771). Feces, urine and plasma samples were collected for metabolite analysis by HPLC with radiochemical detection and characterization of metabolites were carried out by Liquid Chromatography – Mass Spectroscopy (LC-MS). The profile of metabolites in the dog was qualitatively consistent with the rat with the exception of a minor peak at

Reference ID: 3367720

approximately 46 minutes which was detected in dog urine and plasma. The analysis of rat urine and plasma sample extracts by LC-MS revealed a number of polar metabolite peaks in both the plasma and urine extracts. These polar peaks comprised a very polar peak which could not be characterized. Evidence was found to suggest that the remaining polar peaks were several metabolite series comprising either polyethylene glycol groups or acidic polyethylene glycol groups. These series comprised species which were separated by 44 mass units. The oligomers of polidocanol afforded a series of ions separated by 44 mass units, therefore a pattern of ions separated by 44 mass units was taken as an indication of metabolites related to polidocanol. The polarity of these related species was such that the various series co-eluted together giving rise to a very complicated chromatographic profile. It was apparent that polidocanol was metabolized to a series of progressively more polar products which were readily excreted.

To further characterize the metabolites of polidocanol, following completion of an *in vitro* study to confirm that polidocanol did not compromise microsomal membrane integrity (study 789783), an *in vitro* metabolism study was conducted to investigate the rate and routes of [^{14}C]-polidocanol solution metabolism in hepatic microsomes and S9 fractions prepared from male rat, male dog and mixed gender human liver (study 790228). The study provided evidence of NADPH dependent metabolism across the species and tissue fractions. The radio-HPLC chromatographic profile of [^{14}C]-polidocanol was complex and difficult to interpret however and it was not possible to determine and compare the rate of metabolism and the metabolite profiles in the different species and tissue fractions using this method. Nevertheless, during the course of the incubation period, and in the presence of co-factor, visual examination of the chromatographic profile displayed a change in each of the species and tissue fraction examined in accordance with the following order: human> dog> rat. Increased radioactivity recovered from the aqueous portion of the sample extraction process meant there could have been polar metabolites which may not have been detected using the radio-HPLC method.

The rat, dog and human microsomal and S9 samples from study 790228 were further analyzed by radio-HPLC using reverse phase methods to facilitate metabolite identification by LCMS (study 219463). The results indicated metabolism of [^{14}C]-polidocanol occurred in microsomes incubated for 120 minutes in the presence of co-factors. Very little, or no metabolism of [^{14}C]-polidocanol was observed when microsomes were incubated without co-factors indicating that oxidative metabolism was a key metabolic process. Metabolism was suggested to proceed via non-specific hydroxylation of the dodecyl chain with further hydroxylation and elimination to form the oxidized hydroxyl (keto/enol) metabolite series for polidocanol oligomers. The carboxylic acid metabolite series identified in study 169771 would be formed by oxidative cleavage of the double bond of the enol metabolite (oxidized hydroxyl metabolite). This carboxylic acid metabolite series was not observed in any of the *in vitro* samples analyzed. This was deemed to be due to the fact that metabolism had not proceeded to the same extent in microsomes as was observed in rat and dog *in vivo* (study 169771). The metabolism of [^{14}C]-polidocanol was qualitatively similar in each species with some

Reference ID: 3367720

quantitative differences observed between the 3 species. Due to the oligomeric nature of polidocanol and its metabolites and the analytical challenges this posed, it was not possible to determine if the quantitative differences were genuine species differences or were a consequence of differences in the metabolic competency of the microsomal preparations.

The pharmacokinetic data package was completed with four studies investigating the excretion profile. Three of these studies were conducted in the Beagle dog (study 169572, study 167711, study 175188) and one in the Sprague Dawley rat (study 167706) with the aim of investigating the excretion profile of polidocanol following intravenous administration of [^{14}C]-polidocanol solution and microfoam (dog only). Radioactivity levels in urine, feces, plasma and carcass were determined using LSC. The nature of the radioactivity was analyzed by HPLC. In the rat, the majority of the total radioactivity was eliminated within the first 24 hours post dose. The predominant route of excretion was via the urine followed by feces and expired air. Low levels of radioactivity were detected in the gastrointestinal tract and residual carcass. The majority of radioactivity in urine and feces was postulated to be polar metabolites of [^{14}C]- polidocanol. A similar pattern was observed for the dog and the excretion patterns were similar for the solution and the microfoam.

No non-clinical interaction studies were performed with polidocanol.

Methods of analysis

Development of methodologies for the formulation and analysis of polidocanol in dosing formulations (Study 334496) (GLP)

A study was undertaken to develop and validate an analytical method for the determination of polidocanol in sterile water for injection (WFI) intravenous dose formulations and the use of this method to confirm formulation procedures for polidocanol in intravenous dose formulations at [redacted] (b) (4). The stability and homogeneity of polidocanol in WFI were also investigated.

In the validated method, samples were diluted as necessary in 2-propanol and analyzed by gas chromatography with flame ionization detection (GC-FID). External standardization was employed. Polidocanol has several components and quantification was based on the oligomer peak with the largest area. The chromatographic system was shown to give a linear response over the range 20 to 200 µg/mL polidocanol. The formulation and analytical procedures were found to be satisfactory over the range 0.2 mg/mL to 15 mg/mL in WFI.

Formulations containing 0.2 mg/mL and 15 mg/mL polidocanol in WFI were shown to be accurately and homogeneously prepared. They were also shown to be stable for at least 8 days when stored at 2-8 °C in the dark.

Reference ID: 3367720

Low level sample solutions containing 20 µg/mL polidocanol in 2-propanol were stable for at least 8 days when stored at ambient laboratory temperature in the dark. A level of 150 µg/mL was not found to be as stable with a deviation from day 1 values of -10.5%.

Polidocanol: adaptation of existing methodology for the formulation and analysis of polidocanol in dosing solutions (Study 420616) (GLP)

Experiments were conducted to establish and validate an analytical method for the determination of polidocanol in the vehicle, disodium hydrogen orthophosphate dihydrate (2.4 g/L), potassium dihydrogen phosphate (0.8 g/L) and 4% v/v ethanol, for dosing solutions and the use of this method to confirm formulation procedures for polidocanol in dose formulations prepared at [redacted] (b) (4)

The method was based upon the chromatographic conditions from Analytical Method Number 3449, with samples diluted as necessary in 2-propanol and analyzed by GCFID. External standardization was employed and quantification was based on the polidocanol component oligomer peak with the largest area.

Results indicated the formulation containing 1.0% w/v polidocanol in vehicle to be accurately and homogeneously prepared. The dosing formulation was also shown to be stable for at least 8 days when stored at ambient laboratory temperature in the dark. Sample solutions prepared for analysis were shown to be stable for at least 8 days when stored at ambient laboratory temperature in the dark.

Validation of an analytical method for the determination of polidocanol in rat, dog and human plasma (Study 368478) (GLP)

An analytical method utilizing gas chromatography with flame ionization (GC-FID) for the determination of specific oligomer components of polidocanol was validated in rat, dog and human plasma. The analytical procedure was developed under [redacted] (b) (4) Project No. 368305.

In the method, polidocanol was extracted from plasma by liquid/liquid partition into diethyl ether followed by solid phase clean-up using silica cartridges. The extract is evaporated to dryness and reconstituted in hexane:dichloromethane. Aliquots (1 µL) of these final extracts are analyzed by GC-FID.

The validated concentration range was 20 to 800 µg/mL in rat plasma, 20 to 400 µg/mL in dog plasma and 10 to 200 µg/mL in human plasma. Aliquots of plasma (0.25 mL for rat, 0.5 mL for dog and 1 mL for human) were spiked with polidocanol for calibration standards and quality control samples.

Peak height results were used to construct calibration lines for polidocanol oligomers 5, 6 (dog), 9, 12 and 14. The distribution of each of these oligomers relative to overall

Reference ID: 3367720

polidocanol is shown in Table 1. Limits of detection/assay ranges were based upon data obtained from the largest peak, usually oligomer 9 which is approximately 9% of the total oligomer content.

Relative distribution of polidocanol oligomers (Batch 87815) (study 368478)

Oligomer of polidocanol	5	6	9	12	14
Relative distribution by area (%)	6.21	7.17	9.03	7.32	5.07

System suitability and detector linearity

The chromatographic and detection systems were satisfactory in terms of column efficiency, peak width at half-height, tailing factor, retention time, peak resolution, detector linearity, limit of on-column detection and system precision.

Assay validation

The assay was found to be specific for the determination of polidocanol in rat, dog and human plasma samples, with lower limits of quantification of 20 µg/mL for rat and dog plasma and 10 µg/mL for human plasma.

Assessment of accuracy and precision in rat and human control plasma samples was made on five occasions. Target acceptance criteria for each oligomer were ± 20% accuracy (± 15% oligomer 9) and ≤ 20% precision (≤ 15% oligomer 9). For rat plasma, the accuracy and precision data were acceptable on 4/5 occasions for oligomer 5 at the high and low levels and on 5/5 occasions at the mid level. The data were acceptable on 5/5 occasions for oligomer 9 at the low level and on 4/5 occasions at the mid and high levels. The data were acceptable on 5/5 occasions for oligomer 12 at the low and mid levels and on 4/5 occasions at the high level. The data were acceptable on 3/5 occasions for oligomer 14 at the low level and on 5/5 occasions at the mid and high levels. Sufficient data were collected to demonstrate acceptable accuracy and precision for oligomers 5, 9, 12 and 14 in rat plasma.

For human plasma, the accuracy and precision data were acceptable on 5/5 occasions for both oligomers 5 and 9 at the low and mid-levels and on 4/5 occasions at the high level. The data were acceptable on 3/5 occasions for oligomer 12 at all 3 concentration levels. The data were acceptable on 2/5 occasions for oligomer 14 at the low level, on 3/5 occasions at the mid-level and on 0/5 occasions at the high level. Sufficient data were collected to demonstrate acceptable accuracy and precision for oligomers 5 and 9 but demonstrated to be only semi-quantitative for oligomers 12 and 14 in human plasma.

The recovery of oligomers 5, 9, 12 and 14 from both rat and human plasma was satisfactory.

Reference ID: 3367720

Specificity was not achieved for oligomer 5 in dog plasma, therefore oligomer 6 was chosen as an acceptable alternative. Only 3 occasions were required to demonstrate acceptable accuracy and precision for oligomers 6, 9, 12 and 14 in dog plasma. The recovery of oligomers 6, 9, 12 and 14 from dog plasma was satisfactory.

Stability investigations

Polidocanol was found to be stable in rat and human plasma samples during 1, 2 and 3 freeze/thaw cycles and in dog plasma samples for up to 2 freeze/thaw cycles, in rat, dog and human plasma samples for at least 2 hours when stored at ambient temperature and in extracts from rat, dog and human plasma samples stored at ambient temperature for at least 42 hours. No significant instability of polidocanol in rat, dog and human plasma samples stored for approximately 15 months at approximately -180, -80 and -20°C was observed.

Solutions of polidocanol in methanol were found to be stable for approximately 8 weeks when stored at approximately 4°C.

Partial validation of a liquid chromatography-tandem mass spectrometry (LC-MS-MS) assay for polidocanol in dog plasma (study RD 198/23966GV) (GLP)

This study comprised the partial validation of a liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for polidocanol by the determination of four characteristic oligomers of polidocanol, namely E5, E9, E12 and E14, in dog plasma. Also included was an instrument transfer validation for the use of the method on two different LC-MS-MS instruments. The method was originally developed and validated in human plasma (study RD 198/23887HV).

[redacted] (b) (4) were used as internal standards (IS) and all compounds were extracted from plasma using solid-phase extraction (SPE). Following extraction, samples were subjected to LC-MS-MS analysis in electrospray positive ion mode.

Quality control (QC) and back-calculated standard concentrations were reported for polidocanol and each of the four characteristic oligomers. Polidocanol concentrations were obtained by combining the individual results for the four oligomers and adjusting the result to account for the combined abundance of the four oligomers in the polidocanol mixture. The oligomer concentrations were calculated from the Certificate of Analysis using the Area % values as determined by gas chromatography with flame ionization detection (GC-FID).

Results showed the method was linear for each of the four chosen oligomers over the nominal standard concentration range equivalent to 50 to 10,000 ng/mL polidocanol.

Reference ID: 3367720

Accuracy of the back-calculated standard data was in the range 89.4% to 105.4%, 91.4% to 112.8%, 93.5% to 110.3%, 92.0% to 108.9% and 94.4% to 109.5% for oligomers E5, E9, E12, E14 and polidocanol, respectively.

Replicate analysis of intra-batch dog plasma QC samples on one LC-MS-MS instrument (API 365) showed accuracy of the mean data ranging from 99.2% to 106.2%, 91.7% to 109.6%, 86.2% to 103.8%, 94.5% to 111.2% and 96.1% to 106.3% for E5, E9, E12, E14 and polidocanol, respectively. Assay imprecision (coefficient of variation) for the same intra-batch QC samples did not exceed 14.7%.

Replicate analysis of intra-batch dog plasma QC samples on a second LC-MS-MS instrument (API III) showed accuracy of the mean data ranging from 90.6% to 111.8%, 89.5% to 106.9%, 89.1% to 107.4%, 84.6% to 104.1% and 92.0% to 107.2% for E5, E9, E12, E14 and polidocanol, respectively. Assay imprecision (coefficient of variation) for the same intra-batch QC samples did not exceed 11.1%.

Selectivity and sensitivity at the lower limits of quantitation for each oligomer were demonstrated. No significant interference from different dog plasma samples was observed to any of the oligomer or internal standard peaks in the assay.

5.1 PK/ADME

Absorption

Since the proposed route of administration is by intravenous injection, there is no reason to determine absorption, and bioavailability is, by definition, 100%. Therefore no absorption studies have been performed.

Distribution

The tissue distribution of [^{14}C]-polidocanol in the rat following intravenous administration (quantitative whole body autoradiography) (Study 167690) (GLP)

Study Objective
This study was conducted to examine the tissue distribution of total radioactivity in the male albino and pigmented rat following single intravenous administration of [^{14}C]-polidocanol.

Study Design

Five male albino (Sprague Dawley) and 4 male pigmented (Lister Hooded) rats each received a single intravenous administration of [^{14}C]-polidocanol at a target dosage level of 3 mg/kg. One albino animal was humanely killed at 5 minutes and 0.5, 6, 24 and 168

Reference ID: 3367720

hours post-dose. One pigmented animal was killed at 6, 24, 168 and 336 hours post-dose.

Immediately prior to sacrifice, a whole blood sample was collected and aliquots removed for total radioactivity analysis. Immediately following sacrifice, the right eye from each animal was removed and weighed. Each animal was then immediately frozen and embedded in a block of methyl cellulose and sagittal sections (30 µm) taken, freeze dried and applied to phosphor screens, together with a series of calibration standards. After an exposure period of 7 days, the radioactivity present in various organs and tissues was determined by QWBA. Liquid scintillation counting (LSC) of blood and eye samples was also conducted.

The tail from the animal killed at 5 minutes post-dose was blocked, sectioned and analyzed as above.

Results

The concentration of total radioactivity in tissues is given in the Table below.

Reference ID: 3367720

Concentration of total radioactivity in tissues following single intravenous administration of [^{14}C]-polidocanol to male albino (Sprague Dawley, SD) and pigmented (Lister Hooded, LH) rats (µg equivalent/g) (study 167690)

Time-point	5 minutes		0.5 hours		6 hours		24 hours		168 hours		336 hours	
Rat strain	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**
Tissues/organs												
Adrenal gland	11.20	NP	3.88	NP	1.27	1.13	1.09	1.25	0.24	0.90	NP	0.41
Bladder	3.93	NP	2.71	NP	NQ	NQ	0.19	0.14	NQ	0.11	NP	NQ
Blood	3.24	NP	2.85	NP	NQ	0.49	0.15	0.15	0.02	0.03	NP	0.02
Bone Marrow	4.44	NP	2.54	NP	0.68	0.77	0.55	0.81	NQ	0.22	NP	NQ
Brain	2.20	NP	1.26	NP	0.19	0.26	0.14	0.13	0.06	0.09	NP	0.05
Brown fat	8.45	NP	6.43	NP	1.71	2.01	1.49	1.11	0.15	0.28	NP	0.11
Epididymis	1.63	NP	1.70	NP	0.46	0.33	0.21	1.14	0.06	0.51	NP	0.16
Eye	0.47	NP	1.35	NP	0.13	0.15	0.08	0.08	0.02	0.03	NP	0.02
Harderian gland	5.74	NP	5.63	NP	2.53	2.08	1.75	1.56	0.12	0.18	NP	NQ
Heart	7.94	NP	3.86	NP	0.42	0.39	0.23	0.21	0.06	0.09	NP	0.04
Kidney (cortex)	11.58	NP	20.53	NP	0.99	1.03	0.44	0.50	0.06	0.11	NP	0.05
Kidney (medulla)	9.85	NP	23.32	NP	1.00	1.05	0.37	0.47	0.04	0.09	NP	NP
Lachrymal gland	4.79	NP	3.18	NP	0.78	0.74	0.63	0.52	0.13	0.10	NP	0.09
Large intestine wall	5.23	NP	2.86	NP	1.64	2.38	0.68	0.59	NQ	NQ	NP	NQ
Liver	15.18	NP	18.16	NP	4.25	5.34	1.21	1.23	0.14	0.20	NP	0.13
Lung	5.52	NP	3.46	NP	0.61	0.78	0.31	0.26	0.03	0.06	NP	0.02
Lymph node	3.47	NP	2.44	NP	0.42	0.41	0.43	0.38	0.08	0.11	NP	0.03
Pancreas	10.10	NP	4.57	NP	0.60	0.49	0.33	0.34	0.07	0.10	NP	NQ

Time-point	5 minutes		0.5 hours		6 hours		24 hours		168 hours		336 hours	
Rat strain	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**	**SD**	**LH**
Pituitary gland	6.67	NP	4.64	NP	0.55	0.54	0.45	0.33	NQ	NQ	NP	NQ
Preputial gland	4.37	NP	4.69	NP	1.79	NQ	2.29	2.75	0.12	1.59	NP	NP
Prostate	3.81	NP	2.40	NP	0.54	0.32	0.74	0.32	0.09	0.62	NP	NQ
Rectum	5.14	NP	2.20	NP	0.46	0.25	0.98	0.56	NQ	NQ	NP	NQ
Salivary gland	7.82	NP	3.67	NP	0.98	0.91	0.58	0.66	0.16	0.19	NP	0.09
Seminal vesicles	0.93	NP	1.01	NP	0.16	0.36	0.55	0.39	0.46	0.51	NP	NQ
Skeletal muscle	4.11	NP	1.83	NP	0.17	0.18	0.14	0.12	0.03	0.05	NP	NQ
Skin (albino)	1.98	NP	2.02	NP	0.21	0.37	0.23	0.61	0.08	0.34	NP	0.25
Skin (pigmented)	NP	NP	NP	NP	NP	0.43	NP	0.89	NP	0.50	NP	0.24
Small intestine wall	7.84	NP	5.57	NP	2.04	2.25	0.99	1.09	0.23	NQ	NP	NQ
Spinal cord	1.76	NP	1.36	NP	0.31	0.39	0.26	0.21	0.14	0.20	NP	0.11
Spleen	8.97	NP	2.44	NP	0.62	0.74	0.41	0.49	0.06	0.11	NP	NQ
Stomach wall	9.70	NP	4.26	NP	0.71	0.66	0.47	0.49	NQ	NQ	NP	NQ
Testes	0.78	NP	0.74	NP	0.15	0.17	0.12	0.12	0.04	0.07	NP	0.04
Thymus	2.59	NP	2.00	NP	0.31	0.30	0.32	0.25	0.09	0.12	NP	0.06
Thyroid gland	5.53	NP	3.21	NP	0.43	0.50	0.51	0.32	0.07	0.11	NP	NQ
White fat	1.52	NP	1.59	NP	0.54	0.49	1.63	1.28	0.77	1.24	NP	0.51
NP=Not performed, NQ=Not quantified												

Albino animals:

At 5 minutes post-dose, radioactivity was rapidly and widely distributed throughout the tissues. The highest tissue concentrations were observed in the liver, kidney cortex, adrenal, pancreas and kidney medulla (15.18, 11.58, 11.20, 10.10, and 9.85 µg equivalent/g, respectively). Concentrations observed in the stomach wall, spleen, brown fat, heart, small intestine wall and pituitary gland (9.70, 8.97, 8.45, 7.94, 7.84 and 6.67 µg equivalent/g, respectively) were all greater than 2 times the concentration measured in blood (3.24 µg equivalent/g). All other concentrations were similar to or below those measured in blood and were in the range of approximately 2-6 µg equivalent/g. At this time, radioactivity in the tail vein was demonstrated to be associated with the blood vessel membrane, consistent with the known mechanism of action of polidocanol.

At 0.5 hours post-dose, concentrations decreased in most tissues or remained at similar levels. Concentrations increased in the organs of elimination and were correspondingly highest in the kidney cortex, kidney medulla and liver (20.53, 23.32 and 18.16 µg equivalent/g, respectively). The concentration of radioactivity in blood was slightly lower than at 5 minutes post-dose (2.85 µg equivalent/g). Concentrations of radioactivity in the other tissues at this time were in the range 2-6 µg equivalent/g.

At 6 hours post-dose, concentrations decreased significantly from the previous time-point. Concentrations of radioactivity in the kidney medulla and cortex (1.00 and 0.99 µg equivalent/g, respectively) declined at a similar rate to those observed in blood. Concentrations in most tissues fell more slowly than blood and consequently were generally above the blood concentration. Highest concentrations were observed in the liver, harderian gland, small intestine wall, preputial gland, large intestine wall, brown fat and adrenal (4.25, 2.53, 2.04, 1.79, 1.64, 1.71 and 1.27 µg equivalent/g). Most other tissues contained concentrations in the range 0.2-1 µg equivalent/g. The concentration of radioactivity in white fat (0.54 µg equivalent/g) was lower than previously observed. The concentration of radioactivity measured by LSC in the blood was 0.187 µg equivalent/g. The concentration of radioactivity measured by LSC in the eye (0.091 µg equivalent/g) was comparable to the value obtained by QWBA analysis (0.13 µg equivalent/g).

At 24 hours post-dose, concentrations in most tissues continued to decrease with the concentration of 0.15 µg equivalent/g measured in blood. Some tissues contained levels of radioactivity which were greater than were observed at 6 hours post-dose, most notably in the preputial gland and white fat. Highest tissue concentrations were observed in the preputial gland, harderian gland, white fat, brown fat and adrenal gland (2.29, 1.75, 1.63, 1.49 and 1.09 µg equivalent/g, respectively). Concentrations in other tissues were in the range 0.1-1 µg equivalent/g. Concentrations of radioactivity measured by LSC in the blood (0.089 µg equivalent/g) and eye (0.076 µg equivalent/g) were similar to the values obtained by QWBA analysis (0.15 and 0.08 µg equivalent/g, respectively).

Reference ID: 3367720

At 168 hours post-dose, the concentrations of total radioactivity in all tissues were much lower than those seen at 24 hours post-dose. The blood concentration at this time was 0.02 µg equivalent/g, which was less than 1 % of the peak concentration. Concentrations in the majority of tissues were reduced by a similar extent to blood. Concentrations in the white fat and seminal vesicles declined more slowly and represented approximately 50% of peak concentrations at 168 hours post-dose. Highest tissue concentrations were correspondingly noted in these tissues (white fat and seminal vesicles (0.77 and 0.46 µg equivalent/g, respectively)). Concentrations in the remaining tissues were low and in the range of 0.02-0.2 µg equivalent/g.

The composite (albino and pigmented rats) half life of elimination of radioactivity from white fat, blood, kidneys, liver and lung was estimated to be approximately 205, 110, 98, 99 and 82 hours, respectively.

Pigmented animals:

The distribution and elimination of total radioactivity following intravenous administration of [^{14}C]-polidocanol to pigmented animals was similar to that observed in non-pigmented animals. There was no evidence of retention of radioactivity in melanin containing tissues.

Conclusions

Radioactivity was rapidly and widely distributed with peak tissue concentrations generally observed at the first time-point (5 minutes post-dose). Highest concentrations of total radioactivity were observed in the organs of elimination (liver and kidney), with peak concentrations in these tissues observed later than most other tissues (at 0.5 hours post-dose). Initially, elimination of radiolabelled components from most tissues was relatively rapid with concentrations in most tissues at approximately 10 % of peak concentrations by 24 hours postdose. In some tissues, most notably in white fat, elimination of radiolabelled components was considerably slower. By 168 hours post-dose, concentrations were relatively low and in some cases below the limit of reliable detection. There was no evidence of retention of radioactivity in melanin- containing tissues, the pigmented skin and eye.

The tissue distribution of [^{14}C]-polidocanol in the rat following intravenous administration (Study 169609) (GLP)

Study Objective

This study was conducted to examine the tissue distribution of total radioactivity following single intravenous administration of [^{14}C]-polidocanol to Sprague Dawley rats. The nature of radioactivity present in selected tissues was also investigated.

Study Design

Sixteen male rats each received a single intravenous administration of [^{14}C]-polidocanol at a target dose level of 3 mg/kg. Urine and feces was collected quantitatively from the 4

Reference ID: 3367720

animals for the period 0-24 hours post-dose. The excreta samples were not analyzed in this study but were used for chromatographic analysis under study number 169771.

Following dose administration, 4 animals were killed at 5 minutes and 6, 24 and 168 hours postdose and the following tissues and body fluids were retained at necropsy:

Blood, kidney, liver, plasma, renal fat, subcutaneous fat and residual carcass
Levels of total radioactivity were determined in each sample collected (except carcass).

Pooled samples of liver, kidney, subcutaneous fat and renal fat were prepared at each collection time-point. These samples were extracted with organic solvent and analyzed by Thin Layer Chromatography (TLC).

Results

Tissue distribution of total radioactivity

The concentrations of total radioactivity, expressed as µg equivalent/g (/mL) are presented in the Table below.

Concentration of total radioactivity in tissues following a single intravenous administration of [^{14}C]-polidocanol to male rats expressed as µg equivalent/g(/mL) (mean n=4 ± SD) (study 169609)

Time-point	5 minutes	6 hours	24 hours	168 hours
Tissues/organs				
Fat – renal	1.336±0.183	1.444±0.665	1.733±0.364	2.070±0.632
Fat – subcutaneous	1.406±0.127	0.963±0.443	1.318±0.406	1.280±0.253
Kidneys	9.521±1.198	1.434±0.469	0.520±0.100	0.167±0.008
Liver	14.483±1.638	4.878±0.639	1.617±0.115	0.203±0.025
Whole blood	3.957±0.649	0.618±0.062	0.182±0.092	0.022±0.004
Plasma	5.605±0.181	1.025±0.054	0.279±0.027	0.018±0.003

Highest concentrations of total radioactivity were detected in whole blood, plasma and the organs associated with elimination (liver and kidney) at 5 minutes post-dose (the first measurement time). At this time, the concentration of total radioactivity was at its lowest in renal fat. The concentration of total radioactivity in subcutaneous fat remained relatively constant at all time-points investigated (5 minutes to 168 hours post-dose).

By 168 hours post-dose the concentrations of total radioactivity in whole blood and plasma had decreased steadily to just above the limit of reliable measurement. The levels of total radioactivity had also decreased steadily in the liver and kidney but at a slower rate than that observed in whole blood and plasma. At this time, highest

Reference ID: 3367720

concentrations of total radioactivity were observed in renal fat and levels of radioactivity remained relatively constant in the subcutaneous fat.

Sample extraction

Solvent extracts of liver and kidney contained between 51% and 100% of the sample radioactivity. Generally, lower recoveries were obtained from later time-point samples. Solvent extracts of fat samples (subcutaneous and renal) contained between 48.6% and 82.3% of the sample radioactivity and there was no obvious data trend.

TLC analysis
Chromatographic analysis of liver samples contained components with retention factor (Rf) values consistent with polidocanol and later time-points (6, 24 and 168 hours after dosing) contained a range of polar metabolites with Rf values ranging from 0.01 to 0.86. In addition each of the samples contained a non-polar component that chromatographed at the solvent front and was less polar than polidocanol.

Each of the kidney samples contained polidocanol and a range of polar metabolites with Rf values ranging from 0.00 to 0.88. Components of greater polarity were present in the later timepoint samples.

The chromatography of the radioactivity present in fat extracts was compromised by the presence of lipids in the extracts, however TLC chromatograms showed regions of non-polar radioactivity broadly consistent with the Rf of polidocanol. Early time-point fat samples (5 minutes and 6 hours after dosing) also contained minor polar components.

Conclusions
Following intravenous administration of [^{14}C]-polidocanol to rats at 3 mg/kg, the total radioactivity was well distributed throughout the tissues analyzed. Highest concentrations of total radioactivity were detected in whole blood, plasma and the organs associated with elimination (liver and kidney) at 5 minutes post-dose. The concentration of total radioactivity detected in the renal fat and subcutaneous fat remained relatively constant throughout the time-points investigated (5 minutes, 6, 24 and 168 hours post-dose).

The disposition of [^{14}C]-polidocanol in the rat following intravenous administration (Study 167706) (GLP)

Study Objective
This study was conducted to examine the excretion and plasma kinetics of total radioactivity following intravenous administration of [^{14}C]-polidocanol to Sprague Dawley rats. The nature of radioactivity present in plasma and urine was also investigated.

Reference ID: 3367720

Study Design
Each animal received a single intravenous administration of [^{14}C]-polidocanol at a target dose level of 3 mg/kg.

Following dose administration to 5 male rats in Phase 1, samples of urine, feces and expired air (2 animals only) were collected at intervals up to 168 hours post-dose.

Following dose administration to 5 male rats in Phase 2, serial plasma samples were collected at intervals up to 48 hours post-dose.

Following dose administration to 4 male rats in Phase 3, 2 animals were killed at 0.25 hours and 1 hour post-dose and a sample of plasma obtained for chromatographic analysis.

Levels of total radioactivity were determined in each sample collected.

Urine samples collected at 6 and 24 h post dose, feces samples collected for the period 0-24 hours post-dose and plasma samples collected at 0.25 hours and 1 hour post-dose were analyzed by HPLC.

Results
Excretion kinetics from this study are presented later (under Excretion). The concentration of total radioactivity in plasma following intravenous administration of [^{14}C]-polidocanol is shown in the Table below.

Concentration of total radioactivity in plasma following a single intravenous administration of [^{14}C]-polidocanol to male rats expressed as µg equivalent/g (mean results, n=5 animals) (study 167706)

Time-point	5 min	0.25 h	0.5 h	1 h	2 h	4 h	6 h	8 h	24 h	48h
	6.51	3.87	2.94	2.17	1.43	0.68	0.63	0.45	0.19	0.06*

* = Mean includes results calculated from data less than 30 d.p.m above background

The peak mean plasma concentration was observed at 5 minutes post-dose with a value of 6.51 µg equivalent/mL. Thereafter, the mean concentration of total radioactivity declined rapidly to 3.87 µg equivalent/mL at 0.25 hours post-dose and further to 1.43 µg equivalent/mL at 2 hours post-dose. The mean concentration of total radioactivity continued to decline accounting for 0.68 and 0.45 µg equivalent/mL at 4 and 8 hours post-dose, respectively. At the final time-point of 48 hours post-dose, the mean concentration of total radioactivity was 0.06 µg equivalent/mL.

Ether extracts of plasma collected at 15 minutes and 1 hour post-dose contained 48.3% and 12.1% of sample radioactivity, respectively. Given that [^{14}C]-polidocanol is readily extractable from aqueous solution using ether, it is suggested that a greater proportion of polidocanol was present in the 15 minutes sample. Further extraction of the plasma extract residue with methanol yielded an additional 48.3% and 75.1% of the radioactivity

Reference ID: 3367720

in each sample respectively, confirming that at 1 hour, a greater proportion of polar material was present. The samples generated from plasma contained insufficient radioactivity to give meaningful chromatograms and no further analysis was conducted.

The tissue distribution and kinetics of total radioactivity in the rat following intravenous administration of [^{14}C] polidocanol microfoam or solution (Study 171995) (GLP)

Study Objective

This study was conducted to examine the similarities between the tissue distribution and excretion of total radioactivity following intravenous administration of [^{14}C]-polidocanol microfoam or solution to Sprague Dawley rats.

Study Design

Two groups of 20 male animals received an intravenous administration of [^{14}C]-polidocanol formulated either as a microfoam or as a solution at a target dose volume of 0.1 mL of the formulation per animal (equivalent to a target of approximately 0.1 mg of [^{14}C]-polidocanol).

The microfoam was prepared as follows: [^{14}C]-polidocanol (2.95 mL, equivalent to 10.25 mg) was dispensed as an ethanolic solution, and the ethanol evaporated under a steady stream of nitrogen. An appropriate amount of non-radiolabelled polidocanol (191.15 mg) was weighed to which ethanol (0.8 mL) and sodium phosphate buffer (approximately 16 mL) were added and the resultant suspension sonicated until completely dissolved. The non-radiolabelled solution was then transferred to the flask containing [^{14}C]-polidocanol and the flask made up to volume with sodium phosphate buffer. The formulation was [redacted] (b) (4) and the specific activity (0.200 MBq/mg) and concentration (10.0698 mg/mL) determined. A portion of this solution (18 mL) was added to a canister and the canister pressurized to [redacted] (b) (4) bar using a mixture of gasses ([redacted] (b) (4)

Four animals in each group were killed at each of the following time-points:
5 minutes, 0.25, 1, 2 and 6 hours post-dose.

The following organs, tissues and body fluids were retained for each animal: Lungs, blood, plasma, gastrointestinal tract including contents, fat (subcutaneous, approximately 1-2 g sample), heart, kidneys, tail, remaining carcass and liver.

Where produced, urine and feces were collected for each animal, in order to allow for the total dose recovered to be quantified. Cages were washed at each collection and wash retained. The tissue pad that the animals were retained on during the anesthetic period was retained and added to the cage wash (where collected) or to the post mortem (PM) residue. Any tissue used at necropsy was also retained as a PM residue.

The levels of radioactivity were determined in each sample collected.

Reference ID: 3367720

Results

Polidocanol microfoam
The mean recovery of total radioactivity (as % recovered dose) following intravenous administration of [^{14}C]-polidocanol microfoam to rats is shown below.

Recovery of total radioactivity at intervals following single intravenous administration of [^{14}C]-polidocanol microfoam to rats expressed as mean (n=4) % recovered dose (± SD) (study 171995)

Time-point	5 minutes	0.25 hours	1 hour	2 hours	6 hours
Tissues/organs					
Urine	NS	NS	1.26 ± 2.25*	14.31 ± 7.94	30.59 ± 5.87
Feces	NS	NS	0.01 ± 0.02*	2.75 ± 4.32	0.11 ± NA
Cage wash	NS	NS	13.31 ± 3.25	10.56 ± 5.58	7.27 ± 5.04*
PM residue	2.45 ± 2.01*	3.09 ± 1.94	4.49 ± 2.06	5.24 ± 2.91*	4.95 ± 1.59
Fat (subcutaneous)	0.17 ± 0.11	0.14 ± 0.11*	0.25 ± 0.09	0.09 ± 0.04	0.11 ± 0.02*
Heart	0.79 ± 0.03	0.46 ± 0.02	0.23 ± 0.04	0.20 ± 0.03	0.09 ± 0.01
Kidneys	3.01 ± 0.25	2.97 ± 0.30	1.77 ± 0.22	1.23 ± 0.10	0.42 ± 0.05
Liver	32.33 ± 1.94	26.53 ± 2.40	23.88 ± 2.40	19.54 ± 1.92	9.06 ± 1.19
Lungs	0.98 ± 0.03	0.52 ± 0.10	0.47 ± 0.11	0.32 ± 0.05	0.20 ± 0.02
Tail	1.35 ± 0.89	0.98 ± 0.22	0.66 ± 0.26*	0.73 ± 0.33*	1.46 ± 0.64
GI tract (including contents)	9.43 ± 1.05	14.92 ± 4.88	21.23 ± 2.21	25.19 ± 2.17	32.05 ± 1.07
Remaining carcass	42.40 ± 5.67	43.57 ± 1.87	28.76 ± 1.33	17.26 ± 1.13	12.27 ± 2.46*
Whole blood	7.03 ± 0.85	6.79 ± 1.13	3.68 ± 1.12	3.21 ± 0.35	1.45 ± 0.14
TOTAL	99.94 ± 0.05	99.98 ± 0.06	100.01 ± 0.05	99.96 ± 0.02	99.97 ± 0.04

NS = No sample collected, NA = Not applicable

* = Mean includes results calculated from data less than 30 disintegrations per minute (d.p.m) above background.

The concentration of total radioactivity in plasma fell from a mean of 0.8 µg equivalent/mL at 5 minutes post-dose, the first sampling time-point, to a mean of approximately 0.2 µg equivalent/mL at 6 hours post dose.

At 5 minutes and 0.25 hours after dosing, the distribution of total radioactivity was very similar, with the highest amounts found in the residual carcass (36-48% of the recovered dose). The majority of the remaining total radioactivity was found in the liver (23-34%) and gastrointestinal tract (9-22%), including contents. Significant amounts of total radioactivity were also observed in the whole blood, kidneys and tail (site of injection).

Reference ID: 3367720

At 1 hour post-dose, radioactive residues in carcass were reduced (27-30%) with corresponding increases in the amount of radioactivity in the gastrointestinal tract (18-23%). Radioactivity in the liver was at a similar level to that at 0.25 hours (21-26%). Significant recoveries of total radioactivity were also observed in the whole blood and kidneys. Radioactivity in the tail at this time accounted for approximately 1% of the recovered radioactivity.

At 2 and 6 hours post-dose, the proportion of the recovered total radioactivity in the gastrointestinal tract continued to increase, consistent with the elimination of the dose via bile. Similarly, slightly lower recoveries were observed in the liver and carcass. Significant amounts of total radioactivity were also observed in the whole blood, kidneys, urine and feces. Radioactivity in the tail again accounted for approximately 1% of the recovered radioactivity.

Polidocanol solution
The mean recovery of total radioactivity (as % recovered dose) following intravenous administration of [^{14}C]-polidocanol solution to rats is shown below.

Reference ID: 3367720

Recovery of total radioactivity at intervals following single intravenous administration of [^{14}C]-polidocanol solution to rats expressed as mean (n=4) % recovered dose (± SD) (study 171995)

Time-point	5 minutes	0.25 hours	1 hour	2 hours	6 hours
Tissues/organs					
Urine	NS	NS	3.47 ± 3.14	2.82 ± NA	26.96 ± 5.83
Feces	NS	NS	0.46 ± NA	0.02 ± 0.04	0.42 ± 0.57
Cage wash	NS	NS	4.08 ± 2.83	3.83 ± 2.34	6.47 ± 3.86
PM residue	0.71 ± 0.22	0.87 ± 0.27	0.96 ± 0.64	1.08 ± 0.65	2.64 ± 2.86
Fat (subcutaneous)	0.15 ± 0.07	0.75 ± 0.97	0.16 ± 0.02	0.23 ± 0.05	0.09 ± 0.02
Heart	0.80 ± 0.06	0.55 ± 0.10	0.40 ± 0.08	0.26 ± 0.02	0.12 ± 0.02
Kidneys	3.68 ± 0.61	3.09 ± 0.68	2.43 ± 0.16	1.61 ± 0.31	0.52 ± 0.05
Liver	31.27 ± 1.12	22.69 ± 8.57	28.64 ± 2.47	24.96 ± 2.34	10.34 ± 0.73
Lungs	1.14 ± 0.09	0.65 ± 0.16	0.61 ± 0.06	0.54 ± 0.04	0.28 ± 0.04
Tail	0.69 ± 0.51	2.76 ± 1.91	1.17 ± 0.24	1.04 ± 0.30	0.89 ± 0.49
GI tract (including contents)	10.69 ± 0.79	15.17 ± 2.85	26.70 ± 1.76	37.80 ± 1.21	34.25 ± 2.14
Remaining carcass	43.68 ± 2.74	47.82 ± 5.60	27.45 ± 2.82	23.70 ± 1.97	15.29 ± 4.01
Whole blood	7.19 ± 0.46	5.64 ± 1.38	4.57 ± 0.37	4.28 ± 0.26	1.72 ± 0.24
TOTAL	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

NS = No sample collected

NA = Not applicable

* = mean includes results calculated from data less than 30 disintegrations per minute (d.p.m) above background

The concentration of total radioactivity in plasma fell from a mean of 0.962 µg equivalent/mL at 5 minutes post-dose, the first sample time, to a mean of 0.244 µg equivalent/mL at 6 hours postdose.

At 5 minutes and 0.25 hours after dosing, the distribution of total radioactivity was very similar, with the highest amounts found in the residual carcass (40-53% of the recovered dose). The majority of the remaining total radioactivity was found in the liver (10-33%) and gastrointestinal tract (10-18%), including contents. Significant amounts of total radioactivity were also observed in the whole blood, lungs, kidneys and tail (site of injection).

At 1 hour post-dose, radioactive residues in carcass were reduced (24-31%) with corresponding increases in the amount of radioactivity in the gastrointestinal tract (26-29%). Radioactivity in the liver was at a similar level to that at 0.25 hour (25-31%).

Reference ID: 3367720

Significant recoveries of total radioactivity were also observed in the whole blood, kidneys and urine. Radioactivity in the tail at this time accounted for approximately 1% of the recovered radioactivity.

At 2 hours and 6 hours post-dose, the proportion of the recovered total radioactivity in the gastrointestinal tract continued to increase, consistent with the elimination of the dose via bile. Similarly, slightly lower recoveries were observed in the liver and carcass. Significant amounts of total radioactivity were also observed in the whole blood, kidneys and urine. Radioactivity in the tail again accounted for approximately 1% of the recovered radioactivity.

Conclusions

Following intravenous administration of [^{14}C]-polidocanol, formulated as either a microfoam or solution, radioactivity was extensively distributed to the selected tissues analyzed. Radioactivity dispersed from the site of injection at a similar rate following each administration with only 1% remaining in the tail by 15 minutes post-dose. The amount of total radioactivity recovered in each tissue (expressed as % recovered dose) was generally comparable between the two formulations at each of the time-points analyzed.

[^{14}C]-polidocanol has therefore been demonstrated to be distributed in a similar fashion following administration in either the microfoam or solution formulation to rats.

The disposition of [^{14}C]-polidocanol in the dog following intravenous administration (Study 167711) (GLP)

Study Objective

This study was conducted to provide information on the distribution, metabolism and excretion of polidocanol in the dog following intravenous administration of [^{14}C]-polidocanol. The nature of the radioactivity in samples of excreta and plasma was also examined by chromatographic techniques.

Study Design

Four male beagle dogs received a single intravenous administration (10 minute infusion) of [^{14}C]-polidocanol at a target dose level of 2 mg/kg. Urine was collected quantitatively for the periods 0-6 hours, 6-24 hours and then at 24 hour intervals until 168 hours post-dose. Feces were collected quantitatively at 24 hour intervals up to 168 hours post-dose. At the time of each daily collection, any debris (wasted food) was separately removed and the cages washed. The cage wash and any debris were retained.

Whole blood samples were removed from a jugular vein into heparinized tubes at predose, 0 minute (immediately post infusion), 5 minutes, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 hours post-dose. A sample of whole blood was

Reference ID: 3367720

retained separately. Plasma was separated from the remaining sample by centrifugation and the blood cells discarded.

One animal at approximately 5 and 15 weeks and 2 animals at approximately 22 weeks post-dose were humanely killed and retained frozen prior to analysis under study number 169572. The results of these analyses are reported below (under **Excretion)**.

Results

Excretion kinetics data are presented in the following excretion studies. The concentration of total radioactivity in plasma and whole blood is presented below

Concentration of total radioactivity in whole blood and plasma following single intravenous administration of [^{14}C]-polidocanol to male dogs, expressed as µg equivalent/g (whole blood) and µg equivalent/mL (plasma) (mean, n=4) (study 167711)

Time-point	Whole blood	Plasma
0 minute	2.49	3.91
5 minutes	2.58	3.81
0.25 hours	2.33	3.48
0.5 hours	2.12	3.14
0.75 hours	1.88	2.82
1 hours	1.72	2.52
2 hours	1.07	1.57
4 hours	0.47	0.65
6 hours	0.25	0.35
8 hours	0.17	0.23
24 hours	0.08	0.10
48 hours	0.05	0.06
72 hours	0.04	0.04
96 hours	0.03	0.04
120 hours	0.03	0.03
144 hours	0.02	0.02
168 hours	0.02	0.02

The highest mean plasma concentration of total radioactivity was 3.91 µg equivalent/mL and was observed immediately after the end of the infusion. The mean concentration of total radioactivity in plasma then decreased to 1.57 µg equivalent/mL at 2 hours and further to 0.10 µg equivalent/mL at 24 hours post-dose. The mean concentration of total radioactivity continued to decline steadily and by 168 hours post-dose, the last sampling time-point, the mean concentration of total radioactivity in plasma was 0.02 µg equivalent/mL.

Reference ID: 3367720

The mean concentration of total radioactivity in whole blood showed a similar kinetic pattern to that found in plasma, although at lower levels. The peak mean concentration of total radioactivity in whole blood was observed at 5 minutes after the end of the infusion and was 2.58 µg equivalent/g. Thereafter the mean concentration fell to 1.07 µg equivalent/g at 2 hours and further to 0.08 µg equivalent/g at 24 hours post-dose. The decline in mean concentration continued steadily and by 168 hours post-dose, the last sample time-point, the mean concentration of total radioactivity in whole blood was 0.02 µg equivalent/g.

Extracts of pooled plasma collected at 15 minutes and 2 hours post-dose contained low amounts of radioactivity and components present were not detectable above background in the radio- HPLC chromatograms. The recovery of radioactivity from the HPLC column during analysis of the extracts indicated the presence of both polar and non-polar components.

Conclusions

Following intravenous administration of [^{14}C]-polidocanol to male dogs, the majority (approximately 60%) of the administered dose was eliminated via the urine. There was evidence of biliary elimination with approximately 22% of the administered dose being recovered in feces. Recovery of total radioactivity was 87-90% at 168 hours post dose. The low recovery may be due to losses through expired air or retention of radiolabelled dose in the animal.

Total radioactivity was steadily eliminated from whole blood and plasma over the sampling period, with low levels still detectable at 168 hours post-dose. The mean whole blood:plasma ratio of total radioactivity was approximately 0.7-0.8, indicating that radioactivity was mainly associated with plasma.

The disposition and distribution of [^{14}C]-polidocanol in the dog following intravenous administration (Study 169572) (GLP)

Study Objective

Plasma kinetics, metabolism and excretion of [^{14}C]-polidocanol in the dog were investigated in study 167711. The administered dose was not fully accounted for however, and a new study was conducted to provide additional information on the excretion of radioactivity and to investigate possible tissue retention of radiolabelled material following intravenous administration of [^{14}C]- polidocanol.

Study Design

Two male beagle dogs received a single intravenous administration (10 minute infusion) of [^{14}C]-polidocanol at a target dose level of 2 mg/kg. Following dose administration, samples of urine, feces and expired air (up to 24 hours post-dose) were collected from each animal and the levels of radioactivity present determined, at intervals up to 168 hours post-dose. The animals were sacrificed at 1 week and 2 weeks post-dose and a

Reference ID: 3367720

number of key tissues retained separately from each animal. Four male beagle dogs that had been previously dosed as above under study number 167711 were killed at approximately 5 and 15 weeks (one animal at each time) and at approximately 22 weeks (2 animals).

The following organs, tissues and body fluids were retained separately from each animal and analyzed for total radioactivity:

Blood (animals 1 and 2 only), plasma (animals 1 and 2 only), kidney, renal fat, liver, subcutaneous fat, lung and residual carcass.

Results

Excretion kinetics from the study are presented below (under Excretion).

The concentrations of total radioactivity in selected tissues, organs and body fluids, expressed as
µg equivalent/g are presented below

Concentration of total radioactivity in tissues at 1, 2, 5, 15 and 22 weeks following single intravenous administration of [^{14}C]-polidocanol to male dogs, expressed as µg equivalent/g (study 169572)

Time-point	1 week	2 weeks	5 weeks	15 weeks	22 weeks	
Organ/ tissue						
Renal fat	0.272	0.209	0.036	0.009*	0.020*	0.034
Subcutaneous fat	0.232	0.231	0.036	0.025*	0.012*	0.026
Kidneys	0.431	0.209	0.039	0.008*	0.002*	0.003*
Liver	0.227	0.057	0.019	0.005*	0.004*	0.006*
Lungs	0.125	0.182	0.008*	0.000*	0.011	0.025*
Whole blood	0.036	0.015	NA	NA	NA	NA
Plasma	0.051	0.016	NA	NA	NA	NA

* = Results calculated from data below the limit of reliable determination of the assay (30 d.p.m)

NA = Not applicable

The concentrations of total radioactivity in selected tissues (except lungs) were highest in the animal killed at 1 week post-dose (the first measurement time). Tissue concentrations of total radioactivity ranged from 0.125 to 0.431 µg equivalent/g, and were significantly higher than the concentration present in whole blood and plasma (plasma concentration 0.051 µg equivalent/g).

Reference ID: 3367720

At 2 weeks, the concentration in whole blood and plasma had decreased to close to the limit of reliable determination. Concentrations had also decreased in kidneys, renal fat and liver (0.209, 0.209 and 0.057 µg equivalent/g, respectively), but were slightly elevated in lung and similar in subcutaneous fat compared to 1 week post-dose.

By approximately 5 weeks post-dose, concentrations of radioactivity were much lower in all tissues measured, and by 15 weeks post-dose, all tissues were at or below the limit of reliable determination. At the final collection time (approximately 22 weeks post dose), the levels of radioactivity in all tissues were again at or below the limit of reliable determination.

Conclusions
Low and persistent levels of radioactivity were present in the tissues examined, however levels decreased over the measurement period and were at the limit of reliable measurement by approximately 15 and 22 weeks post-dose.

The tissue distribution of total radioactivity in the dog following intravenous administration of [^{14}C]-polidocanol microfoam (Study 175188) (GLP)

Study Objective
This study was conducted to examine the tissue distribution of total radioactivity following intravenous administration of [^{14}C]-polidocanol microfoam to Beagle dogs.

Study Design
Twelve male dogs each received a single intravenous administration of [^{14}C]-polidocanol microfoam at a target dosage level of 2 mg/kg polidocanol.

The microfoam was prepared as follows: [^{14}C]-polidocanol (0.110 g) and non-radiolabelled polidocanol (2.1379 g) were dissolved in ethanol (9 mL). Phosphate buffer was added to obtain a final target concentration of 1% polidocanol. Equal volumes of the solution (15 mL) were transferred into microfoam generating canisters. Canisters were pressurized for 30 seconds using a gas mixture (b) (4) . Density of the microfoam produced from each canister was determined gravimetrically. Only canisters that produced microfoam within the density range (b) (4) were used in the study.

The dose was administered by bolus injection via the cephalic vein. Target dose volumes were adjusted according to the microfoam density in order to obtain a target dosage level of 2 mg/kg polidocanol. Dose volumes were in the range of 1.38-1.69 mL/kg.

Two animals were killed at each of the following time-points:1, 24 and 96 hours and 1, 2 and 4 weeks post-dose

Reference ID: 3367720

The following organs, tissues and body fluids were retained for each animal: Blood, lungs, heart, plasma, injection site (vein and surrounding tissues), renal fat, kidneys, spleen, liver, subcutaneous fat

Following dose administration, urine and feces were collected daily from animals scheduled for the 168 hours post-dose sacrifice. Cages were washed at each collection time and the wash retained.

Levels of total radioactivity were measured in all samples collected.

Results
The concentrations of total radioactivity in selected tissues, organs and body fluids, expressed as µg equivalent/g are presented below.

Concentration of total radioactivity in tissues at 1, 24 and 96 hours, and at 1, 2 and 4 weeks following single intravenous administration of [14C]-polidocanol microfoam to male dogs, expressed as µg equivalent/g (mean, n=2) (study 175188)

Time-point	1 hour	24 hours	96 hours	1 week	2 weeks	4 weeks
Organ/ tissue						
Renal fat	2.27	0.52	0.42	0.33	0.21	0.17
Subcutaneous fat	1.36	0.17	0.31	0.28	0.19	0.15
Heart	1.58	0.27	0.13	0.11	0.07	0.05
Injection site	1.28	0.31	0.12	0.08*	0.05*	0.13*
Injection site tissues	0.37	0.11	0.24	0.08	0.17*	0.04
Kidneys	7.71	1.32	0.64	0.40	0.31	0.15
Liver	11.59	1.62	0.96	0.78	0.67	0.46
Lungs	2.24	0.47	0.28	0.19	0.12	0.06
Spleen	1.37	0.31	0.27	0.20	0.12	0.07
Whole blood	2.22	0.35	0.09	0.06	0.03	0.02*
Plasma	4.44	0.50	0.13	0.08	0.03	0.01*

*** =** Mean value includes results calculated from data below the limit of reliable determination of the assay (30 d.p.m)

Radioactivity was well distributed to the tissues investigated. The concentration of total radioactivity generally decreased over time, with the highest concentrations in each

Reference ID: 3367720

tissue observed at the first sampling period (1 hour post-dose) and the lowest concentrations observed in most tissues at the final sampling period (4 weeks post-dose).

At 1 hour post-dose, the highest concentrations of total radioactivity were observed in the liver and kidneys, with mean values of 11.59 and 7.71 µg equivalent/g, respectively. The mean concentrations of total radioactivity observed in the tissues surrounding the injection site and the injection site itself (cephalic vein) were 0.37 and 1.28 µg equivalent/g, respectively, suggesting no specific accumulation in the injection site area. The mean concentrations of radioactivity in whole blood and plasma at this time were 2.22 and 4.44 µg equivalent/g, respectively. The mean concentration of total radioactivity in the remaining tissues ranged from 1.36-2.27 µg equivalent/g.

At 24 hours post-dose, the concentration of total radioactivity had decreased in all tissues. The highest concentrations were observed in the liver and kidneys, with mean values of 1.62 and 1.32 µg equivalent/g, respectively. The mean concentrations of radioactivity in whole blood and plasma at this time were 0.35 and 0.50 µg equivalent/g, respectively. The mean concentration of total radioactivity in the remaining tissues ranged from 0.11-0.52 µg equivalent/g.

By 96 hours post-dose, radioactivity had decreased in most of the tissues, with the exception of the subcutaneous fat, which showed an increase in concentration (mean of 0.31 µg equivalent/g). The mean concentrations of radioactivity in whole blood and plasma at this time were 0.09 and 0.13 µg equivalent/g, respectively. The mean concentration of total radioactivity in the remaining tissues ranged from 0.12-0.96 µg equivalent/g.

At 1 week post-dose, radioactivity had progressively decreased, with liver and kidneys still showing the highest values (mean values of 0.78 and 0.40 µg equivalent/g, respectively). Subcutaneous and renal fat showed mean values of 0.28 and 0.33 µg equivalent/g, respectively. Mean concentrations of radioactivity in whole blood and plasma at this time were 0.06 and 0.08 µg equivalent/g, respectively.

From 2 weeks onwards, the concentration of radioactivity progressively decreased further, with liver and kidneys still showing the highest values. Subcutaneous and renal fat still showed high concentrations suggesting retention of radioactivity in these tissues.

The radioactivity was eliminated slowly in the tissues examined with terminal half-life values (calculated in the 168-672 hour interval) of approximately 650, 603, 582, 446, 359, 337, 326 hours for liver, renal fat, subcutaneous fat, heart, spleen, kidneys and lungs, respectively.

<u>Conclusions</u>
Radioactivity was well distributed in the tissues investigated following intravenous administration of [^{14}C]-polidocanol to dogs as a microfoam. Total radioactivity levels

Reference ID: 3367720

were higher in organs associated with metabolism (liver and kidneys). Subcutaneous and renal fat were shown to retain some radioactivity.

In vitro binding of [^{14}C]-polidocanol to the plasma proteins and the blood cells of rat, dog and man (Study 167727) (GLP)

Study Objective
This study was conducted to determine the *in vitro* binding of [^{14}C]-polidocanol to the plasma proteins of rat, dog and man at various concentrations and to determine the blood:plasma concentration ratio and binding to rat, dog and human blood cells.

Study Design
The association of [^{14}C]-polidocanol with blood cells of male rats, male dogs, and male human volunteers was determined at concentrations of 0.01, 0.1, 1, 10 and 100 µg/mL, following incubation at approximately 37ºC for approximately 30 minutes.

The binding of [^{14}C]-polidocanol to plasma proteins of male rats, male dogs and male human volunteers was determined by ultracentrifugation at concentrations of 0.1 and 100 µg/mL.

Results
The association of [^{14}C]-polidocanol with blood cells of male rats, male dogs and male human volunteers, at target concentrations of 0.01, 0.1, 1, 10 and 100 µg/mL is presented in the Table below.

Association of [^{14}C]-polidocanol at various concentrations with rat, dog and human blood cells (study 167727)

Target concentration (µg/mL)	% Associated with blood cells		
	Rat	Dog	Human
0.01	20.9	9.5	2.6
0.1	13.7	6.3	1.3
1	12.2	7.3	3.2
10	16.6	5.4	7.0
100	22.8	9.9	8.2

The plasma to whole blood ratio is presented in the following Table.

Reference ID: 3367720

Plasma to whole blood ratios for rat, dog and human blood (study 167727)

Target concentration (µg/mL)	Plasma:whole blood ratio		
	Rat	Dog	Human
0.01	1.44	1.56	1.80
0.1	1.57	1.64	1.83
1	1.60	1.60	1.79
10	1.52	1.63	1.72
100	1.40	1.58	1.70

Association of [^{14}C]-polidocanol with blood cells was relatively low and varied slightly between species. Blood cell association was ranked human < dog < rat (ranges of 1-8%, 5-10% and 12- 23%, respectively).

The plasma to whole blood ratio was 1.4-1.6 in the rat, approximately 1.6 in the dog and 1.7-1.8 in man.

The *in vitro* plasma protein binding of [^{14}C]-polidocanol in male rats, male dogs and male human volunteers, at target concentrations of 0.1and 100 µg/mL is presented below

Protein binding of [^{14}C]-polidocanol at various concentrations in rat, dog and human plasma (study 167727)

Target concentration (µg/mL)	Replicate	% Protein bound		
		Rat	Dog	Human
0.1	A	29.8	74.2	47.9
	B	38.0	72.3	43.9
	Mean	33.9	73.2	45.9
100	A	15.5	55.0	2.0
	B	31.5	39.7	1.9
	Mean	23.5	47.4	1.9

The plasma protein binding of [^{14}C]-polidocanol decreased with increasing concentration in each species.

Reference ID: 3367720

The extent of protein binding also varied between species, with the highest degree of binding observed in the dog at both concentrations investigated. From lowest to highest, protein binding was ranked; rat < man < dog at the lower concentration (34%, 46% and 73%, respectively), although at 100 µg/mLprotein binding was higher in rat than in man (2%, 24% and 47% for man, rat and dog, respectively).

Conclusions
The extent of blood cell association was relatively low, independent of the concentration of [^{14}C]-polidocanol used and varied from 1-23%, with the species ranked; human < dog < rat. The degree of plasma protein binding decreased with increasing concentration of [^{14}C]-polidocanol. The extent of protein binding also varied between species and was higher in the dog than in the other two species. Protein binding was ranked; rat < man < dog at the lower concentration and man < rat < dog at the higher concentration.

Metabolism (inter-species comparison)

Investigation of the nature and identity of radiolabelled components present in biological samples obtained from rats and dogs following an intravenous administration of [^{14}C]-polidocanol (Study 169771) (GLP)

Study Objective
The tissue distribution of [^{14}C]-polidocanol in the rat following intravenous administration was investigated during study 169609. The disposition of [^{14}C]-polidocanol following intravenous administration in rats and dogs was investigated during study 167706 and study 167711, respectively, and these studies also included limited metabolite profiling. This study investigated the biotransformation of polidocanol in the rat and dog in greater detail using samples retained during the above studies.

Study Design
A reversed phase HPLC method was developed which allowed the resolution of polidocanol from more polar metabolites and which was compatible with LC-MS. Metabolite profiles were produced for urine, feces and plasma samples generated from the rat and dog studies. In addition metabolites were characterized by LC-MS and LC-MS(MS).

Results
The quantification of radiolabelled components in urine, plasma and feces from rats and dogs that had received an intravenous administration of [^{14}C]-polidocanol is shown in the next two Tables.

Reference ID: 3367720

Quantification of radiolabelled components from rats that had received an intravenous administration of [^{14}C]-polidocanol (study 169771)

Animal Numbers	Sample	Timepoint (h)	Component	Retention Time (min)	Component as:	
					% TRR	% Dose**
3-5	Urine	0–24		2.0	19.0	8.82
				4.2	12.5	5.80
				18.5*	64.6	29.99
11	Plasma	0.25		2.10	11.9	0.536
				20.70*	46.2	2.082
				47.97	3.0	0.135
			Polidocanol	58.79	39.0	1.758
12	Plasma	0.25		1.89	23.4	0.958
				18.99*	35.5	1.454
				47.89	2.4	0.098
			Polidocanol	58.49	33.3	1.364
13	Plasma	1		1.89	39.5	1.024
				20.82*	28.2	0.731
				48.23	1.3	0.034
			Polidocanol	58.40	17.7	0.459
14	Plasma	1		1.90	37.8	0.990
				22.70*	27.3	0.715
			Polidocanol	58.41	15.2	0.398
3-5	Feces	0–24		1.90	27.1	6.85
				24.60*	23.5	5.94
				48.40	3.9	0.99
			Polidocanol	58.20	9.2	2.33

* Retention time of the largest peak in a range of peaks
** Plasma data expressed as µg equiv/g

Quantification of radiolabelled components from dogs that had received an intravenous administration of [^{14}C]-polidocanol (study 169771)

Animal Numbers	Sample	Timepoint (h)	Component	Retention Time (min)	Component as:	
					% TRR	% Dose**
1-4	Urine	0-24		2.09	10.6	6.46
				20.79*	77.4	47.19
				46.29	1.5	0.91
				48.19	0.6	0.37
			Polidocanol	58.59	1.8	1.1
1	Plasma	0.25		2.01	6.8	0.238
				46.11	9.5	0.333
				48.01	11.3	0.396
			Polidocanol	58.41	70.1	2.454
2	Plasma	0.25		1.89	12.1	0.393
				22.69*	23.8	0.774
				48.09	5.2	0.169
			Polidocanol	58.69	46.2	1.502
				67.49	1.6	0.052
				70.29	2.1	0.068
3	Plasma	0.25		1.99	9.3	0.316
				20.90*	2.1	0.071
				46.10	6.0	0.204
				48.10	11.1	0.377
			Polidocanol	58.30	58.9	2.003
4	Plasma	0.25		2.01	9.4	0.353
				22.30*	16.3	0.613
				26.81	2.0	0.075
				48.11	23.2	0.872
			Polidocanol	58.30	49.2	1.850
1-4	Feces	0–48		1.91	8.7	1.82
				25.11*	17.9	3.75
				46.41	2.3	0.48
				48.41	7.7	1.61
			Polidocanol	58.11	22.5	4.71

* Retention time of the largest peak in a range of peaks
** Plasma data expressed as µg equiv/g

Urine samples were prepared for HPLC analysis by centrifugation and the recovery of radioactivity was 96.0% and 91.9% for rat and dog, respectively. Extracts of plasma samples contained between 80.3% and 100% total radioactive residue (TRR). The lowest values in the range were obtained for the later time-point samples from the rat. The extraction of radioactivity from feces with organic solvent yielded 59.1% TRR from dog feces and 63.7% TRR from rat feces.

Radio-HPLC chromatograms generated from rat urine, plasma and fecal extract each contained individual peaks at approximately 2 and 48 minutes and a range of peaks between the retention times of approximately 8 and 30 minutes. In addition, plasma (0.25 and 1 hour post-dose) and fecal extracts both contained polidocanol at a retention

Reference ID: 3367720

time of approximately 58 minutes. An additional peak was present in rat urine at a retention time of approximately 4.2 minutes.

The profile of metabolites in the dog was qualitatively consistent with the rat with the exception of a minor peak at approximately 46 minutes which was detected in dog urine and plasma.

The analysis of rat urine and plasma sample extracts by liquid chromatography-mass spectrometry revealed a number of polar metabolite peaks. These comprised a very polar peak which, because of its very polar nature, was not characterized. Evidence was found to suggest that the remaining polar peaks were several metabolite series comprising either polyethylene glycol groups or acidic polyethylene glycol groups. These series comprised species which were separated by 44 mass units. The oligomers of polidocanol afforded a series of ions separated by 44 mass units, therefore a pattern of ions separated by 44 mass units was taken as an indication of metabolites related to polidocanol. The polarity of these related species was such that the various series co-eluted together giving rise to a very complicated chromatographic profile. It was considered unlikely that any single peak represented more than 2-3% of the dose.

Conclusions

The development of a reverse phase HPLC method for the characterization of polidocanol and its metabolites in biological samples has allowed the relative polarity of polidocanol metabolites to be assessed. It is apparent that polidocanol is metabolized to a series of progressively more polar products which are readily excreted.

Effect of polidocanol on the membrane integrity of human liver microsomes assessed by glucose-6-phosphatase and UDPglucuronosyltransferase activity (Study 789783) (GLP)

Study Objective

This study was conducted in preparation for an *in vitro* metabolism study. Due to the known mechanism of action of polidocanol as a sclerosant and a non-ionic surfactant, it was considered possible that it could compromise the integrity of the microsomal membrane thus affecting its own metabolism. This study aimed to address whether polidocanol impacted on human liver microsomal integrity.

Study Design

The potential for polidocanol to impact on the membrane integrity of human liver microsomes was to be examined by assessing the activation of latent glucose-6-phosphatase (G6Pase) and UDP-glucuronyltransferase (UGT) activity in human hepatic microsomes. The catalytic activity of these enzymes resides on the luminal surface of the endoplasmic reticulum or microsomal vesicle. Transport of substrates or formation of a ‘pore’ is required for access to the enzyme. For substrates of G6Pase and UGT that are not transported across the microsomal membrane, such as mannose-6-phosphate

Reference ID: 3367720

and 1-napthol, *in vitro* catalysis by microsomes that have been disrupted (e.g. by treatment with a surfactant) is greater than catalysis by intact microsomes.

The possible effects of polidocanol in causing disruption to the microsomal membranes by measuring increased metabolism of selected G6Pase and UGT substrates was intended to be compared to the effects of the detergent Brij 58; an agent known to disrupt membranes.

In this study an appropriate method could not be established for an assay determining G6Pase activity by measuring inorganic phosphate liberation.

Activation of UGT was assessed successfully however by measuring the formation of 1-naphthol glucuronide following incubation of microsomes with, or without, polidocanol. Aliquots of microsomes were pre-incubated with polidocanol at concentrations of 0.5, 5 or 50 µM for 30 minutes on ice. Microsomes were also pre-incubated with Brij 58 (an agent known to disrupt membranes) as a positive control, or water, as a negative control. Heat deactivated microsomes were also used as a negative control.

Following the 30 minute pre-incubation an aliquot of pre-incubate was removed and added to a tube containing 1-naphthol, magnesium chloride and potassium phosphate buffer . Reactions were performed in triplicate at a temperature of 37°C and were initiated by the addition of [^{14}C]-uridine 5′ diphosopho glucuronic acid. Reactions were terminated after 30 minutes by the addition of ice cold acetonitrile. Samples were centrifuged and then analyzed by HPLC.

Results

The Table below presents the results for the formation of 1-naphthol glucuronide following exposure of human liver microsomes to increasing concentrations of polidocanol.

Reference ID: 3367720

Assessment of 1-naphthol glucuronidation following exposure of human liver microsomes to increasing concentrations of polidocanol (study 789783)

Treatment	Formation of 1-naphthol glucuronide			
	nmol/min/mg protein	Mean +/- SD nmol/min/mg protein	% of vehicle control	Mean +/- SD % of vehicle control
Negative control (dH_2O)	6.11 5.96 6.66	6.24 +/- 0.37	97.84 95.42 106.74	100.00 +/- 5.96
Polidocanol (0.5 µM)	6.48 5.90 5.63	6.00 +/- 0.43	103.79 94.53 90.21	96.18 +/- 6.94
Polidocanol (5 µM)	6.59 6.07 5.87	6.17 +/- 0.37	105.50 97.17 94.01	98.90 +/- 5.94
Polidocanol (50 µM)	7.35 6.73 6.76	6.95 +/- 0.35	117.74 107.77 108.28	111.26 +/- 5.62
Brij 58 (0.025%, w/v)	17.45 17.48 17.49	17.48 +/- 0.02	279.56 279.99 280.20	279.92 +/- 0.33
Heat inactivated microsomes	0.00 0.00 0.00	0.00 +/- 0.00	0.00 0.00 0.00	0.00 +/- 0.00

In control incubations, the mean rate of 1-naphthol glucuronide formation was 6.24 nmol/min/mg protein. Polidocanol did not produce any increase in the rate of 1-naphthol glucuronide at concentrations of 0.5 µM and 5 µM (approximately 96% and 99% of negative control, respectively). As increased UGT activity is suggestive of microsomal membrane disruption, the results from this study suggest that polidocanol does not disrupt microsomal integrity at 0.5 µM and 5 µM. At the highest polidocanol concentration (50 µM), a minor increase in UGT activity was observed (approximately 111% of negative control activity), suggesting the possibility of slight disruption to microsomal integrity. This increase was not thought to be of significance. The positive control, Brij 58 (0.025%, w/v), was conducted in parallel to polidocanol and increased activity to approximately 280%, demonstrating that disruption to the microsomal membrane increases UGT activity in this assay. This confirmed the suitability of this method.

Conclusions

Polidocanol produced no, or minor, impacts on microsomal membrane integrity at the concentrations of 0.5, 5 and 50 µM in this study when assessed using the UGT assay. This result provided confidence that *in vitro* microsomal metabolism studies could be conducted at these concentrations without compromising microsomal membrane integrity.

Reference ID: 3367720

The comparative metabolism of [^{14}C]-polidocanol in rat, dog and human liver microsomes and S9 fraction (Study 790228) (GLP)

Study Objective

This *in vitro* metabolism study was designed to investigate the rate and routes of metabolism of [^{14}C]-polidocanol in the presence and absence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) in hepatic microsomes and S9 fractions prepared from male Sprague Dawley rat, male Beagle dog and mixed gender human liver.

Study Design

Incubations were performed, in triplicate at 37°C. Reactions contained ^{14}C]-polidocanol (50 µM) and either microsomes or S9 fraction (1 mg/mL) in a final volume of 2.5 mL of potassium phosphate buffer (50 mM, pH 7.4). Following a period of pre-warming, reactions were initiated by the addition of a standard NADPH-generating system. Reactions were also conducted in the absence of the NADPH-generating system. Incubations were terminated after 0, 30, 60, and 120 minutes by removing an aliquot (500 µL) from the appropriate vial and transferring it into diethyl ether (3 mL). At 120 minutes, the remaining sample (approximately 500 µL) was mixed with the appropriate volume (approximately 3 mL) of diethyl ether.

A liquid/liquid extraction procedure was then conducted on the time-course samples by placing them into a tube rotator for approximately 15 minutes, followed by centrifugation for approximately 10 minutes. Following centrifugation, three distinct layers were formed; the bottom layer which was the aqueous portion, the protein layer in the centre and the solvent layer at the top containing [^{14}C]-polidocanol (sample extract). A hexane bath was then used to freeze the protein and aqueous layers to allow the sample extract to be poured into clean glass vials. The solvent layer was then dried under a stream of nitrogen gas. Once the aqueous layer and protein layers had defrosted (aqueous phase), the procedure was conducted a further twice, with the sample extracts combined into a single glass vial. Dried extracts were reconstituted in 500 µL of mobile phase and duplicate aliquots (10 µL) removed for analysis by liquid scintillation counting (LSC) to establish the recovery of radioactivity, calculated as a percentage of the administered dose. The extracts were then equally transferred into two labeled HPLC vials and stored at approximately -80ºC prior to being taken for radio-HPLC analysis. The remaining aqueous phase was transferred to scintillation vials and taken for analysis by LSC.

In addition to the microsomal and S9 fraction incubations, control incubations containing heat deactivated microsomal protein and incubation buffer only were also conducted to assess the stability of the test item ([^{14}C]-polidocanol). In parallel incubations, the metabolic competence of the microsome and S9 preparations (1 mg/mL) was determined by incubation of [^{14}C]-testosterone.

Reference ID: 3367720

Results

Radioactive recoveries were calculated from each of the incubation samples following the extraction process. The aqueous phase containing the microsomal or S9 protein remaining at the end of the extraction process was also analyzed.

The radioactive recoveries obtained from the microsomal and S9 extracts were predominately above 80%. In analysis of the aqueous phase, the highest level of recovery of radioactivity was observed in the 120 minute samples.

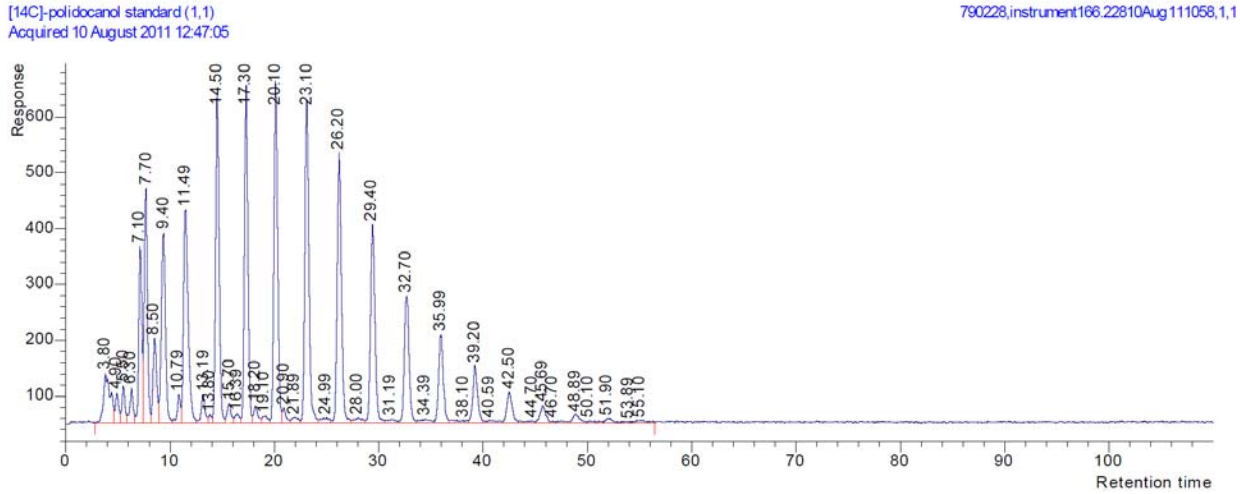
In the rat and dog microsomal incubations, the recovery of radioactivity observed in the aqueous phase increased from approximately 3% at 0 minutes to a maximum of approximately 10% at 120 minutes. In the human microsomal incubations, the recovery in the aqueous phase increased to a maximum level of approximately 19%. This was only observed in the incubations conducted in the presence of cofactor. The effect was not observed in the incubations conducted in the presence of heat deactivated protein or with incubation buffer only. This effect was also observed in the S9 fraction incubations although to a lesser degree. This may be indicative of the formation of polar metabolites which were not soluble in the solvent phase of the extraction process.

A representative radio HPLC chromatogram of the [^{14}C]-polidocanol HPLC standard is presented in the first Figure. Forty-two peaks were integrated and considered to be associated with [^{14}C]-polidocanol. In the extracts derived from incubations in the absence of cofactor and in the control incubations (heat deactivated protein and incubation buffer only), the number of peaks ranged between 38 and 42 with very little change in the chromatographic profile or peak size. This indicated that [^{14}C]-polidocanol was stable under the incubation conditions used.

In the incubation samples containing cofactor NADPH, changes in the chromatographic profile were apparent over time. The very distinctive shape of the radio-HPLC chromatograms displaying the [^{14}C]-polidocanol oligomers was lost following incubation time with the greatest change observed at 120 minutes (next three Figures). As this occurred in samples containing cofactor, the changes were deemed to be due to metabolism of [^{14}C]-polidocanol. In the rat (2nd Figure), dog (3rd Figure) and human (4th Figure) microsomal samples, there was increased formation of small quantifiable peaks between the main oligomer peaks which displayed high responses and also additional peak formation, specifically around the retention time of 40-50 minutes. The additional peaks observed towards the end of the analytical run time were indicative of more polar components. Using a qualitative approach only, the change in the profile with formation of new peaks and reduction in others, was most apparent in the human, then the dog followed by the rat showing the smallest change out of the three species examined in this study. In the S9 incubations, the observed changes also occurred; but to a lesser extent. The order of metabolic activity was consistent with the microsomal profiles with the human S9 fraction displaying the greatest change in chromatographic profile. Due to the analytical method employed and the presence of radioactivity remaining in the aqueous phase of the extract process, it is possible that the chromatographic profiles are not complete in terms of displaying the metabolites of [^{14}C]- polidocanol.

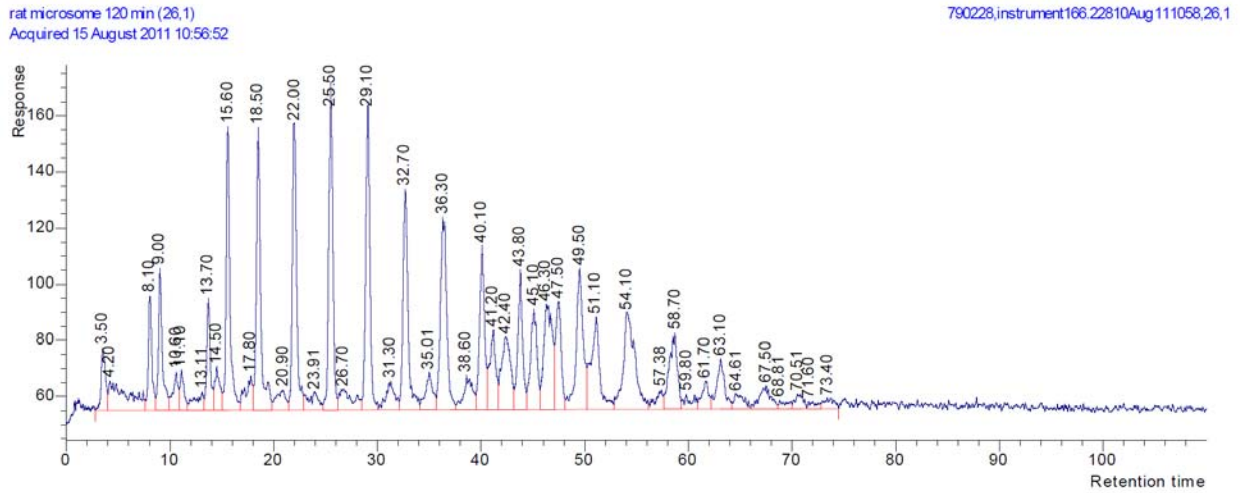
Reference ID: 3367720

[^{14}C]-Polidocanol HPLC standard prepared from dosing solution (study 790228)



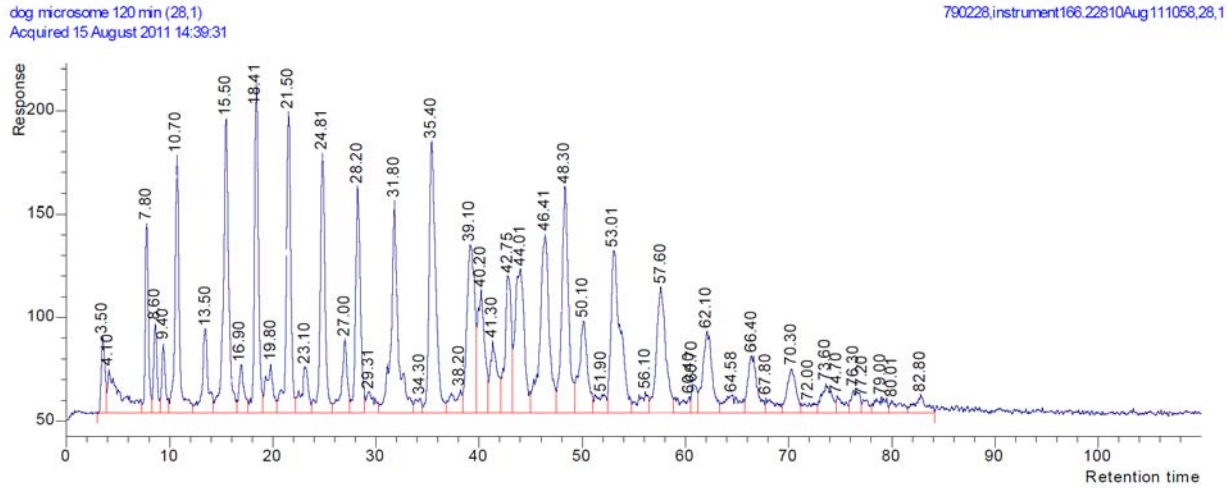
Total peak number = 42

[^{14}C]-Polidocanol (50 µM) incubation with rat hepatic microsomes in the presence of cofactor (120 minutes) (study 790228)



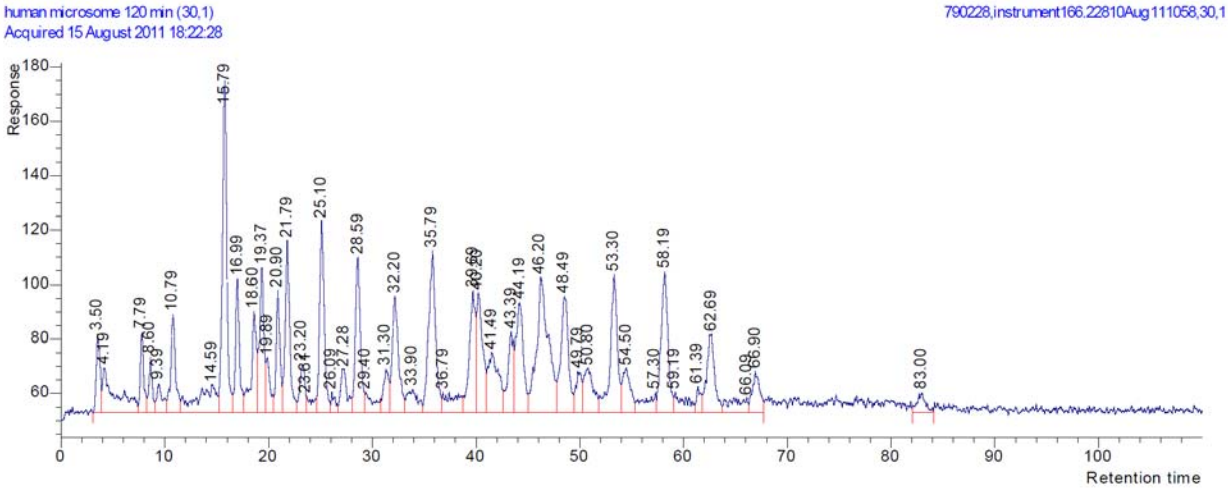
Total peak number = 44

[^{14}C]-Polidocanol (50 µM) incubation with dog hepatic microsomes in the presence of cofactor (120 minutes) (study 790228)



Total peak number = 48

[^{14}C]-Polidocanol (50 µM) incubation with pooled human hepatic microsomes in the presence of co factor (120 minutes) (study 790228)



Total peak number = 45

Incubation of rat, dog and pooled human microsome and S9 liver fractions with the positive control [^{14}C]-testosterone resulted in the disappearance of parent compound (maximum approximately 91% loss of parent, recorded at 60 minutes) over 120 minutes incubation period, with the appearance of a number of polar metabolites. One of the metabolites formed was identified by co- chromatography with the authentic standard 6β-hydroxytestosterone. The presence of the polar metabolites indicated that the tissue fractions were viable and suitable for the assessment of the metabolism of [^{14}C]-polidocanol.

Conclusions

Following incubation of [^{14}C]-polidocanol (50 µM) with microsomes and S9 fractions prepared from male rat, male dog and mixed gender human liver tissue, evidence of NADPH dependent metabolism was observed across the species and tissue fractions. However, the chromatographic profile of [^{14}C]-polidocanol was difficult to interpret and, therefore, it was not possible to determine and compare the rate of metabolism and the metabolite profiles in the different species and tissue fractions employed in this study using the radio-HPLC method employed. During the course of the incubation period, in the presence of the cofactor NADPH, visual examination of the chromatographic profile displayed a change in each of the species and tissue fraction examined in accordance to the following order: human> dog> rat. However, due to increases in the radioactivity content recovered from the aqueous portion as a result of the extraction process, there could be polar metabolites which may not have been detected using the analytical method employed in this study.

Investigation of the nature and identity of radiolabelled metabolites in rat, dog and human microsomes incubated with [^{14}C]-polidocanol (study 219463) (non-GLP)

Study objective

The objective of this study was to investigate the nature and identity of the metabolites of polidocanol formed when [^{14}C]-polidocanol was incubated with hepatic microsomes and the S9 fraction from rat, dog and human sources and to use this information to suggest a metabolic pathway for [^{14}C]-polidocanol.

Study outline

Rat, dog and human microsomal and S9 incubates from Study 790228 were transferred to this study and analyzed by HPLC with concurrent radiodetection. The reverse-phase HPLC method used for analysis of ex vivo samples in Study 169771 was used for the initial analysis of microsome and S9 incubates. The gradient used in this method was modified to improve metabolite resolution and to aid metabolite identification. Method development was conducted using the human microsome sample. This sample was chosen as it exhibited the highest extent of metabolism in the initial analytical run. Samples were analyzed by LC-MS, using the developed method, with concurrent radiodetection achieved by splitting the post-column eluant via an in-line splitter such that approximately 25% of the flow was directed to the mass spectrometer and the remainder to the radiodetector.

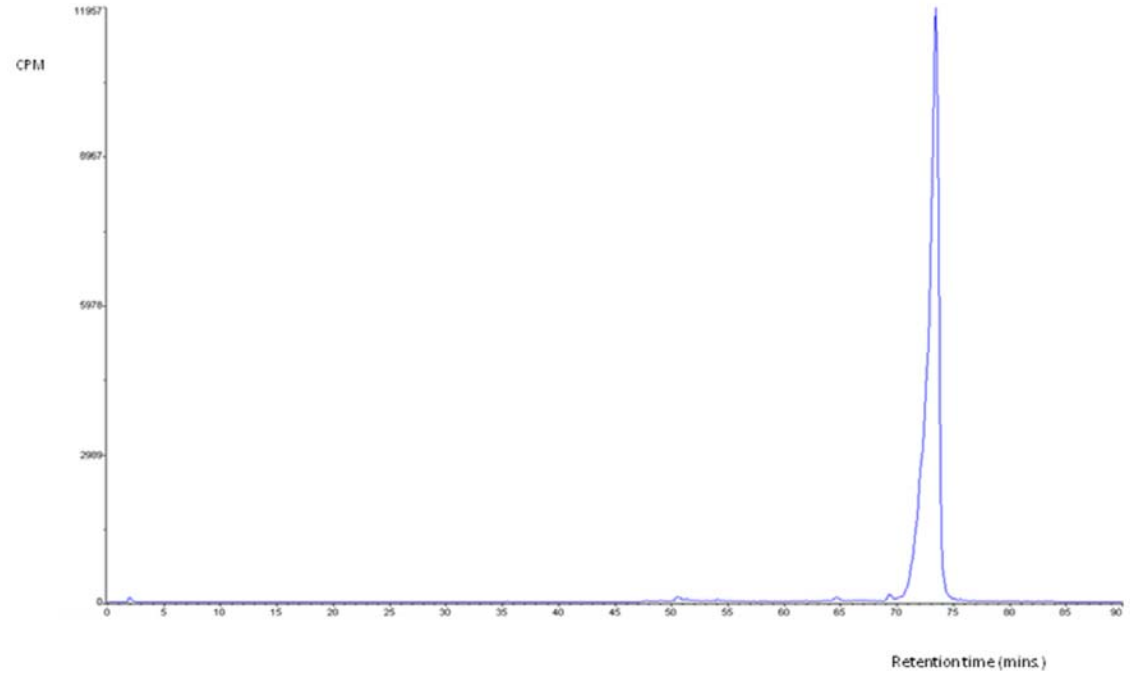
Results

Chromatographic analysis of microsome and S9 samples incubated with [^{14}C]-polidocanol

Reference ID: 3367720

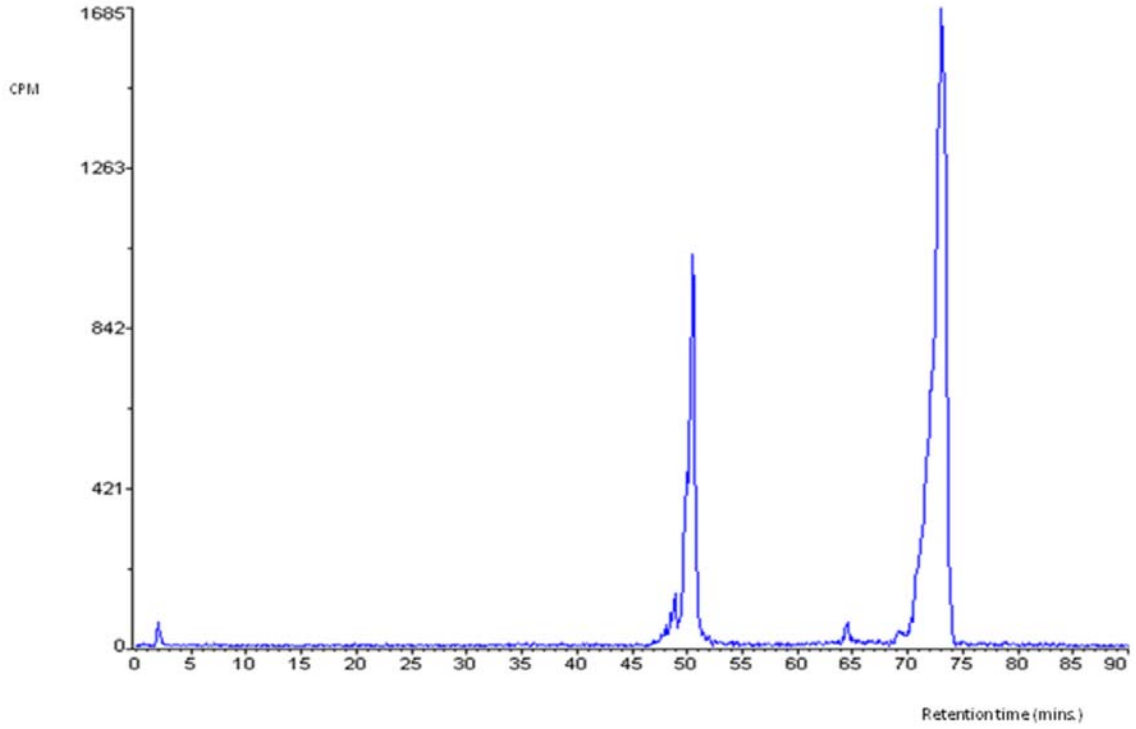
Microsomal incubates (with and without cofactor) and S9 incubates from all species at T0 showed no significant components present other than the parent polidocanol cluster (retention time 70-75 minutes, see Figure below for a representative chromatogram).

[^{14}C]-Polidocanol + Rat Microsomes - Incubation Time 0 minutes (study 219463)

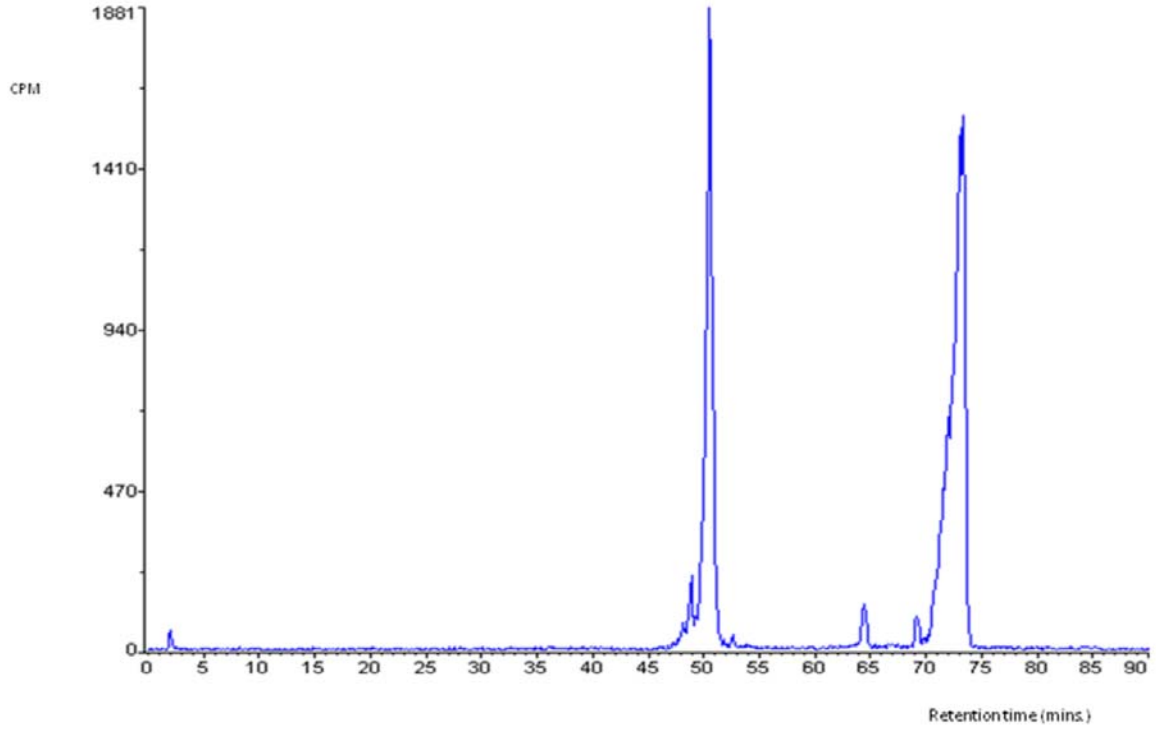


Following incubation for 120 minutes, metabolism was observed in each of the rat, human and dog microsome samples with added cofactor (NADPH generating system). A large multi-component region of metabolites concentrated at a retention time of approximately 50 minutes was observed in the radio chromatogram. The radio chromatograms for rat, human and dog microsomes incubated with [^{14}C]-polidocanol for 120 minutes are shown in the following three Figures.

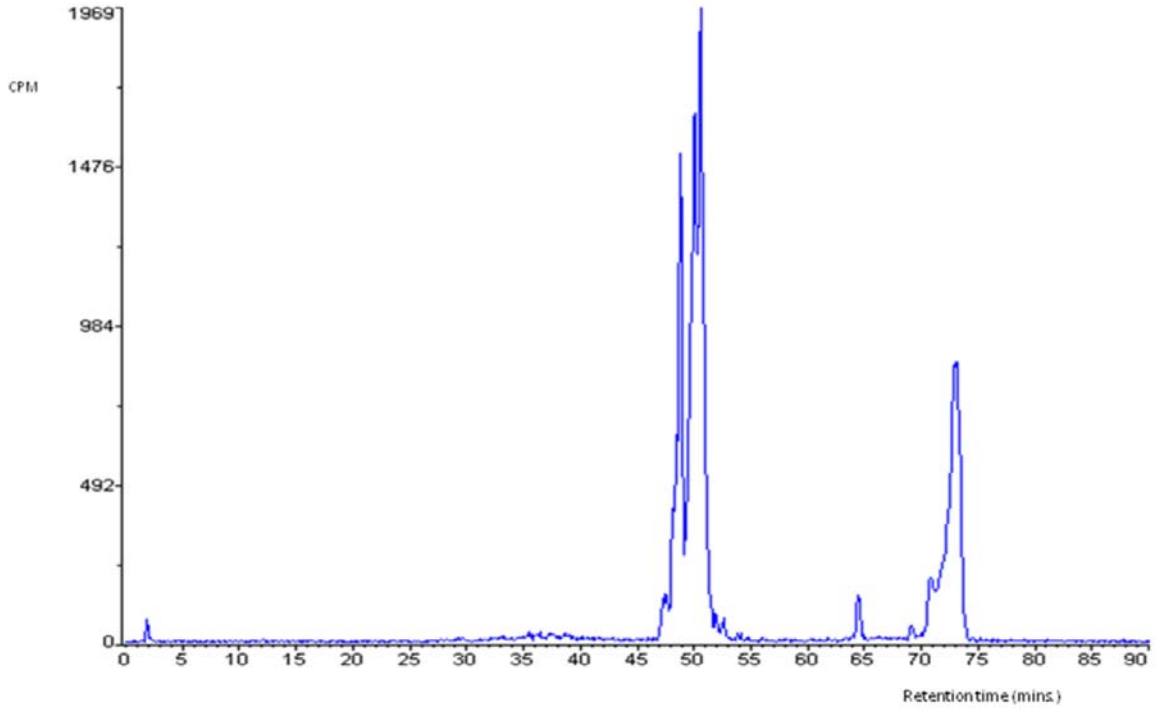
[^{14}C]-Polidocanol + Rat Microsomes - Incubation Time 120 minutes (study 219463)



[^{14}C]-Polidocanol + Dog Microsomes - Incubation Time 120 minutes (study 219463)



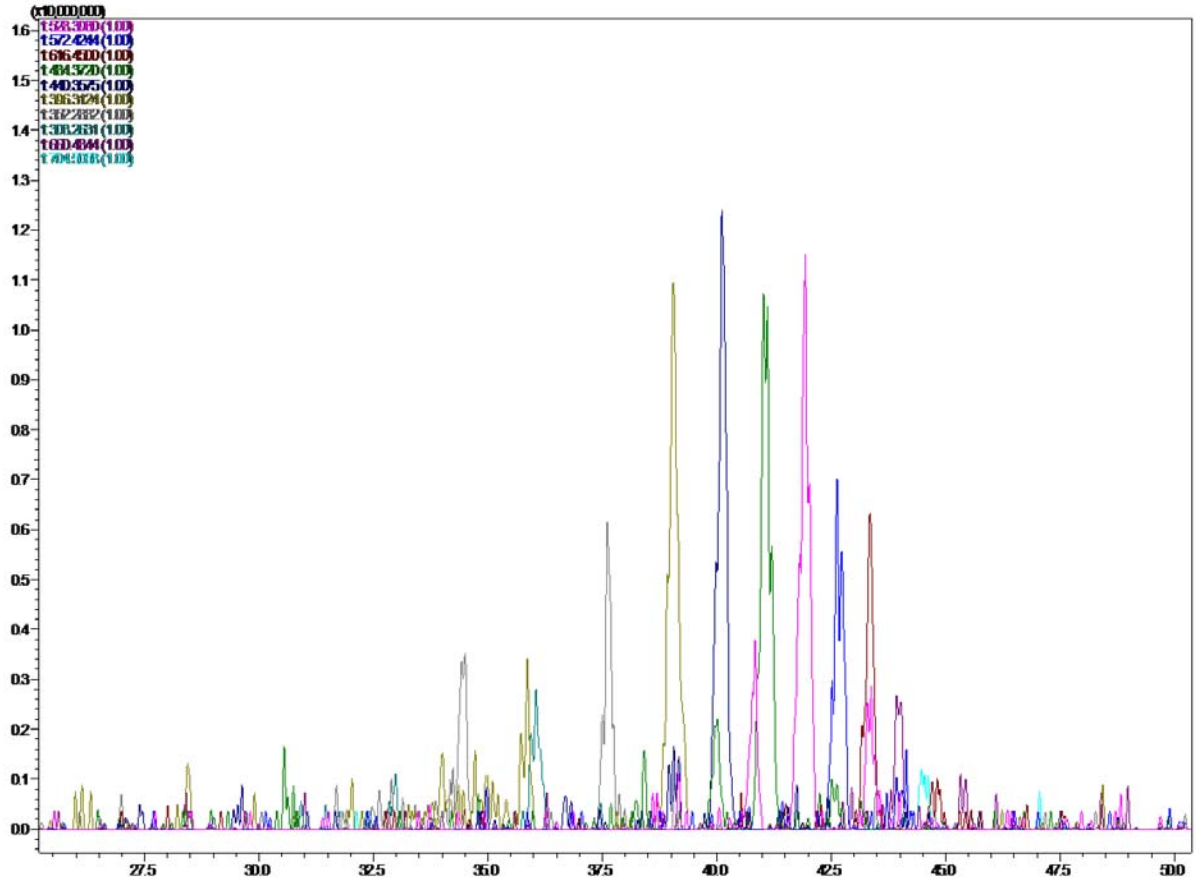
[^{14}C]-Polidocanol + Human Microsomes- Incubation Time 120 minutes (study 219463)



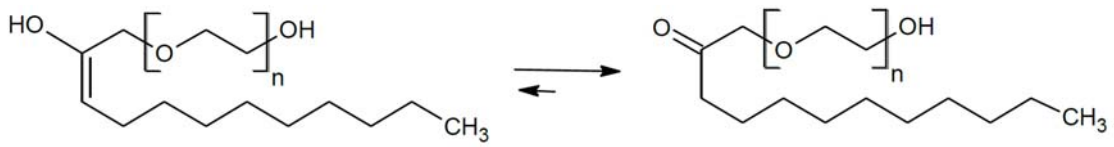
Very little or no metabolism of [^{14}C]-polidocanol was observed for the microsome samples which had no cofactor added suggesting oxidative metabolism was the key metabolic process involved across all of the species. The S9 fraction showed a lower extent of oxidative metabolism in the rat and dog samples compared with the corresponding microsomal incubations, and a minute trace of metabolism present in the human S9 sample.

LCMS analysis of human microsomes
From the extracted ion chromatogram (Figure 9) it is clear that for several of the extracted masses there are multiple components exhibiting the same mass.

[^{14}C]-Polidocanol + Human Microsomes - Incubation Time 120 minutes: Extracted Ion Chromatogram Showing Series of Oxidized hydroxyl (enol) [^{14}C]-Polidocanol Metabolite Masses (study 219463)



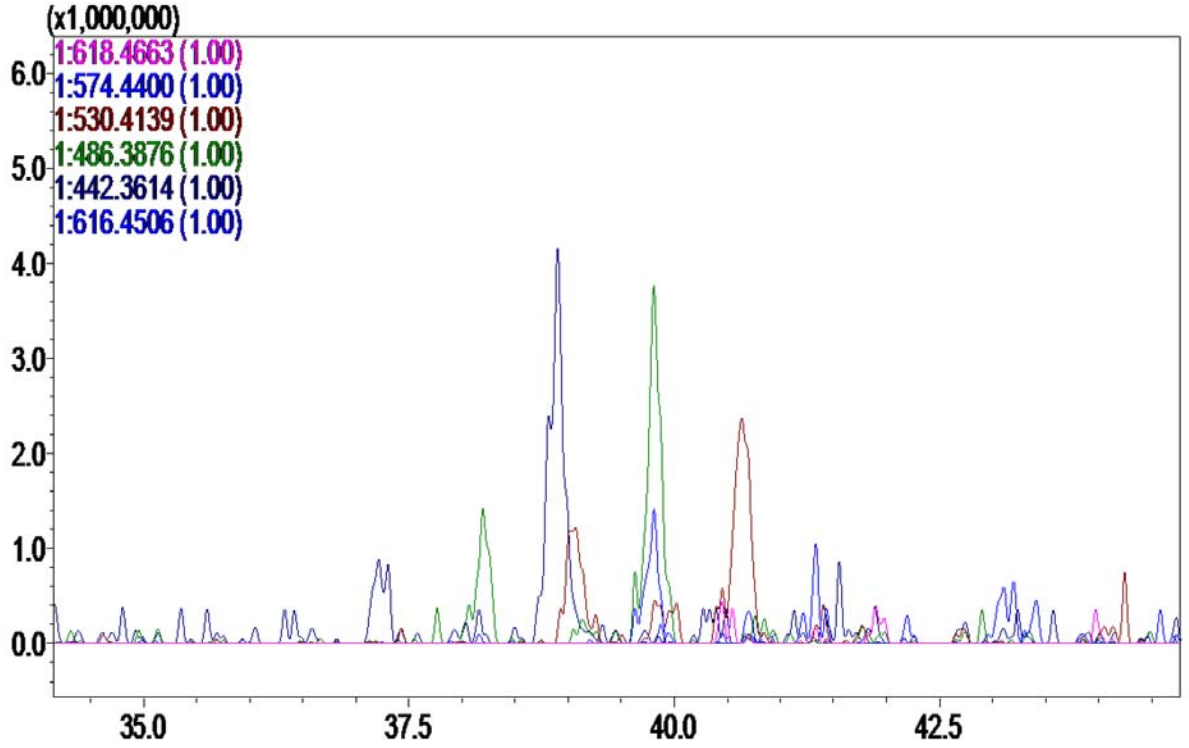
This is indicative of oxidation at different positions in the dodecyl chain. The general structure of the proposed metabolite series formed is shown below where the position of oxidation is nonspecific. This oxidized hydroxyl compound would exist as an equilibrium between the keto and enol forms (see below).



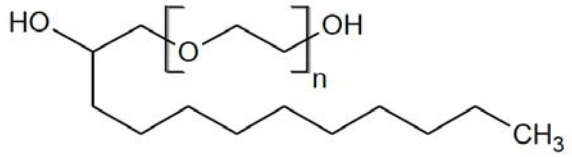
The mass spectra and MS/MS fragmentation for a selection of these ions showed a loss of water indicating that the oxidation had occurred on the dodecyl chain and not on the glycol side chain. Oxidation of the glycol portion would furnish the corresponding series of terminal carboxylic acids. The theoretical fragmentation of the terminal carboxyl containing compound would occur through the loss of 44 amu corresponding to the carboxyl functional group, and this is not observed in the mass spectra for each of the sequence of compounds in the cluster at 30-45 minutes.

A series of mono-hydroxylated metabolites was also observed to be present in the human microsome sample in the region of interest between 30-45 minutes. The level of these metabolites was lower than for the oxidized hydroxy series. The extracted ion chromatogram for each of the series is shown below.

[^{14}C]-Polidocanol + Human Microsomes - Incubation Time 120 minutes: Extracted Ion Chromatogram Showing Series of Hydroxylated [14C]-polidocanol Metabolite Masses (study 219463)



The general structure of this series of compounds is shown below and again the existence of multiple peaks for several of the masses suggests non specific oxidation.

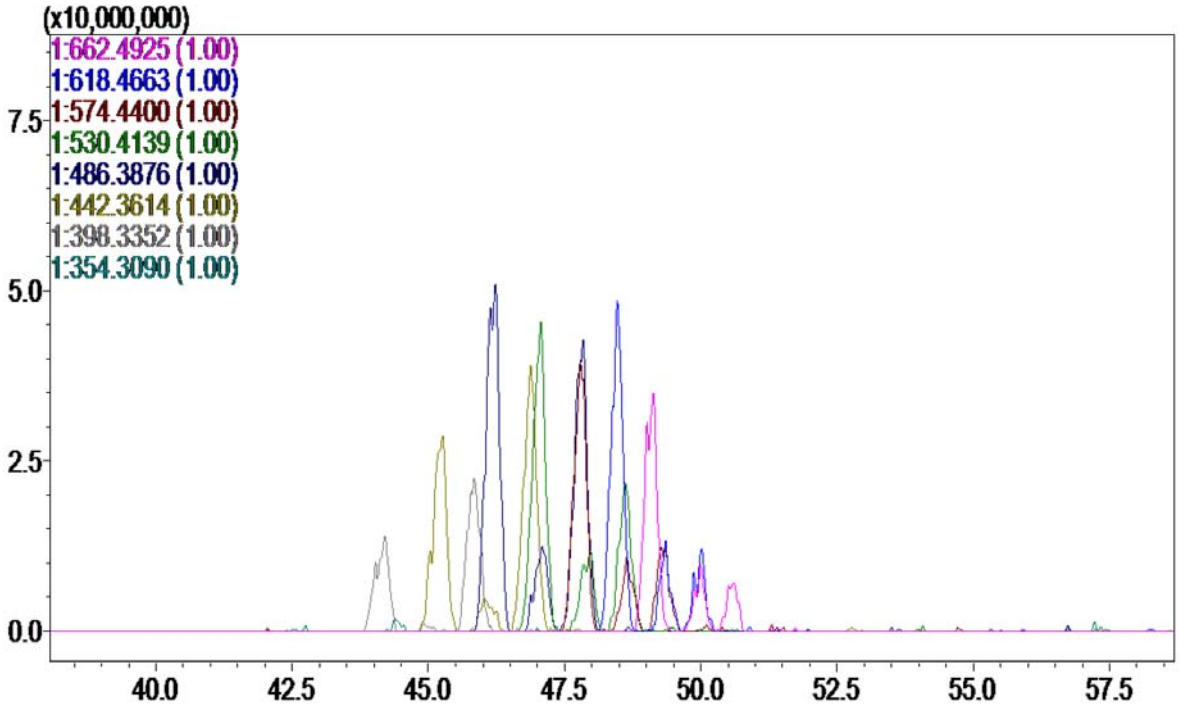


LCMS analysis of dog microsomes

Dog microsomes showed the presence of the mono hydroxylated metabolite series. Extracted ion chromatogram for each of the mono hydroxylated metabolites is shown in the Figure below.

Reference ID: 3367720

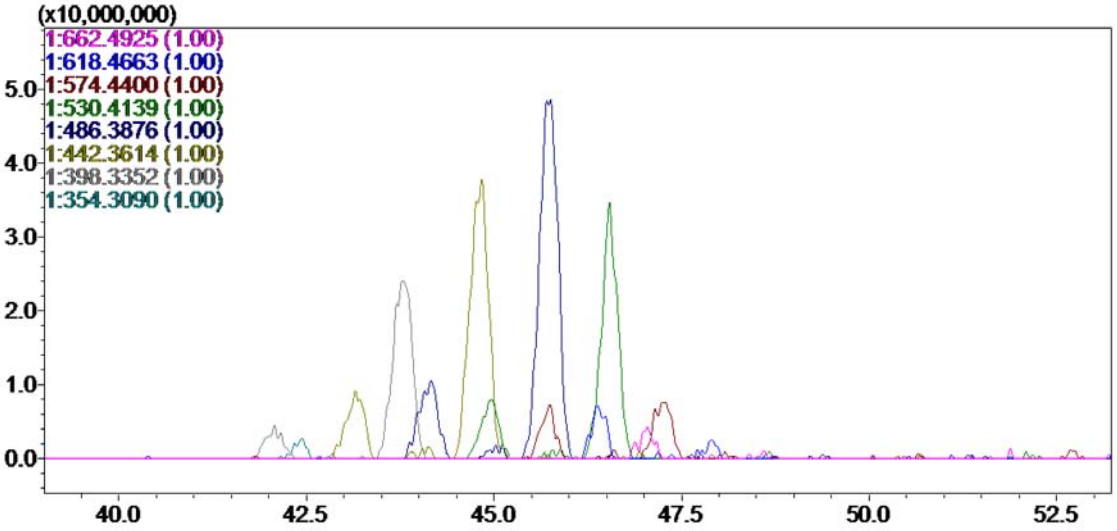
[^{14}C]-Polidocanol + Dog Microsomes - Incubation Time 120 minutes: Extracted Ion Chromatogram Showing Series of Hydroxylated [14C]-polidocanol Metabolite Masses (study 219463)



LCMS analysis of rat microsomes

Similarly for the rat microsome sample the mono hydroxylated metabolite series was observed (extracted ion chromatogram shown in the Figure below but only traces of the oxidized hydroxyl series were observed. It is possible that this difference between the human microsomes and the rat and dog is down to the extent of metabolism observed being lower in the case of the rat and the dog when compared to the human *in vitro* samples.

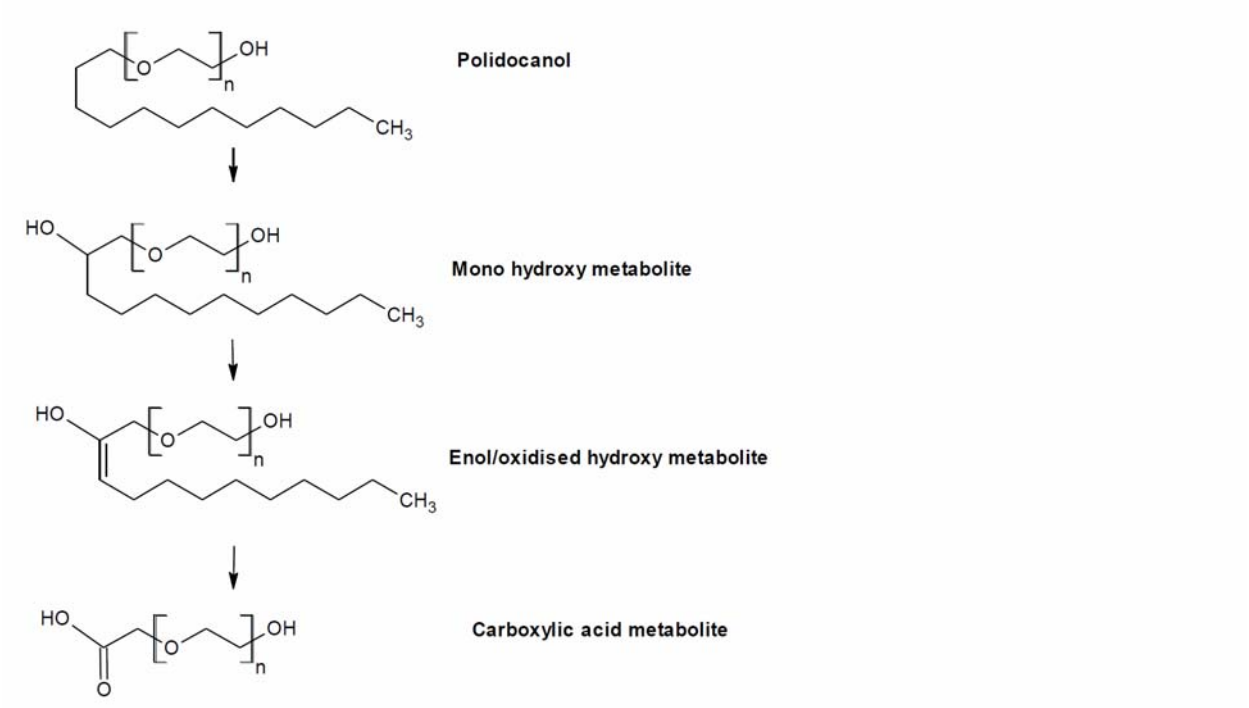
[^{14}C]-Polidocanol + Rat Microsomes - Incubation Time 120 minutes: Extracted Ion Chromatogram Showing Series of Hydroxylated [14C]-polidocanol Metabolite Masses (study 219463)



Postulated biotransformation of [^{14}C]-Polidocanol

A postulated metabolic pathway for [^{14}C]-polidocanol is presented in the following scheme based on the data obtained in this study and extrapolating to the observed metabolism *in vivo* determined in study 169771. The initial stage of metabolism is the hydroxylation of the dodecyl chain producing the hydroxyl metabolite which was observed in all species but which was more prevalent in rat and dog. Further hydroxylation and elimination furnishes the oxidized hydroxyl (keto/enol) metabolite series prevalent in the human microsome samples. The carboxylic acid metabolite series determined in study 169771 would be obtained by oxidative cleavage of the double bond of the enol metabolite (oxidized hydroxyl metabolite). This carboxylic acid metabolite series was not observed in any of the *in vitro* species samples analyzed under this study (219463). This was deemed to be due to the fact that metabolism had not proceeded to the same extent in microsomes as was observed in rat and dog *in vivo* (study 169771). While quantitative differences in the metabolism were observed between the 3 species, the metabolic pathway was qualitatively similar in each species. It was not possible to determine if the quantitative differences were genuine species differences or were a consequence of difference in the metabolic competency of the microsomal preparations.

Proposed metabolism of polidocanol (representative structures depicted)



Conclusions
The results indicated metabolism of [^{14}C]-polidocanol occurred in microsomes incubated for 120 minutes in the presence of co-factors. Only the cluster of peaks corresponding to [^{14}C]-polidocanol oligomers was detected in the T0 samples. Very little, or no metabolism of [^{14}C]-polidocanol was observed when microsomes were incubated without co-factors. These results suggested that oxidative metabolism was a key metabolic process. While some oxidative metabolism was observed in the S9 fractions this was limited, with only trace evidence of metabolism in the human S9 fraction.

Excretion

The disposition of [^{14}C]-polidocanol in the rat following intravenous administration (Study 167706) (GLP)

Study Objective
This study was conducted to examine the excretion and plasma kinetics of total radioactivity following intravenous administration of [^{14}C]-polidocanol to male Sprague Dawley rats. The nature of radioactivity present in plasma and urine was also investigated.

Study Design
Each animal received a single intravenous administration of [^{14}C]-polidocanol at a target dose level of 3 mg/kg.

Following dose administration to 5 rats in phase 1 of the study, samples of urine, feces and expired air (2 animals only) were collected at intervals up to 168 hours post-dose.

Following dose administration to 5 rats in phase 2 of the study, serial plasma samples were collected at intervals up to 48 hours post-dose.

Following dose administration to 4 rats in phase 3 of the study, 2 animals were killed at 0.25 hours and 1 hour post-dose and a maximal sample of plasma obtained for chromatographic analysis.

Levels of total radioactivity were determined in each sample collected.

Urine samples collected at 6 hours and 24 hours post-dose, fecal samples collected for the period 0-24 hours post-dose and plasma samples collected at 0.25 hours and 1 hour post-dose were analyzed by High Performance Liquid Chromatography (HPLC).

Results

Excretion of total radioactivity
The cumulative recovery of total radioactivity following intravenous administration of [14C]-polidocanol to male rats is shown in the next two Tables.

Reference ID: 3367720

Cumulative excretion of total radioactivity (including expired air) following a single intravenous administration of [^{14}C]-polidocanol to male rats (expressed as % administered dose) (study 167706)

Urine	Animal 1	Animal 2	Mean
6 hours	35.52	27.97	31.74
24 hours	56.43	53.64	55.03
48 hours	57.36	54.88	56.12
72 hours	57.67	55.20	56.43
96 hours	57.88	55.38	56.63
120 hours	58.04	55.50	56.77
144 hours	58.23	55.61	56.92
168 hours	58.37	55.69	57.03

Carcass	Animal 1	Animal 2	Mean
168 hours	3.13	3.05	3.09

Total	Animal 1	Animal 2	Mean
6 hours	35.52	27.97	31.74
24 hours	93.13	92.92	93.02
48 hours	96.16	96.71	96.44
72 hours	96.90	97.67	97.29
96 hours	97.21	98.00	97.61
120 hours	97.45	98.21	97.83
144 hours	97.74	98.40	98.07
168 hours	101.39	102.00	101.69

Feces	Animal 1	Animal 2	Mean
24 hours	27.32	30.88	29.10
48 hours	28.46	32.17	30.32
72 hours	28.64	32.49	30.56
96 hours	28.74	32.63	30.69
120 hours	28.80	32.72	30.76
144 hours	28.88	32.77	30.83
168 hours	28.94	32.83	30.88

Cage wash	Animal 1	Animal 2	Mean
24 hours	1.11	0.11	0.61
48 hours	1.26	0.35	0.81
72 hours	1.26	0.35	0.81
96 hours	1.26	0.35	0.81
120 hours	1.28	0.36	0.82
144 hours	1.30	0.38	0.84
168 hours	1.36	0.39	0.88

Cumulative excretion of total radioactivity (excluding expired air) following a single intravenous administration of [^{14}C]-polidocanol to male rats (expressed as % administered dose) (study 167706)

Urine	Animal 3	Animal 4	Animal 5	Mean	SD
6 hours	10.33	31.23	30.65	24.07	11.90
24 hours	42.26	41.85	55.15	46.42	7.56
48 hours	43.56	42.90	56.34	47.60	7.58
72 hours	43.86	43.28	56.72	47.95	7.60
96 hours	44.07	43.51	56.97	48.18	7.61
120 hours	44.33	43.67	57.12	48.37	7.58
144 hours	44.52	43.82	57.36	48.57	7.62
168 hours	44.65	43.92	57.47	48.68	7.62
Feces	**Animal 3**	**Animal 4**	**Animal 5**	**Mean**	**SD**
24 hours	23.70	33.34	18.80	25.28	7.40
48 hours	28.39	34.81	20.57	27.92	7.13
72 hours	28.75	35.06	20.82	28.21	7.14
96 hours	28.98	35.18	20.94	28.37	7.14
120 hours	29.40	35.25	21.03	28.56	7.15
144 hours	29.67	35.30	21.13	28.70	7.13
168 hours	29.77	35.36	21.19	28.77	7.14

Cage wipe	Animal 3	Animal 4	Animal 5	Mean	SD
96 hours	NC	0.11	NC	0.11	NA
Cage wash	**Animal 3**	**Animal 4**	**Animal 5**	**Mean**	**SD**
24 hours	2.93	1.44	1.77	2.05	0.78
48 hours	3.28	1.56	1.91	2.25	0.91
72 hours	3.33	1.61	1.92	2.29	0.92
96 hours	3.47	1.61	1.92	2.33	1.00
120 hours	3.60	1.62	1.96	2.39	1.06
144 hours	3.63	1.62	2.00	2.42	1.07
168 hours	3.67	1.68	2.08	2.48	1.05
Cage debris	**Animal 3**	**Animal 4**	**Animal 5**	**Mean**	**SD**
168 hours	1.61	0.86	-	1.24	NA
GI tract	**Animal 3**	**Animal 4**	**Animal 5**	**Mean**	**SD**
168 hours	0.16	0.24	0.12	0.17	0.06

Reference ID: 3367720

Carcass	Animal 3	Animal 4	Animal 5	Mean	SD
168 hours	5.40	4.44	3.04	4.29	1.19
Total	**Animal**	**Animal 4**	**Animal 5**	**Mean**	**SD**
6 hours	10.33	31.23	30.65	24.07	11.90
24 hours	68.89	76.63	75.72	73.75	4.23
48 hours	75.23	79.27	78.82	77.77	2.21
72 hours	75.94	79.95	79.46	78.45	2.19
96 hours	76.52	80.41	79.83	78.92	2.10
120 hours	77.33	80.65	80.11	79.36	1.78
144 hours	77.82	80.85	80.49	79.72	1.66
168 hours	85.26	86.61	83.90	85.26	1.36

NA = Not applicable, NC = Not collected, SD = Standard deviation

Following intravenous administration, the majority of the total radioactivity was eliminated within the first 24 hours post-dose. In all animals, the predominant route of excretion was via the urine. Mean cumulative recoveries of 55.03% of the administered dose for Animals 1 and 2 and 46.42% of the administered dose for Animals 3-5 being excreted by 24 hours post-dose. At 168 hours post-dose, the mean cumulative recovery of total radioactivity in the urine was 57.03% (Animals 1 and 2) and 48.68% (Animals 3-5) of the administered dose.

Excretion of total radioactivity via feces was also rapid but proportionately lower than urine with cumulative means of 29.10% (Animals 1 and 2) and 25.28% (Animals 3-5) of the administered dose being excreted via this route at 24 hours post-dose. At 168 hours post-dose, the mean cumulative recovery of total radioactivity in feces was 30.88% (Animals 1 and 2) and 28.77% (Animals 3-5). The excretion of total radioactivity via feces was considered likely to be due to the biliary elimination of the test material. Radioactivity detected in the gastrointestinal tract and residual carcass represented 3-5% of the administered dose.

A significant proportion of radioactivity was eliminated through expired air, accounting for a cumulative mean of 9.49% of the administered dose at 72 hours post-dose, suggesting that a portion of the test material may be metabolically unstable.

Recovery of the dose was quantitatively complete with mean recoveries of 101.69% (including expired air) and 85.26% (excluding expired air) over the 168 hour collection period. The excretion of total radioactivity was rapid in all animals with means of 93.02% (Animals 1 and 2) and 73.75% (Animals 3-5) recovered in the first 24 hours post-dose.

Reference ID: 3367720

Nature of the radioactivity in urine and feces
Ether extracts of pooled urine contained 8.7% of the sample radioactivity. The extracted urine was reduced to dryness under a stream of nitrogen and reconstituted in a mixture of methanol, dichloromethane and hexane. This extract contained 27.7% of the sample radioactivity. The ether extract was analyzed by HPLC and the chromatogram was consistent with the chromatogram obtained from [^{14}C]-polidocanol, confirming the principle that ether will extract unchanged parent material. The second extract of urine contained multiple components with retention times between approximately 25 minutes and 80 minutes. These retention times were later than those observed for [^{14}C]-polidocanol and this suggests that these metabolites were more polar than polidocanol oligomers. This was further supported by the recovery of radioactivity from the HPLC column. The column recovery of the ether extract was 65.3% whereas the column recovery of the second extract was 31.8%. This confirmed that the second extract contained more polar metabolites and some of this polar material was not eluted from the HPLC column.

Ether extracts of pooled feces samples contained 23.5% of the sample radioactivity. The extracted residue was further extracted with acetonitrile followed by methanol, and these extracts contained 16.9% and 17.9% of the sample radioactivity, respectively. The radio-HPLC chromatogram obtained from ether extracts contained multiple components with retention times ranging from 2.5 minutes to 50 minutes. Components with retention times of less than 25 minutes were considered to be consistent with polidocanol oligomers. The radio-HPLC chromatograms generated from acetonitrile and methanol extract of feces contained multiple components which were more polar than polidocanol oligomers. The recovery of radioactivity from the HPLC column during the analysis of ether, acetonitrile and methanol extracts was 56.5%, 35.6%, 22.6%, respectively. This supported the conclusion that the more polar solvents extracted metabolites of increasing polarity from the fecal matrix and some of the extracted polar material was not eluted from the HPLC column.

Conclusions
Following intravenous administration of ^{14}C]-polidocanol to rats at 3 mg/kg, radioactivity was rapidly excreted with the majority of the dose eliminated by 24 hours post-dose. The major route of excretion was via urine (44-58%), although biliary elimination via feces (21-35%) was also significant. Elimination of radioactivity as volatile components in expired air represented approximately 10% of the administered dose. Low levels of radioactivity (3-5%) were retained in the carcass of animals at 168 hours post-dose. Chromatographic analysis of urine and feces indicated extensive metabolism of the administered dose into more polar components prior to excretion. Only low quantities of radioactivity in urine (approximately 4% of the administered dose) were present as unchanged [^{14}C]-polidocanol.

Reference ID: 3367720

The disposition of [^{14}C]-polidocanol in the dog following intravenous administration (Study 167711) (GLP)

Study Objective

This study was conducted to provide information on the distribution, metabolism and excretion of polidocanol in the dog following intravenous administration of [$_{14}$C]-polidocanol. The nature of the radioactivity in samples of excreta and plasma was also examined by chromatographic techniques.

Study Design

Four male beagle dogs received a single intravenous administration (10 minute infusion) of [^{14}C]-polidocanol at a target dose level of 2 mg/kg. Urine was collected quantitatively for the periods 0-6 hours and 6-24 hours post-dose and then at 24 hour intervals until 168 hours postdose. Feces were collected quantitatively at 24 hour intervals up to 168 hours post-dose. At the time of each daily collection, any debris (wasted food) was separately removed and the cages washed. The cage wash and any debris were retained.

Whole blood samples were removed from a jugular vein into heparinized tubes at predose, 0 minute (immediately post infusion), 5 minutes, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 48, 72, 96, 120, 144 and 168 hours post-dose. A sample of whole blood was also retained. Plasma was separated from the remaining sample by centrifugation and the blood cells discarded.

At approximately 5 and 15 weeks post-dose, one animal was humanely killed and retained frozen prior to analysis under (b) (4) Project No. 169572. The results of these analyses are reported in the Distribution section above. Plasma and whole blood kinetics are also reported in the same section (study 167711).

Results

The cumulative recovery of total radioactivity following intravenous administration of [^{14}C]-polidocanol to male dogs is summarized below.

Reference ID: 3367720

Cumulative excretion of total radioactivity following single intravenous administration of [^{14}C]-polidocanol to male dogs (expressed as % administered dose) (mean, n=4) (study 167711

Urine	Mean	SD
6 hours	46.74	4.21
24 hours	60.97	3.37
48 hours	62.29	3.53
72 hours	62.80	3.46
96 hours	63.04	3.39
120 hours	63.19	3.36
144 hours	63.33	3.37
168 hours	63.41	3.35
Feces	**Mean**	**SD**
24 hours	15.94	3.36
48 hours	20.94	1.58
72 hours	21.42	1.51
96 hours	21.65	1.47
120 hours	21.74	1.47
144 hours	21.81	1.47
168 hours	21.85	1.47

Cage wash	Mean	SD
24 hours	1.40	1.56
48 hours	1.84	1.92
72 hours	2.15	2.27
96 hours	2.40	2.26
120 hours	2.56	2.29
144 hours	2.71	2.24
168 hours	3.20	2.58
Total	**Mean**	**SD**
6 hours	46.74	4.21
24 hours	78.31	5.07
48 hours	85.07	2.09
72 hours	86.36	1.60
96 hours	87.09	1.45
120 hours	87.50	1.44
144 hours	87.84	1.55
168 hours	88.47	1.37

Following intravenous administration of [^{14}C]-polidocanol, the predominant route of excretion was via the urine accounting for a mean of 60.97% (range 56-65%) of the administered dose at 24 hours post-dose. Total radioactivity was excreted more slowly throughout the remainder of the urine collection period, with a mean cumulative recovery of 63.41% (range 59-67%) recovered by 168 hours post-dose.

Excretion of total radioactivity via feces was lower with a mean of 15.94% (range 11-18%) of the administered dose being excreted via this route at 24 hours post-dose. At 168 hours postdose, the mean recovery of total radioactivity in feces was 21.85% (range 21-24%). The excretion of total radioactivity via feces is likely to be due to the biliary elimination of the test material or related metabolites.

At 168 hours post-dose, the recovery of total radioactivity was not quantitatively complete (range 87-90%, mean value 88.47%). It is possible that some total radioactivity may have been excreted via expired air, and that a small portion of the dosed radioactivity may remain in the body at 168 hours post-dose. This was further investigated under study 169572.

Reference ID: 3367720

Radio-HPLC chromatograms were obtained from extracts of 0-6 hour urine in ether and in methanol. These contained multiple polar and non-polar peaks, some of which were consistent with the retention times seen in the parent material. This indicates the presence of unchanged and metabolized components in the urine sample.

Extracts of pooled feces (collected 0-24 hours post-dose) were obtained in ether, acetonitrile and methanol, which were all analyzed by HPLC. The extracts contained low amounts of radioactivity and components present were not detectable above background in the radio-HPLC chromatograms. The recovery of radioactivity from the HPLC column during analysis of the extracts indicated the presence of both polar and non-polar components.

Conclusions

Following intravenous administration of [^{14}C]-polidocanol to male dogs, the majority (approximately 60%) of the administered dose was eliminated via the urine. There was evidence of biliary elimination with approximately 22% of the administered dose being recovered in feces. Recovery of total radioactivity was 87-90% at 168 hours post-dose. Chromatographic analysis of urine showed the presence of components which co-chromatographed with polidocanol oligomers in the initial ether extractions. Further, more polar components were resolved in the second extract indicating that metabolism of polidocanol was occurring. The proportion of total radioactivity recovered in each extract of the feces samples also suggested the presence of polar material, likely to be due to the metabolism of the parent compound.

The disposition and distribution of [^{14}C]-polidocanol in the dog following intravenous administration (Study 169572) (GLP)

Study Objective

Plasma kinetics, metabolism and excretion of [^{14}C]-polidocanol in the dog were investigated in study 167711. The administered dose was not fully accounted for however, and a new study was conducted to provide additional information on the excretion of radioactivity and to investigate possible tissue retention of radiolabelled material following intravenous administration of [^{14}C]-polidocanol.

Study Design

Two male beagle dogs received a single intravenous administration (10 minute infusion) of [^{14}C]-polidocanol at a target dose level of 2 mg/kg. Following dose administration, samples of urine, feces and expired air (up to 24 hours post-dose) were collected from each animal and the levels of radioactivity present determined, at intervals up to 168 hours post-dose. The animals were sacrificed at 1 week and 2 weeks post-dose and a number of key tissues retained.

The following organs, tissues and body fluids were retained separately from each animal and analyzed for total radioactivity: Blood, plasma, kidney, renal fat, liver, subcutaneous fat, lung and residual carcass.

Reference ID: 3367720

Results

The recovery of total radioactivity following intravenous administration of [^{14}C]-polidocanol to male dogs is shown in below.

Cumulative excretion of total radioactivity following a single intravenous administration of [^{14}C]-polidocanol to male dogs (expressed as % administered dose) (study 169572)

Urine	Animal 1	Animal 2
24 hours	22.84	58.75
48 hours	23.63	60.20
72 hours	24.01	60.56
96 hours	24.17	60.76
120 hours	24.31	60.88
144 hours	24.48	61.00
168 hours	24.58	61.09
Subtotal	*24.58*	*61.09*

Cage wash	Animal 1	Animal 2
24 hours	0.33	0.25
48 hours	4.84	0.27
72 hours	4.89	0.29
96 hours	4.93	0.30
120 hours	5.06	0.32
144 hours	5.09	0.32
168 hours	5.40	0.33
Subtotal	*5.40*	*0.33*

Feces	Animal 1	Animal 2
24 hours	15.79	21.06
48 hours	20.62	23.61
72 hours	21.99	24.31
96 hours	22.48	24.67
120 hours	22.65	24.84
144 hours	22.82	25.04
168 hours	23.05	25.08
Subtotal	*23.05*	*25.08*

Expired air	Animal 1	Animal 2
6 hours	2.27	2.30
12 hours	0.83	0.15
18 hours	0.30	0.04
24 hours	0.21	0.02
Subtotal	*3.61*	*2.51*
Total	***56.64***	***89.01***

The rates and routes of excretion of total radioactivity were similar to that seen in the previous study (167711), however there was an incomplete urine collection from animal 1, which arose from a technical problem with the collection apparatus during the first 24 hours post-dose. Including radioactivity recovered in expired air, the resulting recovery of total radioactivity at 168 hours was approximately 57% and 89% of the administered dose for animals 1 and 2, respectively. Recovery in the first study (167711) where no expired air was collected ranged from 87-90% of the administered dose.

Urine excretion accounted for 24.6% and 61.1% of the administered dose in animals 1 and 2, respectively, compared to 59-67% in the first study (167711). The majority of this (approximately 59% in Animal 2) was recovered in the first 24 hours post-dose. Excretion of total radioactivity via feces was lower accounting for approximately 23%

Reference ID: 3367720

and 25% of the administered dose, compared to 21-24% in the first study (167711). The excretion of total radioactivity via feces is likely to be due to the biliary elimination of the test material or related metabolites.

Excretion of total radioactivity via expired air was relatively low, accounting for 3.6% and 2.5% of the administered dose for animals 1 and 2, respectively. The majority (approximately 2.3%) was recovered in the first 6 hours post-dose.

Conclusions

The excretion pattern observed in this study was essentially the same as seen previously in study 167711, although some loss of urine occurred in animal 1. Of the radioactivity not recovered in excreta by 168 hours post-dose, approximately 3% can be accounted for as radioactivity excreted via expired air, probably as $^{14}CO_2$, suggesting that up to 93% of the dose could be excreted by 7 days post-dose.

The tissue distribution of total radioactivity in the dog following intravenous administration of [^{14}C]-polidocanol microfoam (Study 175188) (GLP)

Study Objective

This study was conducted to examine the tissue distribution and excretion of total radioactivity
following intravenous administration of [^{14}C]-polidocanol microfoam to Beagle dogs.

Study Design

Twelve male dogs each received a single intravenous administration of [^{14}C]-polidocanol microfoam at a target dose level of 2 mg/kg polidocanol.

The microfoam was prepared as follows: [^{14}C]-polidocanol (0.110 g) and non-radiolabelled polidocanol (2.1379 g) were dissolved in ethanol (9 mL). Phosphate buffer was added to obtain a final target concentration of 1% polidocanol. Equal volumes of the solution (15 mL) were transferred into microfoam generating canisters. Canisters were pressurized for 30 seconds using a gas mixture of (b) (4) . Density of the microfoam produced from each canister was determined gravimetrically. Only canisters that produced microfoam within the density range (b) (4) were used in the study.

The dose was administered by bolus injection via the cephalic vein. Target dose volumes were adjusted according to the microfoam density in order to obtain a target dose level of 2 mg/kg polidocanol. Dose volumes were in the range of 1.38-1.69 mL/kg.

Two animals were killed at each of the following time-points:

1, 24 and 96 hours post-dose and 1, 2 and 4 weeks post-dose

Reference ID: 3367720

The following organs, tissues and body fluids were retained for each animal:

Blood, lungs, heart, plasma, injection site (vein and surrounding tissues), renal fat, kidneys, spleen, liver, subcutaneous fat

Following dose administration, urine and feces were collected daily from animals scheduled for the 168 hours post-dose sacrifice. Cages were washed at each collection time and the wash retained.

Levels of total radioactivity were measured in all samples collected.

Results
Tissue distribution data are presented in the Distribution section (study 169572).

The cumulative excretion of total radioactivity is summarized below.

Cumulative excretion of total radioactivity following single intravenous administration of [^{14}C]-polidocanol microfoam to male dogs (expressed as % administered dose) (study 175188)

Sample	Time-point	Animal 7	Animal 8	Mean
Urine	24 hours	62.07	50.70	56.38
	48 hours	63.26	52.12	57.69
	72 hours	63.86	52.57	58.21
	96 hours	64.32	52.84	58.58
	120 hours	64.71	53.00	58.86
	144 hours	64.88	53.08	58.98
	168 hours	65.03	53.21	59.12
Feces	24 hours	25.81	20.57	23.19
	48 hours	28.21	24.65	26.43
	72 hours	29.00	25.73	27.37
	96 hours	29.75	26.25	28.00
	120 hours	30.20	26.72	28.46
	144 hours	30.43	26.91	28.67
	168 hours	30.68	27.08	28.88
Cage wash	24 hours	2.20	1.87	2.04
	48 hours	2.87	2.27	2.57
	72 hours	3.10	2.39	2.74
	96 hours	3.27	2.54	2.91
	120 hours	3.34	2.61	2.98
	144 hours	3.39	2.66	3.02
	168 hours	3.89	2.97	3.43
Total		**99.60**	**83.26**	**91.43**

Reference ID: 3367720

Conclusions
The rates and routes of excretion of total radioactivity were similar to that seen when [^{14}C]-polidocanol was administered intravenously as a solution (Study 169572). The main route of excretion was via urine, with a mean of 59.1% of the administered dose recovered by 168 hours post-dose. Excretion was fairly rapid with a mean of 56.4% of the administered dose recovered in the first 24 hours.

The mean recovery of the administered dose in feces was 28.9% in the 0-168 hour post-dose period. Low levels of radioactivity (approximately 0.2% of the dose) were still excreted in feces at later time-points suggesting a slow rate of excretion of the residual drug-related radioactivity.

The mean recovery of total radioactivity at the end of the 168 hour period was 91.4%.

DISCUSSION AND CONCLUSIONS

Quantitative whole body autoradiography demonstrated that radioactivity derived from [14C]-polidocanol solution was distributed widely to all organs and tissues in the rat with peak tissue concentrations generally observed at the first time-point (5 minutes post-dose) (study 167690). Highest concentrations of total radioactivity were observed in the organs of elimination (liver and kidney), with peak concentrations in these tissues observed later than most other tissues (at 0.5 hours post-dose). Initially, elimination of radiolabelled components from most tissues was relatively rapid with concentrations in most tissues at approximately 10 % of peak concentrations by 24 hours post-dose. In some tissues, most notably in white fat, elimination of radiolabelled components was considerably slower. By 168 hours post-dose, concentrations were relatively low and in some cases below the limit of reliable detection. There were no obvious differences between radioactivity retention in tissues from albino or pigmented rats.

This profile was confirmed by combustion / scintillation counting analysis for rat liver kidney and fat samples taken at intervals up to 1 week after dosing (study 169609). The nature of the radioactivity was investigated by TLC separation and found to represent unchanged polidocanol and several more polar metabolites.

The distribution of [^{14}C]-polidocanol was similar when administered intravenously either as a microfoam or solution formulation to rats (study 171995).

Following intravenous administration of [^{14}C]-polidocanol solution to the dog, radioactivity was well distributed to the selected tissues with peak concentrations generally observed at the first time-point (1 week post-dose) (study 169572). Tissue concentrations were significantly higher than the concentrations present in whole blood and plasma. By approximately 5 weeks postdose, concentrations of radioactivity were much lower in all tissues measured, and by 15 weeks post-dose, all tissues were at or below the limit of reliable determination.

Reference ID: 3367720

Radioactivity was also shown to be well distributed in tissues following intravenous administration of [^{14}C]-polidocanol to dogs as a microfoam (study 175188). Total radioactivity levels were higher in organs associated with elimination (liver and kidneys). Subcutaneous and renal fat were shown to retain some radioactivity. Radioactivity was still present in some tissues at low levels for 5 weeks after dosing and only went below or approached the set limit of quantification at about 22 weeks after dose administration.

Plasma protein equilibrium studies indicated that the binding of radioactivity associated with polidocanol was relatively low and decreased with increasing concentration (study 167727). The extent of protein binding also varied between species and was higher in the dog than in the rat and man. Protein binding was ranked; rat < man < dog at the lower concentration and man < rat < dog at the higher concentration.

Whole blood:plasma ratio for total radioactivity was in the range 0.7 to 0.8 indicating that blood cells had a lesser affinity for the polidocanol material than plasma, even though plasma protein binding was not marked (study 167711).

Analysis of biological samples obtained from rats and dogs following intravenous administration of [^{14}C]-polidocanol, indicated that polidocanol is metabolized and metabolites with more polar characteristics are formed, giving rise to a very complicated chromatographic profile (study 169771). Mass spectrometry suggested that these metabolites comprised either of polyethylene glycol groups or acidic polyethylene glycol groups. These series consisted of species which were separated by 44 mass units, which would correspond to -CH_2-CH_2-O - moieties, an indication of metabolites related to polidocanol. It was apparent that polidocanol was metabolized to a series of progressively more polar products which were readily excreted.

The metabolism of polidocanol was further investigated in an *in vitro* metabolism study (study 790228). There was extensive time-dependant metabolism of [^{14}C]-polidocanol solution following incubation with rat, dog and human liver microsomal and S9 fractions in the presence of NADPH (study 790228). Using a qualitative assessment only, metabolism appeared to be more extensive with the human than animal species and microsomal metabolism was greater than S9 for each species. However, the chromatographic profile of [^{14}C]-polidocanol was difficult to interpret and, therefore, it was not possible to determine and compare the rate of metabolism and the metabolite profiles in the different species and tissue fractions employed.

The rat, dog and human microsomal and S9 samples from study 790228 were further analyzed by radio-HPLC using reverse phase methods to facilitate metabolite identification by $LCMS_n$ (study 219463). The results indicated metabolism of [^{14}C]-polidocanol occurred in microsomes incubated for 120 minutes in the presence of co-factors. Very little, or no metabolism of [^{14}C]-polidocanol was observed when microsomes were incubated without co-factors indicating that oxidative metabolism was a key metabolic process. Metabolism was suggested to proceed via non-specific hydroxylation of the dodecyl chain with further hydroxylation and elimination to form the oxidized hydroxyl (keto/enol) metabolite series for polidocanol oligomers. The carboxylic

Reference ID: 3367720

acid metabolite series identified in study 169771 would be formed by oxidative cleavage of the double bond of the enol metabolite (oxidized hydroxyl metabolite). This carboxylic acid metabolite series was not observed in any of the *in vitro* samples analyzed. This was deemed to be due to the fact that metabolism had not proceeded to the same extent in microsomes as was observed in rat and dog *in vivo* (study 169771). The metabolism of [^{14}C]-polidocanol was qualitatively similar in each species with some quantitative differences observed between the 3 species. Due to the oligomeric nature of polidocanol and its metabolites and the analytical challenges this posed, it was not possible to determine if the quantitative differences were genuine species differences or were a consequence of differences in the metabolic competency of the microsomal preparations. Despite the analytical challenges of quantifying polidocanol metabolites, it was concluded that the interspecies profiles were sufficiently similar to qualify the toxicology species choice.

Excretion of the various metabolites from the body was shown to be via the kidney, liver (bile) or by expiration since radioactivity was detected in urine, feces and the breath of rats (study 167706) and dogs (study 169572, study 167711, study 175188). The predominant route of excretion was via the urine followed by feces and then expired air. Low levels of radioactivity were detected in the gastrointestinal tract and residual carcass. The majority of radioactivity in urine and feces was postulated to be polar metabolites of [^{14}C]-polidocanol. Recovery of radioactivity was never quantitatively complete at the end of study consistent with retention of a small amount of polidocanol-related material in the body for relatively long periods. In the dog, excretion patterns were shown to be similar for the solution and for the microfoam.

5.2 Toxicokinetics

(included in toxicity studies)

Reference ID: 3367720

6 General Toxicology

A program of studies was conducted to evaluate the toxicology of polidocanol solution and polidocanol endovenous microfoam (PEM). When injected into a vein, the microfoam formulation of polidocanol fills the entire vein, thus ensuring maximum contact between the active substance and the epithelial wall, resulting in denudation of the endothelium and collapse of the veins in contact with the microfoam. Hence, repeated intravenous dosing of the microfoam into the same position in the vein is not possible. Further, the physico-chemical properties of the microfoam formulation are such that it has proven physically impossible to administer polidocanol microfoam at dose levels where the adverse effects of polidocanol are known to occur, especially in rodent species. Consequently, the majority of the toxicological studies have been conducted using the polidocanol solution formulation, with appropriate bridging study to the microfoam formulation. PEM NF1, the intended commercial product, was used in one of the pivotal repeat dose toxicity studies in dogs (KMWW-0006). The levels of impurities in the batches of polidocanol drug substance used in the non-clinical toxicology studies were within the ICH controlled specification limits.

The toxicological program is described below and identifies the formulation(s) used in each study. All studies in the Table were conducted in accordance with GLP.

Toxicological program for PEM

Study type and duration	Study number(s)	Route of administration	Species	Formulation administered
Single dose toxicity	256916 257050	IV	Rat and dog	Microfoam
Dose range finding study (7 days)	660364	IV	Rat	Solution
Dose range finding study (3 days)	660390	IV	Dog	Solution
Repeat-dose toxicity (28 days)	660474 660495	IV	Rat and dog	Solution
Repeat dose toxicity (23 & 22 days)	662450 KMWW-0006	IV	Dog	Solution/microfoam (662450) & micro-foam (KMWW-0006)
Genotoxicity (in vitro)	765060 765076 765626	Not applicable	Bacterial and mammalian cells	Solution
Genotoxicity (in vivo)	765081	IV	Mouse	Solution

(cont.)

Reference ID: 3367720

Study type and duration	Study number(s)	Route of administration	Species	Formulation administered
Male fertility	493003	IV	Rat	Solution
Female fertility	493019	IV	Rat	Solution
Developmental toxicity	492869 493265	IV	Rat and rabbit	Solution
Peri and postnatal development	493134	IV	Rat	Solution
Local tolerance	577030	IV	Rabbit	Solution/microfoam
Antigenicity	833398	IP and IV	Guinea pig	Solution

6.1 Single-Dose Toxicity

Polidocanol microfoam: Evaluation of dosing regimes to administer polidocanol microfoam to rats (Study 256916) (GLP)

Objective
The objective of this study was to determine the tolerability of rats to the administration of polidocanol microfoam and to monitor acute responses to doses of microfoam equivalent to at least 3 mg/kg body weight and incremental doses up to a maximum tolerable dose.

Methods
In total, groups of three Sprague-Dawley rats were dosed intravenously with microfoam prepared locally from a 1% polidocanol solution made up in a sodium phosphate buffer containing 4% ethanol. The administration of polidocanol microfoam to rats was via an implanted femoral cannula with the tip of the cannula ending 30 mm cranial to the point of entry into the caudal vena cava. As this was a preliminary investigation, the dosing schedule was adapted during the study based on the acute toxic responses noted following treatment of the initial group. The initial dose volume was 0.5 mL microfoam. The gas phase of the microfoam comprised (b) (4)

Respiratory rate and respiratory pattern were recorded for all animals both prior to dosing and at regular post-dosing intervals. ECG recordings were taken for some animals.

Results
Initially 4 different dose levels (Groups 1-4) were employed, however as a consequence of the number of deaths observed, the study was extended to include another 2 dose levels (Groups 5 and 6). The dosing schedule and outcomes for Groups 1 to 4 are described as follows: The dosing schedule and necropsy findings for Groups 5 and 6 follow below.

Reference ID: 3367720

Treatment and outcome of rats dosed with polidocanol microfoam (study 256916)

Group no.	Animal no.	Microfoam dosed (mL)	Total volume administered (mL)	Animal body weight (g)	Microfoam (mg/kg)	Outcome	Mortality rate (%)
1	1	0.5 x 1	0.5	289.5	0.218	Died 1 minute post dose	33 (1/3)
	2	0.5 x 1*	0.4	275.5	0.184	Completed	
	3	0.5 x 1	0.5	324.4	0.195	Completed	
2	4	0.25 x 2	0.5	241.7	0.262	Died 2 minutes post second dose	100 (3/3)
	5	0.25 x 2	0.5	283.7	0.223	Died 3 minutes post second dose	
	7	0.25 x 1	0.25	279.3	0.113	Died 5 minutes post dose	
3	6	0.1 x 4	0.4	276.3	0.242	Died 5 minutes post fourth dose	67 (2/3)
	8	0.1 x 4	0.4	278.7	0.240	Died 2.5 minutes post fourth dose	
	9	0.1 x 5	0.5	261.8	0.319	Completed	
4a	10	0.08 x 2	0.16	323.0	0.043	Died 0.5 minutes post second dose	100 (1/1)
4b	11	0.05 x 2	0.1	289.0	0.030	Completed	0 (0/2)
	12	0.05 x 2	0.1	305.0	0.029	Completed	

Group no.	Animal no.	Microfoam dosed (mL)	Total volume administered (mL)	Animal body weight (g)	Microfoam (mg/kg)	Necropsy findings
5	13	0.25 x 2	0.5	382	0.134	No abnormalities detected
	14	0.25 x 2	0.5	371	0.138	No abnormalities detected
	15	0.25 x 2	0.5	371	0.138	No abnormalities detected
6	16	0.1 x 5	0.5	365	0.019	Reddening of the lungs
	17	0.1 x 5	0.5	371	0.019	Reddening of the lungs
	18	0.1 x 5	0.5	325	0.021	Reddening of the lungs. Pelvic dilation in te right kidney. Abnormal shaped heart.

Seven of the 12 rats dosed in Groups 1 to 4 died very soon after dosing. The remaining 5 survived the dosing and were euthanized at the time point dictated by the protocol. Signs of cardio-respiratory insufficiency, including cyanosis (blue-tinged mucous membranes) and increased respiratory rate were observed in rats surviving one dose increment. Even when these signs had abated before the administration of the next increment, survival rate was still reduced after administration of a further dose.

Due to the unexpected mortality rate the study was extended to include histopathological examination in an attempt to elucidate the cause of death. An additional 6 animals were dosed (Groups 5 and 6) using treatment levels that had previously resulted in death. No mortalities occurred in these additional animals, despite the fact that the same treatments had caused death in the first batch of animals. It was speculated that this difference was due to the inherent variability in dose administration and the heavier body weights of the second batch of animals (325-382g) compared to the first batch of animals (242-324 g). It was considered that larger animals were likely to be less susceptible to a given degree of blockade of the pulmonary circulation. It was noticeable however that all these animals showed signs of respiratory distress.

Reference ID: 3367720

No necropsy or histopathology findings were noted which could be related to treatment with the microfoam.

Conclusion
The administration of polidocanol microfoam to rats via an implanted femoral cannula could only be tolerated at a dose level that was difficult to administer with accuracy (0.1 mL administered as 0.05 mL x 2 injections). Higher dose levels resulted in several mortalities in the first phase of the study.

Based on the results of this small study and on morphological and morphometric considerations, the pulmonary vasculature of the rat appeared to be unsuitable for the safety evaluation of polidocanol microfoam. The study suggested that use of the femoral cannulated rat and probably similar sized species, for the investigation of the effects of polidocanol microfoam was inappropriate.

Assessment of effect of intravenous administration of 1% polidocanol microfoam to dogs to obtain MTD level (Study 257050) (GLP)

Objective
The objective of the study was to investigate clinical reactions following intravenous administration of 1% polidocanol microfoam to unconscious and then conscious dogs to determine the maximum tolerated dose (MTD) for subsequent toxicity studies.

Methods
Polidocanol microfoam was prepared locally from a 1% polidocanol solution made up in a sodium phosphate buffer containing 4% ethanol. The gas phase of the microfoam comprised (b) (4) The animals received weekly incrementally increasing amounts of the microfoam. Polidocanol microfoam was administered as a bolus injection either via the saphenous vein or the cephalic vein. The study was divided into 3 phases (described in the following 4 Tables). Phase I was conducted in unconscious dogs and was used to determine the dose level appropriate for administration to the conscious dogs. Phase II was conducted to establish the single dose MTD in conscious dogs, while Phase III was conducted to establish the maximum tolerated repeat dose in conscious dogs. Animals were observed for clinical signs. Blood samples were obtained via the jugular vein, pre-treatment and prior to necropsy for clinical pathology.

Reference ID: 3367720

Phase I - Treatment schedule (animals under anesthesia) (study 257050)

Animal No.	1% Polidocanol microfoam	Vessel injected	Day
1	0.67 mL/kg (total vol. 6.2 mL)	Right saphenous vein	1
	2.66 mL/kg (total vol. 25.3 mL)	Left saphenous vein	8
2	1.33 mL/kg (total vol. 11.4 mL)	Right saphenous vein	2
	3.33 mL/kg (total vol. 30.0 mL)	Left saphenous vein	9
3	2.0 mL/kg (total vol. 15.2 mL)	Right saphenous vein	2
	4.0 mL/kg (total vol. 31.2 mL)	Left saphenous vein	9

Phase II - Treatment schedule (conscious animals) (study 257050)

Animal No.	1% Polidocanol microfoam	Vessel injected	Day
4	2.0 mL/kg (total vol. 18.2 mL)	Right cephalic vein	1
	3.33 mL/kg (total vol. 31.0 mL)	Left cephalic vein	8
	4.66 mL/kg (total vol. 45.2 mL)	Left saphenous vein	15
	6.0 mL/kg (total vol. 60.0 mL)	Right saphenous vein	22
5	2.66 mL/kg (total vol. 26.1 mL)	Right cephalic vein	2
	4.0 mL/kg (total vol. 40.0 mL)	Left cephalic vein	9
	5.32 mL/kg (total vol. 53.7 mL)	Left saphenous vein	16

Phase III - Treatment schedule (conscious animals) (study 257050)

Animal No.	1% Polidocanol microfoam	Vessel injected	Day
6	5.0 mL/kg (total vol. 52 mL)	Left cephalic vein	1
	5.0 mL/kg (total vol. 54 mL)	Right cephalic vein	8
	5.0 mL/kg (total vol. 54.5 mL)	Left saphenous vein	15
	5.0 mL/kg (total vol. 55.5 mL)	Right saphenous vein	22
	5.0 mL/kg (total vol. 60 mL)	Tail vein	57
7	5.32 mL/kg (total vol. 53.2 mL)	Left cephalic vein	2
	5.32 mL/kg (total vol. 54.8 mL)	Right cephalic vein	9
8	4.0 mL/kg (total vol. 44 mL)	Left cephalic vein	1
	4.0 mL/kg (total vol. 44 mL)	Right cephalic vein	8
	4.0 mL/kg (total vol. 44.8 mL)	Left saphenous vein	15
	4.0 mL/kg (total vol. 46 mL)	Right saphenous vein	22

Summary of 1% polidocanol microfoam administration (study 257050)

Time in study	Phase I Animal Number			Phase II Animal Number		Phase III Animal Number		
	1	2	3	4	5	6	7	8
Week 1	0.67 mL/kg (9.2 kg)	1.33 mL/kg (8.6 kg)	2.0 mL/kg (7.6 kg)	2.0 mL/kg (9.1 kg)	2.66 mL/kg (9.8 kg)	5.0 mL/kg (10.4 kg)	5.32 mL/kg (10 kg)	4.0 mL/kg (11 kg)
Week 2	2.66 mL/kg (9.5 kg)	3.33 mL/kg (9.0 kg)	4.0 mL/kg (7.8 kg)	3.33 mL/kg (9.3 kg)	4.0 mL/kg (10 kg)	5.0 mL/kg (10.8 kg)	5.32 mL/kg (10.3 kg)	4.0 mL/kg (11 kg)
Week 3	-	-	-	4.66 mL/kg (9.7 kg)	5.32 mL/kg (10.1 kg)	5.0 mL/kg (10.9 kg)	-	4.0 mL/kg (11.2 kg)
Week 4	-	-	-	6.0 mL/kg (10 kg)	-	5.0 mL/kg (11.1 kg)	-	4.0 mL/kg (11.5 kg)
Week 5	-	-	-	-	-	-	-	-
Week 6	-	-	-	-	-	-	-	-
Week 7	-	-	-	-	-	-	-	-
Week 8	-	-	-	-	-	5.0 mL/kg (12.0 kg)	-	-

Results

In Phase I, 3 dogs were treated under anesthesia and the polidocanol microfoam administered ranged from 0.67 mL/kg to 4.0 mL/kg. Clinical signs were observed at the 3.33 mL/kg level although treatment at 4.0 mL/kg did not produce abnormal clinical signs.

In Phase II, 2 conscious animals were treated with weekly incrementally increasing amounts of polidocanol microfoam ranging from 2.0 mL/kg to 6.0 mL/kg. Minor clinical signs noted at 5.32 mL/kg were more marked at 6.0 mL/kg.

In Phase III, 3 further animals were treated for 4 consecutive weeks at 4.0 mL/kg, 5.0 mL/kg or 5.32 mL/kg. The animal treated with 5.32 mL/kg received 2 administrations before having to be removed from the study. The remaining 2 animals received 4 doses of 4.0 mL/kg or 5.0 mL/kg. The study indicated that the test item could be administered via the peripheral veins, via left and right cephalic vein and left and right saphenous vein.

Administration via the tail vein was not successful and the dog used had to be removed from the study. Following a single exposure the entire tail became inflamed and subsequently hardened with thrombus. Although this is the anticipated local reaction to the injection of microfoam, the rich vascularity of the tail led to microfoam becoming trapped throughout the tail and subsequent complete tail vein occlusion. This finding confirmed that this site was an unsuitable route for the administration of microfoam.

The left cephalic vein in one dog (Dog No. 6) had not recanalized after 8 weeks and further injections in that vein were not possible, indicating the efficacy of the polidocanol microfoam. It was concluded a maximum of 4 peripheral administration sites were available using the dog.

Reference ID: 3367720

Necropsy findings indicated reactive changes at the injection sites, vena cava and in some lymph nodes were related to treatment with polidocanol microfoam.

Histological findings of thrombus formation and changes in the endothelial cells lining the veins at the site of injection of polidocanol microfoam and also in the vena cava in some animals were recorded. Inflammatory reaction was apparent in tissues surrounding some veins.

A mild increase in urea levels was noted for Animals No. 7 and 8 following treatment with 1% polidocanol microfoam. However, there were no other related changes in markers of renal function. Animal No. 7 showed an increase in creatine phosphokinase following treatment suggestive of skeletal muscle damage. This may be related to the condition of the right paw which led to the premature removal of Animal No. 7 from the study.

Increased levels of white blood cells and neutrophils were noted in Animal No. 7 following treatment with the microfoam. This marked neutrophilia was probably related to the infected paw.

Animal No. 3 and Animal No. 6 both had mild neutrophilia which may have been related to mild infection.

There were no other significant changes in clinical pathology data to indicate systemic effects following treatment with 1% polidocanol microfoam.

Conclusion
1% polidocanol microfoam was administered as an intravenous bolus to both conscious and unconscious dogs to determine the MTD for subsequent toxicity studies. The microfoam was not trapped within the injected vein. This enabled microfoam to escape from the injection site and for the maximum systemic exposure to be achieved. No post-injection compression was employed during this study. The findings from this study indicated that the MTD for the conscious dog was 5.0 mL/kg per week when administered over a 4 week period into alternating saphenous and cephalic veins. The MTD for a single injection was 6.0 mL/kg.

6.2 Repeat-Dose Toxicity

Polidocanol 7 day intravenous infusion dose range finding study in rats (Study 660364) (GLP)

Objective
This study was conducted to investigate the toxicity of intravenous administration of polidocanol solution to rats for 7 days.

Reference ID: 3367720

Methods

Four groups of 5 male and 5 female Sprague-Dawley rats were administered 0, 5, 15 or 30 mg polidocanol/kg/day by intravenous (bolus) administration via a surgically implanted femoral vein catheter for 7 consecutive days. All the animals were observed at least twice daily and body weights and food consumption were recorded. Laboratory investigations of hematology and clinical chemistry were undertaken on Day 8.

Results

There were no clinical signs observed for animals in Groups 1, 2 or 3 (0, 5 and 15 mg/kg/day, respectively) that were considered to be related to treatment with polidocanol. For animals in Group 4 (30 mg/kg/day) subdued behavior, changes in respiration rate/pattern (slow, irregular, labored) and red staining on the animal's cage bedding were observed. Body weight and food consumption were unaffected.

There was a tendency towards a decrease in red blood cell parameters (Hb, RBC and Hct) and an increase in white blood cell parameters (MCHC, MCV, eosinophils and platelets) for animals dosed at 30 mg/kg/day (Group 4). There were no changes in the biochemical data that were considered to be related to treatment with polidocanol.

There were no organ weight changes noted that were considered to be related to treatment with polidocanol. There were no necropsy findings that were considered to be related to treatment with polidocanol.

To determine that 30 mg/kg/day was the maximum dosage level that could be successfully administered over 7 days, three additional dosage levels of 100, 60 and 45 mg/kg/day were given to single animals. All 3 additional dosage levels proved to be lethal: Animal 1 (100 mg/kg) died during dosing on Day 1 and Animals 42 (60 mg/kg) and 21 (45 mg/kg) were killed *in extremis* on Day 1 of dosing.

Conclusion

The daily intravenous (bolus) administration of polidocanol via a surgically implanted femoral vein catheter at a dosage level of 30 mg/kg for 7 consecutive days to Sprague-Dawley rats was associated with moderate clinical signs which included: subdued behavior, red staining on the animal's cage bedding and changes in respiration rate/pattern (slow, irregular, labored). There was also a tendency towards changes in a number of the hematological parameters (reductions in RBC, Hb and Hct and increases in white blood cell parameters (MCHC, MCV, eosinophils and platelets). Dosage levels of 5 and 15 mg/kg/day were not associated with any treatment related effects.

From the data obtained in this study it was recommended that the dosage levels for a subsequent 28 day toxicity study in the same species by this route of administration should not exceed 30 mg polidocanol/kg/day.

Reference ID: 3367720

Polidocanol: 28 day intravenous toxicity study in rats followed by a 14 day recovery period (Study 660474) (GLP)

Objective
This study was conducted to investigate the toxicity and toxicokinetic profile of polidocanol solution following daily intravenous (bolus) injections in Sprague-Dawley rats for a minimum of 28 consecutive days.

Methods
Fifteen male and 15 female (10 main study and 5 recovery) animals were assigned to each of 4 dosage groups and dosed at 0, 3, 9 and 27 mg polidocanol/kg/day for 28 consecutive days, via surgically implanted femoral vein catheters. The dosing period was followed by a 14 day post dose recovery period to determine the reversibility of any effects observed.

All the animals were observed at least twice daily for signs of ill health or reaction to treatment. In addition, on the days of treatment the animals were also observed immediately post dose and approximately 1 hour post dose. Body weights were recorded pre and post surgery and twice weekly thereafter, whilst food consumption was measured and recorded weekly. Ophthalmoscopy examinations were undertaken once pretrial, during Week 4 and Recovery Week 2. Laboratory investigations of hematology, clinical chemistry and urinalysis were undertaken at the end of the 28 day dosing period and 14 day recovery period. Blood samples for toxicokinetic investigations were obtained from all animals on Days 1 and 28.

On completion of either the 28 day dosing period, subsequent 14 day post dose recovery period or at the time of premature sacrifice, the animals were killed and subjected to a detailed necropsy and organ weight analysis. Groups 1 and 4 (0 and 27 mg/kg/day) were also subjected to a detailed histological evaluation. In addition, the vena cava of animals in Groups 2 and 3 was also evaluated.

Results
There were 7 mortalities during the course of the study, 6 of which were in Group 1 (0 mg/kg/day) and therefore were all considered to be procedural related and could not be attributed to treatment with polidocanol.

Subdued behavior and irregular respiration immediately post-dose and red staining on the animal's cage bedding approximately 1 hour post-dose were observed for the majority of the animals across the dose groups. Mastication, salivation and changes in respiration rate (labored and slow) were observed for the majority of animals in Groups 2, 3 and 4 (3, 9 and 27 mg/kg/day, respectively), immediately post-dose and were generally no longer evident 1 hour post-dose. These signs were infrequently observed for the Group 2 animals throughout the dosing period. There were a few incidences of what were considered to be possible convulsive episodes for animals throughout the treatment groups. The convulsive episodes were not considered to be related to treatment but possibly to the rate of injection (when undertaken manually) of the vehicle

Reference ID: 3367720

or test solutions. There were no effects on body weight or food consumption. There were no ophthalmoscopic changes following treatment with polidocanol.

A reduction in red blood cell parameters (Hb, RBC, Hct) for Group 4 (27 mg/kg/day) male animals was noted at the end of the 4 week treatment period. There were no hematological changes noted following the 2 week recovery period. There were no biochemical changes noted that were considered to be related to treatment with polidocanol. Blood pigments were noted to be present in the urine for animals in all treatment groups at the end of the 4 week treatment period. Following the 2 week recovery period, there was still evidence that blood pigments were present in the urine but the incidence and severity was decreased when compared to the main study values.

Toxicokinetic samples from Groups 2, 3 and 4 (3, 9 and 27 mg/kg/day) were analyzed for 4 oligomers of polidocanol (oligomers 5, 9, 12 and 14) on Days 1 and 28. At the timepoints analyzed (30 min, 1, 2, 4 and 24 h post-dose) the samples were found to give results below the lower limit of quantification for each of the oligomers: 4.977 and 7.465 µg/mL for Days 1 and 28, respectively for oligomer 5; 3.619 and 1.809 µg/mL for Days 1 and 28, respectively for oligomer 9; 2.933 µg/mL for oligomer 12 and 4.063 µg/mL for oligomer 14. Due to available sample volume, however, these lower limits of quantification were effectively higher for individual samples. Although no polidocanol peaks were observed in any of the samples obtained, with increased sample volume or at timepoints prior to 30 minutes post-dose, quantifiable data may possibly have been obtained.

There were no organ weight changes noted that were considered to be related to treatment with polidocanol.

There were no necropsy findings observed that were considered to be related to treatment with polidocanol. Histological changes including vasculitis, intimal proliferation and thrombus formation were apparent in the vena cava of the majority of animals. Minor increases in incidence and/or severity of vasculitis and intimal proliferation were apparent in some treated animals compared to the Control animals (Group 1). There were no consistent differences apparent between the Control and treated animals after the 14 day post-dose recovery period.

Conclusion

The daily intravenous (bolus) administration of polidocanol for 28 consecutive days at dosage levels of 9 and 27 mg/kg/day (Groups 3 and 4, respectively) was associated with moderate clinical signs which included: mastication, salivation and changes in respiration rate (labored and irregular). A dosage level of 3 mg/kg/day (Group 2) was associated with the same clinical signs, however, the incidences were infrequently observed. A dosage level of 3 mg/kg/day was considered to be the No Observed Adverse Effect Level (NOAEL).

Reference ID: 3367720

Polidocanol: intravenous infusion maximum tolerated dose study in dogs (Study 660390) (GLP)

Objective
This study was conducted to investigate the maximum tolerated dosage of polidocanol solution in the Beagle dog following intravenous (bolus) administration.

Methods
Two male and 2 female dogs were assigned to Groups 1 and 2. Group 1 animals (1 and 3) were given increasing doses at 1, 5, 10 and 15 mg polidocanol/kg/day, each for a 3 day period, to determine the maximum tolerated dose (MTD). A further dose at 20 mg/kg was given to each animal on a single occasion. The dogs were catheterized via the external jugular vein using a polyurethane cannula attached to a venous access port. The test material was administered as a solution by intravenous (bolus) injection via the venous access port. Each dose escalation was separated by a 1 day rest period. Group 2 animals (2 and 4) were given the maximum tolerated dosage, i.e. 15 mg/kg/day as determined by the Group 1 animals, for 7 consecutive days. The animals were observed at least twice each day for signs of ill health or reaction to treatment.

Body weights were recorded at regular intervals throughout the study, while food consumption was measured and recorded daily. Laboratory investigations of hematology and clinical chemistry were undertaken on all animals twice pretrial (pre and post surgery), after each dose level escalation for Group 1 animals and on Day 8 for Group 2 animals.

On completion of each phase of the study, the dogs were sacrificed and subjected to a detailed necropsy. Selected organs/tissues were also taken and weighed.

Results
No adverse reactions to treatment were noted for Group 1 animals dosed at 1 mg polidocanol/kg/day. Dosage levels of 5, 10 and 15 mg/kg/day were associated with retching and vomiting in both animals. A dosage level of 20 mg/kg was associated with severe clinical signs which included: ataxia, lying on pen floor, body tremors, head shaking, subdued behavior, salivation, licking of lips, retching and emesis. There was no effect on body weight or food consumption profiles.

Hematological and biochemical data were unaffected by treatment with polidocanol at dosage levels of 1, 5, 10 and 15 mg/kg/day. At a dosage level of 20 mg/kg/day, a slight reduction in red blood cell parameters (Hb, RBC) and an increase in potassium levels was noted for Animals 1 and 3 (Group 1).

There were no effects on organ weights and there were no necropsy findings noted that were considered to be related to treatment with polidocanol.

On the basis of the results obtained for Group 1, the MTD was considered to be 15 mg/kg/day. Clinical signs noted for Group 2 animals dosed for 7 consecutive days at

Reference ID: 3367720

this dose level included: licking of lips, retching, emesis, body tremors and increased respiration rate (Animal 2). Body weight and food consumption profiles were unaffected by treatment with polidocanol. There was no effect on hematological or biochemical parameters following treatment with polidocanol. There were no necropsy findings noted that were considered to be related to treatment with polidocanol.

Conclusion
The intravenous (bolus) administration of polidocanol at a dose level of 20 mg/kg (single administration) was associated with ataxia, body tremors, head shaking, lying on the pen floor, licking of lips, salivation, retching and emesis, and an increase in potassium levels. Dosage levels of 5, 10 and 15 mg/kg/day (3 and 7 day dosing periods) were associated with retching and emesis. The No Observed Effect Level (NOEL) for this study was considered to be 1 mg/kg/day. From the data obtained in this study, it was recommended that the dosage levels for a subsequent 28 day toxicity study in the same species do not exceed 15 mg polidocanol/kg/day.

Polidocanol: 28 days intravenous toxicity study in dogs followed by a 14 day recovery period (Study 660495) (GLP)

Objective
This study was conducted to investigate the toxicity and toxicokinetic profile of polidocanol solution following daily intravenous (bolus) injections in Beagle dogs for at least 28 consecutive days, via a surgically implanted venous access port.

Methods
Three male and 3 female Beagle dogs were each assigned to 4 dose groups that received 0, 2, 6 and 15 mg/kg/day (Groups 1, 2, 3 and 4, respectively) for 28 consecutive days via a surgically implanted venous access port. An additional 2 male and 2 female animals were assigned to Groups 1 and 4 and were retained for a 14 day post dose observation period to determine the reversibility of any effects observed.

All animals were observed at least twice daily for any signs of ill health or reaction to treatment. Body weights were measured twice weekly, while food consumption was measured and recorded daily. Ophthalmoscopy was undertaken once pretrial, towards the end of the dosing and post dose recovery periods. Laboratory investigations of hematology, clinical chemistry and urinalysis were undertaken twice pretrial (pre and post surgery) and towards the end of the dosing and recovery periods. Blood samples for blood level studies were obtained from all animals on Days 1 and 28 of the dosing period. On completion of the 28 day dosing period or subsequent 14 day post-dose observation period, all the animals were subjected to a detailed necropsy, organ weight analyses and a full histological evaluation.

Results
There were no adverse clinical signs observed for animals in Groups 1 and 2 (0 and 2 mg/kg/day, respectively). For animals in Groups 3 and 4 (6 and 15 mg/kg/day,

Reference ID: 3367720

respectively) body tremors, licking of lips, retching and emesis during and up to approximately 1 h post-dose were observed throughout the 28 day dosing period. There were no effects on body weight or food consumption profiles that were considered to be related to treatment with polidocanol. There were no ophthalmoscopy findings that were considered to be related to treatment with polidocanol.

There were no hematological or biochemical changes observed that were considered to be related to treatment with polidocanol.

Toxicokinetic samples obtained from Groups 3 and 4 (6 and 15 mg/kg/day, respectively) were analyzed for 4 oligomers of polidocanol (oligomers 6, 9, 12 and 14) on Days 1 and 28. At the timepoints analyzed (30 min, 1, 2, 4 and 24 hours post-dose) the samples were found to give results below the lower limit of quantification for oligomers 6 and 14 (2.898 and 3.074 µg/mL, respectively). A number of the Group 4 (15 mg/kg/day) samples at 30 minutes post-dose were found to be above the limit of quantification for oligomers 9 and 12 (1.825 and 1.479 µg/mL, respectively) on both Days 1 and 28. A small, non-quantifiable peak for oligomer 9 was, however, noted in the remainder of the 30 minute and some 1 hour samples. The trend in the size of these peaks would indicate that at timepoints prior to 30 minute post-dose, more quantifiable data may have been obtained.

There were no changes in the total nitrous oxide values that were considered to be related to treatment with polidocanol.

There was a reduction in lung weights for males in Group 4 and females in Groups 3 and 4, which was still evident in the Group 4 females after the 14 day post-dose observation period. However, in the absence of any absolute histological correlates these changes were considered to be equivocal. At necropsy the lungs of a number of animals after the 28 day treatment period and subsequent 14 day post-dose observation period showed pale or dark foci and lesions. These findings were not considered to be related to treatment.

Histological changes were noted in the lungs, jugular vein and vena cava. In the lungs, inflammatory changes (bronchopneumonia, interstitial pneumonia, inflammatory infiltrates) were apparent in a number of animals; both control and treated. These correlated with the findings noted at necropsy. Vascular thrombosis, unrelated to inflammatory change was seen in a few animals. It occurred in control animals as well as those treated with polidocanol. In the vena cava, vasculitis, intimal proliferation and thrombus formation were present in most animals from all groups in the study. In the jugular vein, vasculitis was present in most animals with thrombus formation in most females and a few males and intimal proliferation in one Group 4 female.

There was no apparent relationship to treatment with polidocanol for any of these histological changes. Similar changes were still apparent in Groups 1 and 4 after the 14 day post-dose recovery period.

Reference ID: 3367720

Conclusion
The intravenous (bolus) administration of polidocanol at dosage levels of 6 and 15 mg/kg/day (Groups 3 and 4, respectively) for 28 consecutive days was associated with moderate clinical signs which included: body tremors, licking of lips, salivation, retching and emesis. There were no histological changes noted which were considered to be related to treatment with polidocanol. A dosage level of 2 mg/kg/day was considered to be the No Observed Effect Level (NOEL).

Multiple dose intravenous toxicity study in dogs with 1% polidocanol microfoam (Study 662450) (GLP)

Objective
This study was conducted to investigate the toxicity and toxicokinetics of polidocanol microfoam following 4 weekly intravenous administrations. Two polidocanol solution groups were also incorporated into the study design to determine whether there were any differences between the polidocanol microfoam or solution formulations.

Methods
Three male and 3 female Beagle dogs were each assigned to 5 dose groups and received 4 intravenous injections at weekly intervals (into different legs on each occasion (via cephalic or saphenous vein)) of polidocanol microfoam or solution at dosage levels of 0, 2.25 (microfoam), 6.75 (microfoam), 2.25 (solution) and 6.75 (solution) mg/kg/dose (Groups 1, 2, 3, 4 and 5, respectively). An additional 2 males and 2 females were assigned to Groups 1, 3 and 5 and were retained for a 2 week post-dose observation period (Table 8). The first and fourth administration for all animals (with the exception of the Group 1 animals) contained [^{14}C]-polidocanol for toxicokinetic sampling analysis purposes.

Study design (study 662450)

Dose group	Treatment (mg polidocanol/kg/dose)	Dose volume (mL/kg)	Animal designations	Animal numbers	
				Male	Female
1 - control solution	0	0.675	Main	1-3	22-24
			Recovery	4-5	25-26
2 - microfoam (low)	2.25	1.5	Main	6-8	27-29
3 – microfoam (high)	6.75	4.5	Main	9-11	30-32
			Recovery	12-13	33-34
4 – solution (low)	2.25	0.225[a]	Main	14-16	35-37
5 – solution (high)	6.75	0.675	Main	17-19	38-40
			Recovery	20-21	41-42

Note: Dose levels assumed that 15ml 1% Polidocanol Solution = 100 ml Microfoam 1% Polidocanol Solution = 10 mg/mL

[a] = On Day 1, dose volume = 0.3 mL/kg which equated to a dose level of 3 mg/kg/dose rather than 2.25 mg/kg/dose

Reference ID: 3367720

In order to prepare the microfoam, polidocanol liquid was transferred to an aerosol canister and the following gas mixture added under pressure to form the foam: (b) (4)

All animals were observed at least twice daily for any signs of ill health or reaction to treatment. Body weights were recorded weekly, whilst food consumption was measured and recorded daily. Ophthalmoscopy examinations and laboratory investigations of hematology, clinical chemistry and urinalysis were undertaken once pretrial and at the end of the dosing and post dose observation periods. For the purpose of toxicokinetic investigations, [^{14}C] labelled polidocanol was administered for the first and fourth doses on Days 1 and 22 and serial blood samples obtained from all animals on Days 1 and 22 to determine polidocanol concentrations.

On completion of the last dose administration (approximately 24 hours post-dose) or at the end of the subsequent 2 week post-dose observation period, all animals were subjected to a detailed necropsy, organ weight analyses and a full histological examination.

Results

There was one death during the course of the study, Animal 15M (Group 4, 2.25 mg/kg solution) was humanely sacrificed on Day 7 following the appearance of adverse clinical signs which included lameness and subdued behavior. The adverse clinical signs and subsequent removal of the animal from the study was not considered attributable to treatment with polidocanol.

There were no adverse clinical signs in animals in Groups 1 and 4 (Control and 2.25 mg/kg solution). Emesis was recorded for the majority of the animals in Groups 2, 3 and 5 (2.25 and 6.75 mg/kg microfoam and 6.75 mg/kg solution, respectively). Retching, salivation and lip licking were also noted for animals in Groups 3 and 5, whilst pale mucous membranes, changes in respiration, ataxia and the inability to support own body weight (stand on all four limbs) were noted for animals in Group 3 only. The frequency and severity of the signs recorded was dose related and were more severe for the animals in Group 3.

Body weight and food consumption profiles were unaffected following treatment with polidocanol (microfoam or solution).
There were no ophthalmoscopy findings noted that were considered to be related to treatment with polidocanol (microfoam or solution).

There were no hematological, clinical chemistry or urinalysis changes noted that were considered to be related to treatment with polidocanol (microfoam or solution).

Toxicokinetic parameter estimation data showed that on both the first and fourth dose administrations, dose related and generally proportional increases were noted for estimates indicative of overall systemic exposure ($AUC_{(0-\infty)}$, $AUC_{(0-60\ min)}$ and Cmax) from dosage levels of 2.25 and 6.75 mg/kg/dose) in groups dosed with either the

Reference ID: 3367720

polidocanol microfoam or solution formulation. Systemic exposure was slightly lower in groups treated with polidocanol microfoam. Clearance was independent of the dose level administered and was slightly higher in microfoam treated groups. No accumulation of polidocanol microfoam or solution was observed following the fourth dose administration, suggesting that the kinetics of polidocanol remain unaltered following repeat weekly administrations.

There were no organ weight changes noted that were considered to be related to treatment with polidocanol.

Necropsy findings at the end of the dosing period included subcutaneous gelatinous thickening, dark, reddened, enlarged and prominent injection sites in all treated groups (microfoam and solution). Reddened injection sites were also noted for the Group 1 (Control) animals. Enlarged and reddened injection sites were still evident after the 2 week post dose observation period in the treated groups.

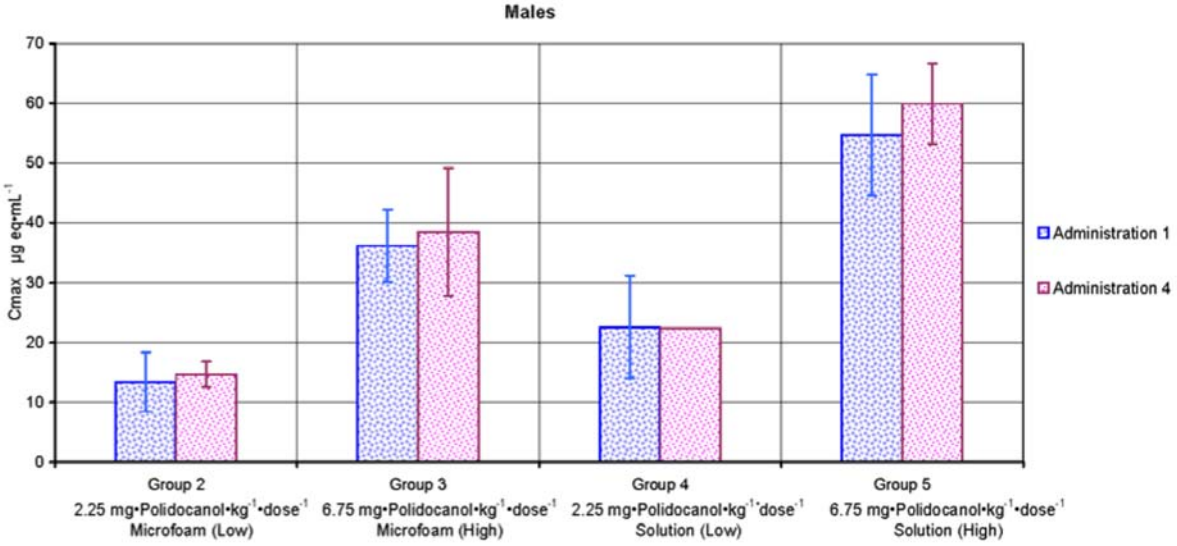
Histological findings noted that were considered to be related to treatment with polidocanol (microfoam or solution) included: thrombus formation, vasculitis and/or perivasculitis, and intimal proliferation. On completion of the 2 week post-dose observation period, thrombus formation, vasculitis and intimal proliferation were still evident.

Conclusion

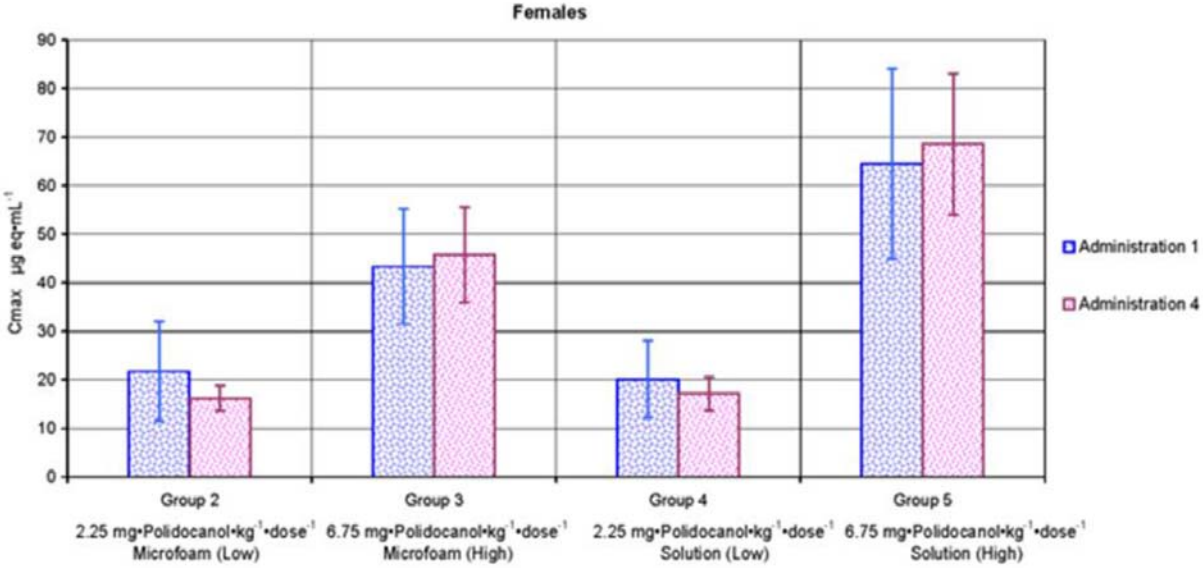
Once weekly intravenous administrations of polidocanol (microfoam or solution), for 4 consecutive weeks at dosage levels of 2.25 and 6.75 mg/kg/dose, was associated with clinical signs, which included: emesis, salivation, lip licking and retching. A dosage level of 6.75 mg/kg/dose with polidocanol microfoam was also associated with ataxia and changes in respiration and (for the majority of female animals) an inability to support their own body weight. Histologically, venous thrombosis, vasculitis and intimal proliferation in the vena cava were noted for animals dosed at 6.75 mg/kg/day polidocanol (microfoam and solution), which can be attributed to the sclerosing nature of polidocanol. Toxicokinetic data indicated that there are no differences in the profile of polidocanol with either the microfoam or solution formulation.

Reference ID: 3367720

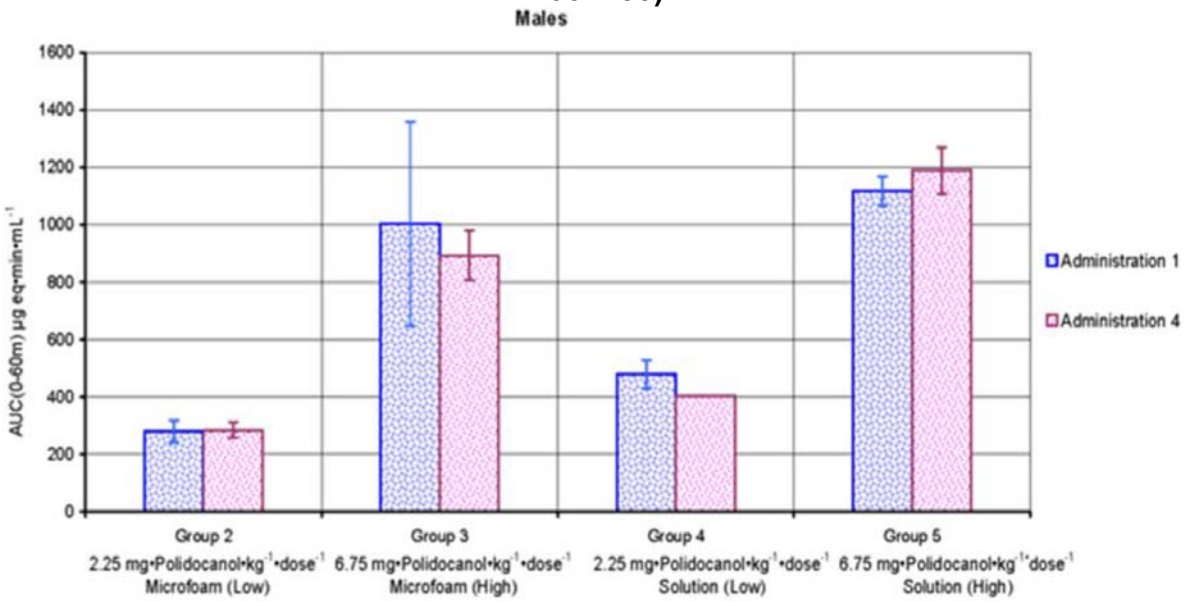
Comparison of [^{14}C]-polidocanol Cmax in plasma following intravenous injection of [^{14}C]-polidocanol microfoam or solution to the Beagle dog (study 662450)



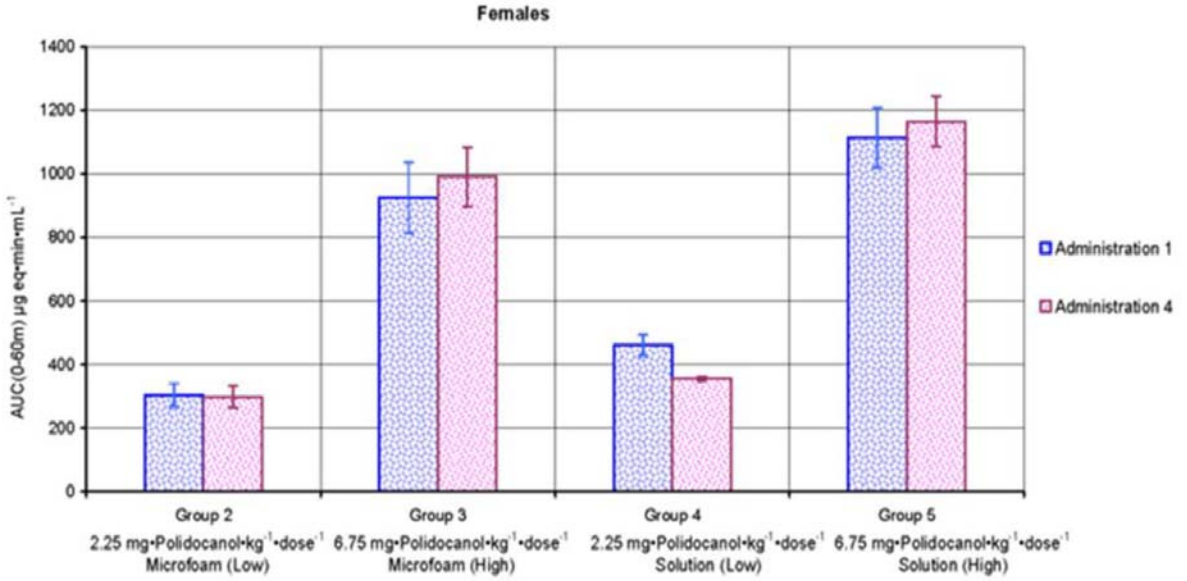
Comparison of [^{14}C]-polidocanol Cmax in plasma following intravenous injection of [^{14}C]-polidocanol microfoam or solution to the Beagle dog (study 662450)



Comparison of [^{14}C]-polidocanol AUC (0-60m) in plasma following intravenous injection of [^{14}C]-polidocanol microfoam or solution to the Beagle dog (study 662450)



Comparison of [^{14}C]-polidocanol AUC (0-60m) in plasma following intravenous injection of [^{14}C]-polidocanol microfoam or solution to the Beagle dog (study 662450)



A pilot dose range finding study of polidocanol-containing Varisolve (PEM) PD microfoam in male beagle dogs (Study KMWW-0007)

Objective

This was a pilot study to investigate the dose range of PEM that was tolerated by male beagle dogs following intravenous injection.

Methods

The gas mixture in the PEM contained oxygen, carbon dioxide, and less than 1% nitrogen. Two different vein models were used; an open vein model (i.e. non-occluded) and a closed vein model where the test article was injected after the vein was occluded through the use of an inflatable venous tourniquet or blood pressure cuff proximal to the site of injection. The vein was occluded for five minutes following the injection. Animals were dosed as shown below.

Dosing schedule (study KMWW-0007)

Group number	Number of males	Vein status	Polidocanol dose level	Dose volume	Dosing regimen	Necropsy day
1	1	Open	6.4 mg/kg	4 mL/kg	Left cephalic on day 1	Day 19
			9.6 mg/kg	6 mL/kg	Right saphenous on day 5	
			12.8 mg/kg	8 mL/kg	Right cephalic on day 8	
			16 mg/kg	10 mL/kg	Left saphenous on day 12	
2	1	Closed	1.6 mg	1 mL	Left cephalic on day 1	Day 19
			4.0 mg	2.5 mL	Right saphenous on day 5	
			8.0 mg	5.0 mL	Right cephalic on day 8	
			12.0 mg	7.5 mL	Left saphenous on day 12	

Group number	Number of males	Vein status	Polidocanol dose level	Dose volume	Dosing regimen	Necropsy day
3	1	Open	12.8 mg/kg	8 mL/kg	Left cephalic on day 15	Day 32
			12.8 mg/kg	8 mL/kg	Right saphenous on day 19	
			12.8 mg/kg	8 mL/kg	Right cephalic on day 22	
			12.8 mg/kg	8 mL/kg	Left saphenous on day 25	
4	1	Closed	12.0 mg	7.5 mL	Left cephalic on day 15	Day 32
			12.0 mg	7.5 mL	Right saphenous on day 19	
			12.0 mg	7.5 mL	Right cephalic on day 22	
			12.0 mg	7.5 mL	Left saphenous on day 25	

Reference ID: 3367720

The following parameters were monitored: mortality, clinical observations, administration site observations, hemoglobin oxygen saturation via pulse oximetry, body weight, clinical pathology (hematology, coagulation and serum chemistry), organ weights, macroscopic pathology and histopathology. The inflammatory response marker ICAM-1 was evaluated by immunohistochemistry. In addition, the concentrations of four characteristic oligomers of polidocanol (E5, E9, E12 and E14) were determined in dog plasma samples obtained at specific times points. A validated method (studies RD198/23887HV and RD198/23966GV), employing high performance liquid chromatography with tandem mass spectrometry detection (LC-MSMS) was used to analyze the samples.

Results

All animals survived until scheduled necropsy. Systemic exposure (i.e., open vein treatment) to the test article (9.6-16 mg/kg [6-10 mL/kg]) resulted in a variety of abnormal clinical signs, including vomiting, retching, ataxia, soft feces, lethargy, pale mucous membranes, and hematuria that resolved within approximately 20 minutes of dose administration. On Day 25, the open vein dog, Animal No. 3001, experienced a transient decrease in hemoglobin oxygen saturation, although the dog's activity may have hampered measurement. The most severe effects were observed following the 16 mg/kg (10 mL/kg) dose. There were no adverse clinical signs following closed vein administration of up to 12.0 mg (7.5 mL) of test article. Animals dosed in open veins experienced slight body weight loss (approximately -3%) and those dosed in closed veins gained weight (approximately 3%). Liver enzymes (ALT and AST) were elevated on Day 19 in the dog dosed with up to 16 mg/kg of test article to open veins. This animal exhibited distended hepatic sinusoids with minimal hemorrhage in sinusoidal and portal regions. Almost all of the treated veins were thrombosed at the time of necropsy regardless of dose or vein status (open or closed). The caudal and cranial vena cava were not thrombosed. The lungs of two animals had microgranulomas consistent with a foreign-body response likely due to embolization. The appearance of these emboli was not consistent with either gas emboli or thromboembolization, however, the source or nature of the material cannot be determined. ICAM-1 reactivity was identified in the inflamed and/or thrombosed segments of the treated saphenous and cephalic veins (and not in any other organs) in the two dogs that were screened after four injections of 12.8 mg/kg (8 ml/kg) of test article to an open vein or 12 mg (7.5 mL) of test article to a closed vein.

The concentration of four characteristic oligomers of polidocanol (E5, E9, E12 & E14) were determined in dog plasma samples. The analysis indicated that systemic exposure (as gauged by $AUC_{0-\infty}$) occurred in all of the dogs following intravenous injection and increased in animals as the polidocanol dosage increased on Day 12 relative to Day 1.

Reference ID: 3367720

A toxicity study of polidocanol-containing Varisolve® (PEM) NF1 microfoam in female beagle dogs (Study KMWW-0006) (GLP)

Objective

This study was conducted to investigate the toxicological potential and toxicokinetics of polidocanol following repeated intravenous administration of PEM NF1 microfoam to a non-occluded vein (i.e. an open vein) and an occluded vein, at least partially, by an inflatable blood pressure cuff placed proximally to the injection site for 5 minutes following each injection (i.e. a closed vein) over a 3 week dosing period and a 2 week recovery period in female beagle dogs.

Methods

The study consisted of three groups (Groups 2, 4, and 5) of four female beagle dogs each and three groups (Groups 1, 3, and 6) of six female beagle dogs each. In all dogs, doses were administered to the left cephalic, right saphenous, right cephalic, and left saphenous veins on Days 1, 8, 15, and 22, respectively. In Groups 1–3, the doses were administered via a slow bolus intravenous injection to a non-occluded (i.e. open) vein. In Groups 4–6, the doses were administered to a closed vein (i.e., occluded, at least partially, by an inflatable blood pressure cuff placed proximally to the injection site for 5 minutes following each injection). Control dogs in Group 1 received 0.9% saline at the dose volume equivalent to the liquid portion of the microfoam administered to Group 3. Dogs in Groups 2 and 3 were administered 1.44 mg/kg and 12.8 mg/kg polidocanol, respectively. Control dogs in Group 4 received 0.9% saline at a dose volume equivalent to that used in Group 6. Dogs in Groups 5 and 6 were injected with 6 and 12 mg of polidocanol, respectively. Four dogs per group were euthanized on Day 23; two dogs per group in Groups 1, 3, and 6 were euthanized on Day 36 after a two-week recovery period. The study design is shown below.

Reference ID: 3367720

Study design (study KMWW-0006)

Group Number	Number of Females	Vein Status	Test Article	Polidocanol Dosage Level[a]	Dosage Volume	Dosing Regimen	Necropsy Day
1	6	Open	0.9% Saline	0 mg/kg	1.2 mL/kg[b]	IV, Left Cephalic Vein on Day 1 IV, Right Saphenous Vein on Day 8 IV, Right Cephalic Vein on Day 15 IV, Left Saphenous Vein on Day 22	4 per sex per group on Day 23 and 2 per sex per group on Day 36
2	4	Open	Polidocanol in PEM NF1 microfoam	1.44 mg/kg	0.9 mL/kg		Day 23
3	6	Open	Polidocanol in PEM NF1 microfoam	12.8 mg/kg	8 mL/kg		4 per sex per group on Day 23 and 2 per sex per group on Day 36
4	4	Closed	0.9% Saline	0 mg	7.5 mL		Day 23
5	4	Closed	Polidocanol in PEM NF1 microfoam	6 mg	3.75 mL		Day 23
6	6	Closed	Polidocanol in PEM NF1 microfoam	12 mg	7.5 mL		4 per sex per group on Day 23 and 2 per sex per group on Day 36

IV = Intravenous
a These represent nominal dosages. The actual polidocanol dosage was calculated based on the density and volume of the foam that was used.
b Represents liquid (15%) component of foam.

The PEM NF1 microfoam two canister system was in a 'ready-to-use' form (product batches 466722 and 479832). Generation of the microfoam was performed just prior to dosing. Each syringe containing microfoam was used within 90 seconds of preparation.

Toxicity was assessed by monitoring clinical observations, administration site observations, ophthalmic and physical examinations, body weights, food consumption measurements, ECG measurements, pulse oximetry (to measure hemoglobin oxygen saturation), clinical pathology (hematology, blood coagulation, serum chemistry, and urinalysis), organ weights, and anatomic pathology. Immunohistochemistry was performed on selected tissues for detection of ICAM-1.

The toxicokinetics of polidocanol were determined following blood collection at a variety of postdose time points on Days 1 and 22. The concentrations of four characteristic oligomers (E5, E9, E12 and E14) were determined using a validated LC-MS-MS method (studies RD198/23887HV and RD198/23966GV). The individual oligomer concentrations were combined to calculate the total polidocanol concentration in each plasma sample.

Reference ID: 3367720

Results
All dogs survived until scheduled euthanasia on Days 23 or 36.

The most severe clinical observations were noted immediately after dosing (Days 1, 8, 15, and 22) in the Group 3 (12.8 mg/kg open vein) dogs. Systemic intravenous administration of PEM NF1 was associated with emesis immediately (1–20 minutes) post-dose in all of the Group 3 dogs. Four out of 6 dogs in Group 3 exhibited moderate to marked ataxia and were lethargic/prostrate for a few minutes following initial dosing on Day 1. In subsequent dosing administrations, at a slower infusion rate, moderate ataxia, lethargy, and a prostrate position occurred in one dog on one occasion and mild ataxia/lethargy occurred in another dog on one occasion. This indicated that the clinical signs observed in the high dose group were associated with rates of microfoam infusion more strongly than with the total dose. Cyanosis (usually indicated by discolored blackish blue tongue and/or gums) was observed within minutes of dose administration on some of the dosing days in the Group 3 dogs. Dilated pupils were observed post-dose in five of six Group 3 dogs on some of the dosing days. Salivation was also noted after dosing on some occasions in two of the Group 3 dogs. Moderate or marked soft feces that sometimes had a mucoid consistency were observed on some of the dosing days in three of the Group 3 dogs. Labored breathing was occasionally noted after dose administration in two Group 3 dogs. Apparent urinary blood was noted postdose in two Group 3 dogs.

One Group 3 dog experienced a more severe reaction to the test article than the others. In addition to the abnormal clinical observations already described above, this dog experienced convulsions after dosing on Days 1 and 8 and tremors on Days 8 and 15. This animal also vocalized on Days 1 and 2 and was noted to be prostrate and lethargic during the scheduled clinical observations on Day 2 (albeit not as severely as on Day 1). On Day 1, this animal experienced apparent respiratory and cardiac arrest (i.e., the dog presented with no pulse or breathing) following dose administration, but recovered following chest compressions and artificial respiration. On Day 8, this dog collapsed following administration of the test article at a rate of 0.5 mL/sec and was administered supplemental oxygen for approximately 10 minutes. In response to these episodes, the rate of dose administration was slowed to 0.25 mL/second for this animal on Days 15 and 22; the slower rate of dose administration was better tolerated by this animal. On Day 22, another Group 3 animal collapsed after dose administration and was administered supplemental oxygen until improvement was observed.

There were no abnormal clinical observations in the Group 1 (open vein) or Group 4 (closed vein) control dogs. Other test article-related abnormal clinical observations were infrequently observed in a few dogs in the other dose groups and included: labored breathing; moderately soft feces; partial or complete disuse of the left forelimb following dosing that resolved following topical application of DMSO and exercise; and apparent blood in the urine.

Many dogs, including the open vein control dogs, experienced episodes of swelling or erythema, usually of mild severity, at the injection sites following dose administration.

Reference ID: 3367720

There were no abnormal physical or ophthalmic examination findings that were related to polidocanol administration. Body weights and food consumption were unaffected by test article administration.

Electrocardiographs obtained on Days 23 and 36 were unaffected by prior polidocanol administration. Hemoglobin oxygen saturation measurements dipped below 80% at several time points in individual dogs (including some in the Group 1 open vein control group) following dose administration, with a general tendency for dogs in Group 3 to be more severely affected.

Intravenous administration of the test article on Days 1, 8, 15, and 22 did not adversely affect hematology, blood coagulation, serum chemistry, or urinalysis on Days 22 or 36.

Organ weights on Days 23 and 36 were unaffected by test article administration. Pathological effects of the polidocanol-containing microfoam were confined to thrombosis at the injection sites, an expected pharmacological effect of this test article. There were no systemic effects; therefore, under the conditions of this study, the anatomic pathology no observable effect level (NOEL) for polidocanol in PEM NF1 was determined to be 12.8 mg/kg polidocanol via open vein treatment and 12 mg polidocanol via closed vein administration to female beagles.

ICAM-1 immunoreactivity was increased in vascular and perivascular tissues on Day 23 at the right saphenous vein administration site in the dogs dosed with the 12.8 mg/kg polidocanol in PEM NF1 to an open vein. This vein served as the administration site on Day 8. Subsequent injections to the right cephalic vein on Day 15 and the left saphenous vein on Day 22 did not result in increased ICAM-1 immunoreactivity. Increased ICAM-1 immunoreactivity at the administration sites was not observed in any of the recovery dogs on Day 36.

The plasma polidocanol concentrations prior to dosing on Days 1 and 22 were below the detection limit in all dogs. The mean plasma polidocanol concentration-time profiles on Days 1 and 22 following intravenous administration of PEM NF1 via either open or closed vein systems were qualitatively similar. With the exception of the first 30 minutes on Day 1, the administration of 1.44 mg/kg to the Group 2 dogs (open vein) yielded higher mean plasma concentrations than administration of approximately 1.42 mg/kg (closed vein) to the Group 6 dogs. Maximal plasma polidocanol concentrations (Cmax) were attained at median times of 0 hours (extrapolated) in the open vein model and 4-5 minutes post-dose in the closed vein model and declined in a multi-phasic manner with mean terminal half-lives ranging from approximately 1 to 3.1 hours. Mean plasma polidocanol concentrations tended to be quantifiable through slightly later times (t_{last}) as the dosage increased and with repeated dosing. Median t_{last} ranged from 5 to 9 hours and from 2 to 4 hours, respectively, for the open and closed vein models. Cmax and AUC_{last} each increased in an apparent dosage proportional manner for both dosing regimens. After administration of comparable dosages of approximately 1.4 mg/kg, the AUC values for the Group 2 open vein dogs on Day 1 exceeded those of the Group 6

Reference ID: 3367720

closed vein dogs by approximately 40%. On Day 22, the AUC values in the Group 2 dogs exceeded those of the Group 6 dogs by nearly 80%. These data, along with the dosage-adjusted AUC values, suggest that overall systemic exposure was somewhat greater following administration of the test article to an open vein as compared to a closed vein. Repeated dosing had no notable effect on either mean Cmax or mean AUClast in either dosing regimen. The volume of distribution (Vz) and clearance (CL) values were comparable for both dosing models (i.e., all were differed by less than a factor of 2).

Conclusion

In some dogs, severe clinical observations followed the systemic administration of 12.8 mg/kg polidocanol. Abnormal administration site observations (swelling and/or erythema usually of mild severity) were often present after dosing in dogs in all test article-treated groups. Although these conditions reversed with time, under the conditions of this study, an overall no observable adverse effects level (NOAEL) could not be determined for either the open or closed vein routes of administration.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Polidocanol: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2*uvr*A

Study no.:	765060
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/31/2000
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch No. 82652, purity 99.0%

Key Study Findings: No mutagenic activity was observed in any of the 5 bacterial strains, in either activation condition. Toxicity was encountered in all strains except TA 1535, and was variable between the different strains. Concentrations inducing toxicity varied from 167 to 5000 μg per plate. No precipitation of the test material was observed. It was concluded that Polidocanol was not mutagenic to *Salmonella typhimurium* or *Escherichia coli*, in either the presence or absence of S9 mix, when tested at concentrations extending into the toxic range.

Reference ID: 3367720

Methods

Strains: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100
Escherichia coli WP2*uvr*A

Concentrations in definitive study: for TA 1535, TA 100 and WP2*uvr*A: 17, 50, 167, 500, 1667 and 5000 μg per plate.

for TA 1537 and TA 98: In the absence of S9 mix, the range was: 1.7, 5, 17, 50, 167, and 500 μg per plate.
In the presence of S9 mix, the range was: 5, 17, 50, 167, 500 and 1667 μg per plate.

Basis of concentration selection: toxicity

Negative control: water

Positive control: 2-aminoanthracene (2-AAN), sodium azide (NaN_3), 9-aminoacridine (9-AA), 2-nitrofluorene (2-NF) and N-ethyl-N-nitro-N-nitrosoguanidine (ENNG)

The positive control substances, except sodium azide, were dissolved in dimethylsulphoxide. Sodium azide was dissolved in sterile, ultra-pure water.

Formulation/Vehicle: water

Study Validity: Strains, incubations, and concentrations tested were all appropriate.

Results

Table 1 Toxicity Test

POLIDOCANOL - revertant colony numbers per plate using strain :- TA 100

Strain	Dose level μg per plate	S-9 mix	Individual revertant colony counts
TA 100	Solvent	-	90
	17	-	107
	50	-	91
	167	-	94
	500	-	112
	1667	-	57
	5000	-	18 STL
	Solvent	+	118
	17	+	109
	50	+	134
	167	+	121
	500	+	117
	1667	+	94
	5000	+	69

\+ With S9
\- Without S9
STL Slightly Thin Lawn

Table 2 First Mutation Assay

Mean Number of Revertant Colonies Per Plate in the Presence of S9 Mix (FLI 090).

Substance	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA (C)
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
WATER	100 µl	10 ± 2	10 ± 5	24 ± 8	102 ± 7	-
Polidocanol	17	10 ± 4	11 ± 4	18 ± 3	101 ± 1	-
	50	7 ± 3	7 ± 5	23 ± 5	98 ± 2	-
	167	7 ± 3	11 ± 2	17 ± 5	116 ± 13	-
	500	8 ± 2	6 ± 6	17 ± 1	86 ± 18	-
	1667	5 ± 1	9 ± 6 (TL)	8 ± 4 (STL)	72 ± 11	-
	5000	10 ± 4	7 ± 4 (TL)	13 ± 2 (TL)	64 ± 2 (STL)	-
Positive controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level µg per plate	2	2	0.5	0.5	20
	Mean ± S.D.	415 ± 39	356 ± 8	568 ± 9	1298 ± 25	-

S.D. Standard Deviation
2AAN 2-Aminoanthracene
TL Thin Lawn
STL Slightly Thin Lawn
C Contaminated

Table 2 (continued) First Mutation Assay

Mean Number of Revertant Colonies Per Plate in the Absence of S9 Mix.

Substance	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA (C)
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
WATER	100 µl	10 ± 4	9 ± 3	22 ± 6	102 ± 5	-
Polidocanol	17	12 ± 4	10 ± 2	16 ± 7	110 ± 2	-
	50	11 ± 5	7 ± 4	16 ± 7	86 ± 9	-
	167	10 ± 2	13 ± 3	8 ± 2	78 ± 13	-
	500	8 ± 3	2 ± 2 (TL)	10 ± 2 (TL)	67 ± 11	-
	1667	10 ± 2	0 ± 1 (TL)	0 ± 0 (TL)	60 ± 7	-
	5000	4 ± 2	0 ± 1 (VTL)	0 ± 0 (TL)	54 ± 4 (STL)	-
Positive controls	Compound	NaN_3	9AA	2NF	NaN_3	ENNG
	Dose Level µg per plate	1	80	1	1	2
	Mean ± S.D.	213 ± 14	5413 ± 171	409 ± 143	681 ± 22	-

S.D. Standard Deviation
NaN_3 Sodium azide
9AA 9-Aminoacridine
2NF 2-Nitrofluorene
ENNG N-Ethyl-N-nitro-N-nitrosoguanidine
TL Thin Lawn
STL Slightly Thin Lawn
C Contaminated
VTL Very Thin Lawn

Table 3 Second Mutation Assay

Mean Number of Revertant Colonies Per Plate in the Presence of S9 Mix (FLI 090).

Substance	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
WATER	100 µl	12 ± 4	16 ± 3	29 ± 3	105 ± 16	10 ± 2
Polidocanol	1.7	-	-	-	-	-
	5	-	20 ± 6	23 ± 7	-	-
	17	13 ± 1	12 ± 5	23 ± 4	113 ± 5	9 ± 4
	50	13 ± 5	14 ± 1	24 ± 2	119 ± 18	7 ± 2
	167	19 ± 6	15 ± 2	23 ± 4	102 ± 16	9 ± 3
	500	8 ± 1	11 ± 1 (TL)	19 ± 4	123 ± 15	15 ± 4
	1667	15 ± 3	7 ± 2 (VTL)	11 ± 3 (STL)	74 ± 7 (STL)	8 ± 2
	5000	10 ± 2	-	-	41 ± 5 (TL)	6 ± 3
Positive controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level µg per plate	2	2	0.5	0.5	20
	Mean ± S.D.	169 ± 9	197 ± 32	732 ± 40	780 ± 28	597 ± 40

S.D. Standard Deviation
2AAN 2-Aminoanthracene
TL Thin Lawn
STL Slightly Thin Lawn
VTL Very Thin Lawn

Table 3 (continued) Second Mutation Assay

Mean Number of Revertant Colonies Per Plate in the Absence of S9 Mix.

Substance	Dose Level µg per plate	TA 1535	TA 1537	TA 98	TA 100	WP2uvrA
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
WATER	100 µl	16 ± 5	10 ± 3	19 ± 3	99 ± 6	13 ± 2
Polidocanol	1.7	-	8 ± 3	13 ± 4	-	-
	5	-	7 ± 3	18 ± 6	-	-
	17	10 ± 2	8 ± 1	16 ± 2	114 ± 9	9 ± 2
	50	14 ± 4	11 ± 4	18 ± 7	95 ± 6	9 ± 4
	167	12 ± 3	7 ± 1 (VTL)	20 ± 3 (TL)	88 ± 6	6 ± 2
	500	14 ± 4	0 ± 0 (VTL)	12 ± 5 (VTL)	103 ± 3	5 ± 2
	1667	5 ± 2	-	-	54 ± 6 (TL)	7 ± 1
	5000	6 ± 3	-	-	37 ± 5 (TL)	8 ± 0 (STL)
Positive controls	Compound	NaN_3	9AA	2NF	NaN_3	ENNG
	Dose Level µg per plate	1	80	1	1	2
	Mean ± S.D.	169 ± 60	4305 ± 144	370 ± 20	860 ± 17	460 ± 25

S.D. Standard Deviation
9AA 9-Aminoacridine
2NF 2-Nitrofluorene
NaN_3 Sodium azide
ENNG N-Ethyl-N-nitro-N-nitrosoguanidine
TL Thin Lawn
STL Slightly Thin Lawn
VTL Very Thin Lawn

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Polidocanol: Mouse Lymphoma Mutation Assay

Study no.: 18740

Study report location: eCTD

Conducting laboratory and location: (b) (4)

Date of study initiation: 3/8/2000

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: Batch No. 82652, purity was given as >99%

Key Study Findings: Polidocanol is very weakly mutagenic in mouse lymphoma L5178Y cells, only in the presence of S9 mix, and only when tested at toxic concentrations (14-17% survival).

Methods

Cell line: tk^+tk^--3.7.2.C mouse lymphoma L5178Y cell

Concentrations in definitive study: Two full mutagenicity assays were performed in the absence and presence of S9 mix. The intention was to carry 4 dose levels

	through to completion of each experiment. The concentrations of Polidocanol tested were as follows (µg/mL): Assay 1 (in the absence of S9 mix[1]): 2, 10, 18, 26, 34, 42, 50 and 58 Assay 2 (in the presence of S9 mix[1]): 5, 15, 25, 35, 45, 55, 65 and 75 Assay 3 (in the absence of S9 mix[2]): 2, 10, 18, 26, 34, 42, 50 and 58 Assay 4 (in the presence of S9 mix[1]): 21, 27, 33, 39, 45, 51, 57 and 63 [1] Experiment using a 4 h exposure period [2] Experiment using a 24 h exposure period
Basis of concentration selection:	toxicity assay
Negative control:	vehicle control cu
Positive control:	The positive controls used in the absence of S9 mix (4 h exposure period) were: 250 µg/mL ethyl methanesulphonate (EMS), a large colony inducer; and 15 µg/mL methyl methanesulphonate (MMS), which usually induces greater numbers of small colonies. When performing an experiment using the extended, 24 h exposure period, these concentrations were reduced to 150 µg EMS/mL and 5 µg MMS/mL In the presence of S9 mix, 3-methylcholanthrene (3-MC), a large and small colony inducer, was used at a concentration of 2.5 µg/mL EMS and 3-MC were dissolved in DMSO, while MMS was dissolved in water.
Formulation/Vehicle:	
Incubation & sampling time:	

Study Validity: Concentrations, incubations and cell line were all appropriate

Results

When exposed to the cells for 4 h in the absence of S9 mix, a concentration of 50 µg Polidocanol.ml^{-1} was lethal. No toxicity occurred at the next lower concentration, 15 µg/ml. In the presence of S9 mix, Polidocanol was slightly less toxic, reducing relative survival to 15.2% at 50 µg.ml^{-1} (150 µg.ml^{-1} was lethal).

When the exposure period in the absence of S9 mix was increased to 24 h, Polidocanol gave a dose-related reduction in relative survival across the range of 0.5 µg/ml to 150 µg/ml. While the latter concentration was lethal, it was noted that the next lower concentration of 50 µg.ml^{-1} resulted in a relative survival of 4.1%, whereas the same concentration exposed to the cells for the shorter, 4 h period was lethal. It is unusual to see such a difference, which may be attributable to a protective effect from the higher serum concentration in the 24 h exposure system.

Reference ID: 3367720

Mutation Assays

The summarized results giving the mean values for the duplicate cultures (and quadruplicate vehicle controls) are presented in the following 4 Tables.

Mouse Lymphoma Mutation Assay
Mutation Test in the Absence of S9 Mix (4 h Exposure)

Chemical	Concentration ($\mu g.ml^{-1}$)	Day 0	Day 2			
		Relative Survival %	Relative Total Growth %	Mutant Fraction ($x\ 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
R_0P	(100 µl added)	100	100	67	0.89	-
EMS	250	99	60	556	0.54	*
MMS	15	68	15	734	2.07	*
Polidocanol	2	91	NPS	-	-	-
	10	95	73	67	1.30	-
	18	89	83	44	1.75	-
	26	86	53	77	1.03	-
	34	54	41	51	4.81	-
	†42	21	9	73	1.88	‡
	50	NPT	-	-	-	-
	58	NPT	-	-	-	-

* = Significant difference in log mutant fraction compared with vehicle control (P < 0.05)
Test for linear trend of mutant fraction with concentration of Polidocanol = not significant (negative slope)
NPS = Not plated - surplus
NPT = Not plated - toxic
† = Data from single surviving culture
‡ = Not included in analysis

Mouse Lymphoma Mutation Assay
Mutation Test in the Presence of S9 Mix

Chemical	Concentration ($\mu g.ml^{-1}$)	Day 0	Day 2			
		Relative Survival %	Relative Total Growth %	Mutant Fraction ($x\ 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
R_0P	(100 µl added)	100	100	86	1.14	-
3-MC	2.5	67	48	1015	1.38	*
Polidocanol	5	87	93	94	1.37	-
	15	66	79	83	1.10	-
	25	51	40	82	0.91	-
	35	25	26	89	1.74	-
	45	14	12	145	0.98	*
	55	6	NPT	-	-	-
	65	NPT	-	-	-	-
	75	NPT	-	-	-	-

* = Significant difference in log mutant fraction compared with vehicle control (P < 0.05)
Test for linear trend of mutant fraction with concentration of Polidocanol = not significant (P = 0.41)
NPT = Not plated - toxic

Mouse Lymphoma Mutation Assay
Mutation Test in the Absence of S9 Mix (24 h Exposure)

Chemical	Concentration ($\mu g.ml^{-1}$)	Day 1	Day 3			
		Relative Survival %	Relative Total Growth %	Mutant Fraction ($x\ 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
R_0P	(200 µl added)	100	100	105	1.26	-
EMS	150	35	22	2779	0.51	*
MMS	5	48	24	2079	1.89	*
Polidocanol	2	66	NPS	NPS	-	-
	10	39	39	99	1.07	-
	18	28	21	127	1.22	-
	26	23	13	115	0.98	-
	34	19	9	132	1.05	-
	42	14	3	167	1.72	-
	50	7	NPT	NPT	-	-
	58	3	NPT	NPT	-	-

* = Significant difference in log mutant fraction compared with vehicle control (P < 0.05)
Test for linear trend of mutant fraction with concentration of Polidocanol = Not significant (P = 0.053)
NPS = Not plated - surplus
NPT = Not plated - toxic

Mouse Lymphoma Mutation Assay
Mutation Test in the Presence of S9 Mix

Chemical	Concentration ($\mu g.ml^{-1}$)	Day 0	Day 2			
		Relative Survival %	Relative Total Growth %	Mutant Fraction ($x\ 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
R_0P	(100 µl added)	100	100	129	1.28	-
3-MC	2.5	78	61	949	1.66	*
Volpo L9	21	64	NPS	NPS	-	-
	27	49	46	113	1.12	-
	33	29	32	159	1.74	-
	39	22	22	175	1.09	-
	45	17	14	220	1.98	*
	51	13	10	202	1.44	*
	57	6	NPT	NPT	-	-
	63	NPT	-	-	-	-

* = Significant difference in log mutant fraction compared with vehicle control (P < 0.05)
Test for linear trend of mutant fraction with concentration of Polidocanol = significant (P = 0.004)
NPS = Not plated - surplus
NPT = Not plated - toxic

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Polidocanol: micronucleus test in bone marrow of CD-1 mice. 0h intravenous dosing and 24h + 48h sampling (Study 765081) (GLP)

Study no: 765081
Study report location: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/9/2000
GLP compliance: yes
QA statement: yes
Drug, lot #, and % purity: Batch No. 82652

Key Study Findings: Polidocanol did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 80 mg/kg in male and female CD-1 mice using a 0 hour intravenous dosing followed by a 24 hour and 48 hour sampling regimen.

Methods

Doses in definitive study: 20, 40 and 80 mg/kg
Frequency of dosing: single dose
Route of administration: intravenous
Dose volume: 10 mL/kg
Formulation/Vehicle: water
Species/Strain: CD-1 mice
Number/Sex/Group: see table below
Satellite groups: none
Basis of dose selection: toxicity tests
Negative control: water 10 mL/kg
Positive control: cyclophosphamide 5mg/kg

Treatment Group	Substance	Treatment and Number of Mice		
		Dose (h)	24 h Sample	48 h Sample
Vehicle Control	10 ml water.kg^{-1}	0	5♂ + 5♀	5♂ + 5♀
Low Dose	20 mg Polidocanol.kg^{-1}	0	5♂	-
Mid Dose	40 mg Polidocanol.kg^{-1}	0	5♂	-
High Dose	80 mg Polidocanol.kg^{-1}	0	5♂ + 5♀	5♂ + 5♀
Positive Control	5 mg Cyclophosphamide.kg^{-1}	0	5♂	-

Study Validity: Doses selected, positive control and incubations are appropriate. The use of water as i.v. vehicle control was not appropriate, and is probably mistakenly reported.

Results

In the micronucleus test, groups of CD-1 mice were dosed intravenously at 0 h with Polidocanol at dosages of 20, 40 and 80 mg.kg-1. Control groups of CD-1 mice were dosed intravenously

Reference ID: 3367720

with the vehicle control 10 ml water.kg^{-1} or the positive control agent, 50 mg cyclophosphamide.kg^{-1}. Bone marrow samples were taken from all groups at 24 h and also at 48 h from the vehicle control and high dosage groups. No animal deaths occurred following dosing. Clinical signs of tail dark, subdued behavior, labored breathing, prostration, convulsions, rolling gait, vocalizing when approached and scabs on tail were observed.

Vehicle Control Group

The numbers of micronucleated bone marrow polychromatic erythrocytes (MN-PCE) in mice dosed with the vehicle, 10 ml water.kg^{-1} averaged 0.06 and 0.05% for the 24 h and 48 h samples respectively. These MN-PCE frequencies conformed to the established in-house control range for vehicle treated mice of the CD-1 strain (=0.00-0. 24% per 10 mice or 0.00-0.28% per 5 mice).

Positive Control Group

Exposure of mice to the positive control agent, 50 mg cyclophosphamide.kg^{-1}, induced large increases in bone marrow micronuclei. The mean MN-PCE frequency for the mice was 1.67%. Bone marrow toxicity accompanied these findings as shown by a suppression of the PCE/NCE ratios.

Test Material Group

There was no indication that Polidocanol induced bone marrow micronuclei in the treated mice. The highest MN-PCE frequency recorded for the test material was in the high dose females where an incidence of 0.06% was observed in both the 24 h and 48 h sample groups. There were indications of bone marrow toxicity in the 24 h sample groups in all 3 dose groups treated with the test material.

Polidocanol Micronucleus Test in Bone Marrow of CD-1 Mice
Summary of Assessment Data

Treatment	Sample (h)	Sex	No. of Mice Scored	Erythrocytes				
				Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			PCE/NCE
				No. of MN-NCE	PCE Analysed	No. of MN-PCE	% MN-PCE	Mean ± S.D.
10 ml water kg^{-1}	24	♂	5	0	10000	4	0.04	1.08 ± 0.16
		♀	5	2	10000	7	0.07	1.20 ± 0.34
		♂♀	10	2	20000	11	0.06	1.14 ± 0.26
10 ml water kg^{-1}	48	♂	5	3	10000	7	0.07	1.03 ± 0.39
		♀	5	2	10000	3	0.03	1.31 ± 0.45
		♂♀	10	5	20000	10	0.05	1.17 ± 0.42
20 mg Polidocanol kg^{-1}	24	♂	5	3	10000	5	0.05	0.81 ± 0.21
40 mg Polidocanol kg^{-1}	24	♂	5	2	10000	4	0.04	0.60 ± 0.20
80 mg Polidocanol kg^{-1}	24	♂	5	2	10000	4	0.04	0.73 ± 0.22
		♀	5	5	10000	6	0.06	0.78 ± 0.20
		♂♀	10	7	20000	10	0.05	0.76 ± 0.20
80 mg Polidocanol kg^{-1}	48	♂	5	1	10000	3	0.03	0.96 ± 0.32
		♀	5	1	10000	6	0.06	1.32 ± 0.17
		♂♀	10	2	20000	9	0.05	1.14 ± 0.30
50 mg Cyclophosphamide. kg^{-1}	24	♂	5	4	10000	167Φ	1.67	0.83 ± 0.16

PCE = Polychromatic erythrocytes
MN-PCE = Micronucleated PCE
NCE = Normochromatic erythrocytes
MN-NCE = Micronucleated NCE
Φ = Positive response in PCE

7.4 Other Genetic Toxicity Studies

3 samples of polidocanol: mouse lymphoma cell mutation assay (Study 765626) (GLP)

Objective
The objective of the study was to compare the potential of three samples of polidocanol to induce forward mutations at the tk_+tk_- locus of mouse lymphoma L5178Y cells.

Methods
Three samples of polidocanol were assayed for mutagenic potential in the mouse lymphoma L5178Y cell line, clone 3.7.2.C, scoring for forward mutations at the thymidine kinase locus: tk^+tk^- to tk^-tk^-. The 3 samples were:

1. (b) (4) Polidocanol, dissolved and diluted in tissue culture medium, as used in the previous mouse lymphoma cell mutation assay, Study 765076 (Sample 1).

2. (b) (4) Polidocanol, supplied as a 2% solution in phosphate buffer/ethanol (Sample 2).

Tests were conducted in the presence of a post-mitochondrial supernatant fraction (S9).obtained from the livers of adult, male rats that, typically, are treated with aroclor.to promote hepatic mixed-function oxidases (Cyt P-450)

Preliminary cytotoxicity tests with the previously untested Samples 2 and 3 showed that polidocanol was toxic at approximately 55 µg/mL. This was slightly more toxic than that previously encountered with Sample 1. Two independent mutation experiments were conducted. In Assay 1, rather less toxicity occurred than that expected with all 3 samples. Results were obtained where the final concentration of Sample 1 in the treatment medium ranged between 46 and 70 µg/mL, while for Samples 2 and 3, results were obtained where the final concentration in the treatment medium ranged between 36 and 60 µg/mL.

In Assay 2, toxicity was similar to that demonstrated previously, and results were obtained where the final concentrations in the treatment medium ranged between 10 and 50 µg/mL for Sample 1 and between 5 and 45 µg/mL for Samples 2 and 3. Positive control cultures were included, and the resultant mutant colonies from these provided proof of adequate recovery of "small" type colonies. Duplicate cultures were carried through the experiments for each treatment point. Vehicle control cultures were also included and were tested in quadruplicate. The results were analyzed for comparison of the log mutant fraction between the vehicle controls and each concentration of polidocanol. The results were also tested for linear trend of mutant fraction with concentration of polidocanol.

Reference ID: 3367720

Results

In both assays, all 3 samples of polidocanol gave weak but statistically significant increases in log mutant fraction in at least the highest concentration assessed. In addition, in all but one occasion, there was a statistically significant linear trend associated with the responses. All the responses were obtained, however, at concentrations resulting in moderate to severe toxicity: that is, in the range 9-26% total cell growth compared to control values.

Conclusion

The 3 samples of polidocanol (Original (b) (4) Polidocanol, (b) (4) Polidocanol and Formulated (b) (4) Polidocanol) were very weakly mutagenic in mouse lymphoma L5178Y cells when tested in the presence of S9 mix, only when tested at toxic concentrations.

8 Carcinogenicity

As the drug product is intended is intended for single or infrequent use, carcinogenicity studies were not required or performed.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Polidocanol: Effects on Male Fertility in Rats

Study no.:	493003
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/14/2001
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch No. 82652 98.9% purity

Key Study Findings: Dosage levels up to 27 mg/kg/day were not associated with any effects on male fertility. Mortality and clinical signs were apparent at 27 mg/kg/day. Although a no-effect level was not established for clinical signs of reaction to treatment in males, the effects at 3 mg/kg/day were confined to mastication in 13 out of 25 animals, and occasional animals with a fixed stare and abnormal respiration.

Reference ID: 3367720

Methods

Doses:	3, 9 and 27 mg/kg/day
Frequency of dosing:	daily, 4 weeks
Dose volume:	10 mL/kg delivered over ~5 min
Route of administration:	intravenous via surgically implanted catheter
Formulation/Vehicle:	sterile water
Species/Strain:	Sprague-Dawley rats (Crl:CD® (SD) IGS BR strain)
Number/Sex/Group:	24-25/sex/group
Satellite groups:	none
Deviation from study protocol:	some animals replaced due to surgical losses

Observations and Results

Mortality

All animals were also checked for viability at the start of and again as late as practical on each day of the study.

A total of 12 animals (1, 2, 2 and 7 at 0, 3, 9 and 27 mg/kg/day, respectively) died or were killed prematurely after the start of treatment.

Clinical Signs

All the animals were examined for signs of reaction to treatment during the day. The onset, intensity and duration of these signs were recorded. In addition to the above, a detailed examination was conducted once weekly. This included appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

At 27 mg/kg/day, all animals showed signs of reaction to treatment which included abnormal respiration (irregular/ labored/ shallow), mastication, a fixed stare, subdued behavior, red staining of the cage, chin rubbing, partial closure of the eyes and increased salivation. Some animals were pale and prostrate. Similar observations have been noted in previous rat toxicology studies with Polidocanol (28 day study, (b) (4) Project No. 660474 and developmental toxicity study (b) (4) Project No. 492869).

At 9 mg/kg/day, there was a similar but lesser reaction with many animals showing mastication and/or a fixed stare, and some animals had abnormal respiration, subdued behavior and partial closure of the eyes.

At 3 mg/kg/day, 13 animals showed mastication, with occasional animals showing a fixed stare and abnormal respiration.

Body Weight

Body weights of all males were recorded at least once prior to commencement of surgery, and then at least once weekly between surgery and commencement of dosing. Male body weights were recorded twice weekly during the treatment period, commencing on the first day of treatment. For clarity/brevity of reporting, only weekly weights are included in the report.

Body weight was essentially similar across all groups.

Reference ID: 3367720

Feed Consumption

Food consumption was recorded weekly for males, commencing one week before the commencement of dosing.

Food consumption was essentially similar across all groups.

Toxicokinetics

Toxicokinetic data was not obtained.

Dosing Solution Analysis

On all sampling occasions, the analyzed concentrations were within the acceptance criteria of ± 10% of nominal concentration, indicating acceptable accuracy of formulation.

Necropsy

All the animals, including those killed or found dead, were subjected to a necropsy examination, which consisted of an external examination (including weight of males) followed by macroscopic examination of the tissues and organs of the cranial (males only) thoracic and abdominal cavities *in situ*. Any gross lesions were described and representative samples were preserved in neutral buffered 10% formalin. Carcasses were discarded following these procedures.

From the males, the following tissues were fixed in 10% formalin (except testes):

Testes (weight recorded individually), fixed in Bouin's fluid
Epididymides (weighed individually)
Seminal vesicles and coagulating gland (weighed)
Prostate gland (weighed)
Pituitary gland
Vena cava

For the females, the reproductive tract was dissected out intact. The uterus was then opened and the contents were examined. The number and location of all implants were recorded and each implant classified as live, a late embryonic death (embryonic remains visible) or an early embryonic death (only early placental remains or a decidual scar visible). The number of corpora lutea graviditatis in each ovary was recorded. Gravid reproductive tracts and their contents were discarded following completion of the examinations.

Microscopic examination was not undertaken for any tissues preserved.

Reference ID: 3367720

Fertility Parameters

Polidocanol Effects on Male Fertility in Rats Mating Performance and Fertility Index

Number of Nights to Positive Mating Sign	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
	Number of Animals (Number of these not becoming pregnant)			
1	1	1	4	3
2	10	12	5	10
3	7	6	8	7
4	5	4	5	2
No clear indication of mating	0	0	1 (1)	1
Median number of nights to positive mating sign	3	2	3	2
Number passing one or more oestruses	1	0	0	0
Number of males paired	23	23	23	23*
Number of siring males	23	23	22	23
Male Fertility Index (%)	100	100	96	100

* = excludes Animal 204 which died after pairing but prior to mating

Polidocanol Effects on Male Fertility in Rats Pregnancy Performance

	Group/ Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Number of animals mated	23	23	23	23*
Number pregnant	23	23	22	23
Pregnancy frequency as %	100	100	96	100
Total corpora lutea graviditatis	362	374	363	356
Total number of implants	352	362	350	353
Pre-implantation loss as %	3	3	4	1
Total live implants (%)	330 (94)	339 (94)	329 (94)	339 (96)
Total dead implants (%)	22 (6)	23 (6)	21 (6)	14 (4)
Total early embryonic deaths (%)	22 (6)	23 (6)	20 (6)	14 (4)
Total late embryonic deaths (%)	0	0	1 (0.3)	0
Mean corpora lutea graviditatis	15.7 ± 2.0	16.3 ± 2.3	16.5 ± 1.5	15.5 ± 1.9
Mean implants	15.3 ± 2.6	15.7 ± 1.8	15.9 ± 1.0	15.3 ± 2.0
Mean live implants	14.3 ± 2.3	14.7 ± 2.1	15.0 ± 1.7	14.7 ± 1.8
Mean dead implants	1.0 ± 1.0	1.0 ± 1.2	1.0 ± 1.2	0.6 ± 0.7
Mean early embryonic deaths	1.0 ± 1.0	1.0 ± 1.2	0.9 ± 1.2	0.6 ± 0.7
Mean late embryonic deaths	0	0	0.05 ± 0.2	0

<u>Conclusions</u>

There was no effect of treatment with Polidocanol on male mating performance and fertility index.

There was no effect of treatment with Polidocanol on pregnancy performance at any of the dose levels tested.

There was no obvious effect of treatment with Polidocanol on the male reproductive organ weights. The slightly increased epididymides weight at 3 mg/kg/day, compared with Control, was considered to have been incidental in the absence of a similar finding at the 2 higher dose levels.

Study title: Polidocanol: Effects on Female Fertility in Rats

Study no.:	493017
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/22/2001
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch No. 82652 98.9% purity

Key Study Findings: Dosage levels up to 27 mg/kg/day were not associated with any effects on female fertility. Although a no-effect level was not established for clinical signs of reaction to treatment in females, the effects at 3 mg/kg/day were confined to mastication in 14 out of 24 animals, and occasional animals with a fixed stare. Although a no-effect level was not established for clinical signs of reaction to treatment in males, the effects at 3 mg/kg/day were confined to mastication in 13 out of 25 animals, and occasional animals with a fixed stare and abnormal respiration.

Methods

Doses:	3, 9 and 27 mg/kg/day
Frequency of dosing:	daily for 2 weeks prior to mating, then throughout the mating period and until Day 6 of gestation
Dose volume:	10 mL/kg delivered over ~5 min
Route of administration:	intravenous via surgically implanted catheter
Formulation/Vehicle:	sterile water
Species/Strain:	Sprague-Dawley rats (Crl:CD® (SD) IGS BR strain)
Number/Sex/Group:	24-25/sex/group
Satellite groups:	none
Deviation from study protocol:	some animals replaced due to surgical losses

Observations and Results

Mortality

All animals were also checked for viability at the start of and again as late as practical on each day of the study.

A total of 4 animals (3 at 9 mg/kg/day and 1 at 27 mg/kg/day) were killed prematurely after the start of treatment, all for animal welfare reasons

Reference ID: 3367720

Clinical Signs

All the animals were examined for reaction to treatment during the day. The onset, intensity and duration of these signs were recorded. In addition to the above, a detailed examination was conducted once weekly. This included appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

At 27 mg/kg/day, all animals showed signs of reaction to treatment which included mastication, a fixed stare, red staining in cage, abnormal respiration (labored/increased/irregular), subdued behavior and chin rubbing. Eight animals also had increased salivation and 7 had partial closure of the eyes. Similar observations have been noted in previous rat toxicology studies with Polidocanol (28 day study, (b) (4) Project No. 660474 and developmental toxicity study, (b) (4) Project No. 492869).

At 9 mg/kg/day, all animals showed mastication and 16 had a fixed stare. Two animals displayed chin rubbing.

At 3 mg/kg/day, 14 animals showed mastication, with 3 animals having a fixed stare.

Body Weight

Body weights of all females were recorded at least once prior to commencement of surgery, and then at least once weekly between surgery and commencement of dosing. Females were weighed daily from commencement of dosing until Day 6 of gestation, and again on Day 13 of gestation. For clarity/brevity of reporting, only weekly weights during the pre-mating period, and weights on Days 0, 6 and 13 of gestation are presented in the report.

Body weight performance was similar across all groups prior to mating and during gestation.

Feed Consumption

Food consumption was calculated weekly for each female until pairing for mating, starting one week before treatment began, and then over Days 0-6 and 6-13 of gestation.

Food consumption was essentially similar across all groups.

Toxicokinetics

Toxicokinetic data was not obtained.

Dosing Solution Analysis

On all sampling occasions, the analyzed concentrations were within the acceptance criteria of ± 10% of nominal concentration, indicating acceptable accuracy of formulation.

Reference ID: 3367720

Necropsy

All the animals, including those killed or found dead after commencement of dosing, were necropsied. This consisted of an external examination followed by macroscopic examination of the tissues and organs of the thoracic and abdominal cavities *in situ*. Any gross lesions were described in terms of location, size, shape, color, consistency, number and any other relevant characteristics. Representative samples of abnormal were taken and fixed in neutral buffered 10% formalin.

The following tissues were also fixed:
- Ovaries
- Uterus (including cervix) and vagina (non-pregnant animals only)
- Vena cava

The reproductive tract was dissected out. The uterus was opened and the contents were examined. The number and location of all implantations were recorded. Each implant was classified as being live, or a late embryonic death (embryonic remains visible) or an early embryonic death (only early placental remains or a decidual scar visible). The number of corpora lutea graviditatis in each ovary was recorded.

Fertility Parameters

Polidocanol Effects on Female Fertility and Early Embryonic Development to Implantation in Rats Mating Performance and Fertility Indices

Number of Nights to Positive Mating Sign	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (3)	3 (9)	4 (27)
	Number of Animals			
1	5	4	4	8
2	6	11	8	3
3	7	6	4	5
4	4	3	3	6
5	1	0	0	0
13	0	0	1	0
14	0	0	1	0
No indication of mating	1	0	2	1
Median number of nights to positive mating sign	3	2	2	3
Number passing one oestrus	0	0	1	0
Number of females paired	24	24	23	23
Number pregnant	24	24	22	22
Female Fertility Index (%)	100	100	96	96

Reference ID: 3367720

Polidocanol Effects on Female Fertility and Early Embryonic Development to Implantation in Rats Pregnancy Performance

	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (3)	3 (9)	4 (27)
Number of animals mated	24	24	23	23
Number pregnant	24	24	22	22
Pregnancy Frequency as %	100	100	96	96
Total corpora lutea graviditatis	376	385	344	359
Total number of implants	352	370	337	351
Pre-implantation loss as %	6	4	2	2
Total live implants (%)	326 (93)	355 (96)	305 (91)	336 (96)
Total dead implants (%)	26 (7)	15 (4)	32 (9)	15 (4)
Total early embryonic deaths (%)	25 (7)	15 (4)	31 (9)	15 (4)
Total late embryonic deaths (%)	1 (0.3)	0	1 (0.3)	0
Mean corpora lutea graviditatis	15.7 ± 2.3	16.0 ± 1.8	15.6 ± 2.2	16.3 ± 1.9
Mean implants	14.7 ± 2.5	15.4 ± 1.9	15.3 ± 2.2	16.0 ± 1.8
Mean live implants	13.6 ± 2.9	14.8 ± 2.1	13.9 ± 3.6	15.3 ± 2.0
Mean dead implants	1.1 ± 1.2	0.6 ± 0.8	1.5 ± 2.3	0.7 ± 0.8
Mean early embryonic deaths	1.0 ± 1.2	0.6 ± 0.8	1.4 ± 2.3	0.7 ± 0.8
Mean late embryonic deaths	0.04 ± 0.2	0	0.05 ± 0.2	0

Means are given ± Standard Deviation
Non pregnant animals are excluded below the double lines

Conclusions

Mating performance, as indicated by the number of nights to a positive mating sign, was similar in all groups. The fertility Index was similar in all groups.

There was no adverse effect of treatment with Polidocanol on pregnancy performance at any of the dosage levels tested.

Dosage levels up to 27 mg/kg/day were not associated with any effects on female fertility. Although a no-effect level was not established for clinical signs of reaction to treatment in females, the effects at 3 mg/kg/day were confined to mastication in 14 out of 24 animals, and occasional animals with a fixed stare.

9.2 Embryonic Fetal Development

Polidocanol: pilot study in rats (Study 492832)

Objective
This study was conducted to assess the suitability of administration of polidocanol to rats by intravenous injection in a subsequent developmental toxicity study.

Methods
Four dosing regimes were assessed, using 3 or 4 rats per regime and a single dose level of 27 mg/kg/day which was already known to be tolerated by rats when administered by infusion following femoral vein catheterization. The 4 regimes administered via the lateral tail vein were:

2.7 mg/mL at 10 ml/kg, preceded and followed by a saline flush
1.35 mg/mL at 20 ml/kg
5.4 mg/mL at 5 ml/kg, mixed with blood prior to dosing
1.35 mg/mL at 20 ml/kg, mixed with blood prior to dosing

Results
For all regimes, all animals were subdued shortly after dosing, with many also prostrate, staggering, and/or breathing with difficulty. Red staining of the cage bedding was also noted for all animals. Animals were dosed for up to 4 days with the first regime, and up to 3 days with the other regimes. After that time, it was not possible to continue dosing as the tail of most animals was dark, swollen or limp.

Conclusion
This study demonstrated that intravenous injection of polidocanol via lateral tail vein for 11 days at a dosage of 27 mg/kg/day, as required for the subsequent developmental toxicity study, is not possible. For all 4 dosing regimes, it was necessary to discontinue dosing after the animals had received 3 or 4 doses due to the marked reaction of the tail and the difficulty in finding the vein. The subsequent development toxicity study should therefore be conducted by infusion following femoral vein catheterization.

Study title: Polidocanol Developmental Toxicity Study in Rats

Study no.:	19467
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/16/2000
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Batch No. 82652 98.9% purity

Reference ID: 3367720

Key Study Findings: There were no effects of treatment with Polidocanol on pregnancy performance, fetal weight, or the incidences of fetal abnormalities and variants.

Methods

Doses:	3, 9, and 27 mg/kg/day
Frequency of dosing:	daily, Days 6-16 of gestation.
Dose volume:	10 mL/kg delivered over ~5 min
Route of administration:	intravenous via surgically implanted catheter
Formulation/Vehicle:	sterile water
Species/Strain:	Sprague-Dawley rats (Crl:CD® (SD) IGS BR strain)
Number/Sex/Group:	25/sex/group
Satellite groups:	none
Deviation from study protocol:	some rats replaced prior to start of dosing due to surgical losses

Observations and Results

Mortality

Except for animals that were replaced prior to commencement of dosing, there were no premature decedents.

Clinical Signs

All the treated animals were examined for signs of a reaction to treatment on each day of dosing. The onset, duration and intensity of any signs were recorded, particular attention being paid to the dosing and post-dosing periods.

At 27 mg/kg/day, all animals showed subdued behavior and had red staining of the cage, and many showed mastication and abnormal respiration (irregular/labored/shallow).

At 9 mg/kg/day, many animals showed subdued behavior and mastication. A single instance of abnormal respiration may have been incidental.

At 3 mg/kg/day, 3 animals showed mastication; a single instance of subdued behavior was considered to be incidental.

Body Weight

Body weights were recorded for study animals on Days 0, 3, 6-17 and 20 gestation but a summary of weights are reported for clarity/brevity.

Body weight performance was essentially similar across all groups.

Reference ID: 3367720

Feed Consumption

Food consumption was recorded daily for study animals commencing on Day 4 of gestation (weighed quantity first offered on Day 3), until necropsy.

Food consumption was essentially similar across all groups.

Toxicokinetics

Toxicokinetics analyses were not conducted.

Dosing Solution Analysis

On both sampling occasions, analyzed concentrations were within the acceptance criteria of ± 10% of nominal, indicating acceptable accuracy of formulation.

Necropsy

The animals were subjected to a gross necropsy, in which the thoracic and abdominal contents were examined macroscopically; any findings were recorded and representative samples of abnormal tissues were taken and fixed in neutral buffered 10% formalin. The reproductive tract was then dissected out and weighed intact prior to the maternal carcasses being discarded.

The uterus was opened and the contents examined. The number of corpora lutea graviditatis in each ovary was recorded and a gross examination of the placentae was conducted. The number and location of all implantation sites in the uterus were recorded. Each implant was classified as being live, a fetal death (death judged to have occurred during the fetal period, after *ca* Day 16 of gestation), a late embryonic death (embryonic remains visible) or an early embryonic death (only early placental remains or a decidual scar visible).

Each live fetus was identified individually within the litter and its weight was recorded. These fetuses were examined for externally visible abnormalities prior to chilling and fixation in chilled fixative. Half the fetuses from each uterus were fixed in methylated ethyl alcohol, the remaining half in Bouins fluid.

<u>Conclusions</u>

At 3 mg/kg/day, obvious maternal effects were confined to mastication observed for 3 out of 24 animals.

There were no effects of treatment with Polidocanol on pregnancy performance, fetal weight, or the incidences of fetal abnormalities and variants.

In conclusion, dosage levels up to 27 mg/kg/day were without effects on the fetus. Although a maternal no-effect level was not established, effects at 3 mg/kg/day were confined to mastication in occasional animals.

Reference ID: 3367720

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Polidocanol Developmental Toxicity Study in Rats Group Mean Pregnancy Performance and Fetal Weights

Parameters	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Number of animals mated	24	24	24	24
Number pregnant at Day 20 necropsy	21	21	23	22
Pregnancy frequency as %	88	88	96	92
Total corpora lutea graviditatis	319	332	365	338
Total number of implants	300	325	347	317
Pre-implantation loss as %	6	2	5	6
Total live implants (%)	284 (95)	311 (96)	333 (96)	306 (97)
Total dead implants (%)	16 (5)	14 (4)	14 (4)	11 (3)
Total early embryonic deaths (%)	15 (5)	14 (4)	14 (4)	11 (3)
Total late embryonic deaths (%)	1 (0.3)	0	0	0
Total foetal deaths (%)	0	0	0	0
Mean corpora lutea graviditatis	15.2 ± 1.6	15.8 ± 1.7	15.9 ± 1.7	15.4 ± 1.6
Mean implants	14.3 ± 1.7	15.5 ± 2.0	15.1 ± 2.1	14.4 ± 2.5
Mean live implants	13.5 ± 1.7	14.8 ± 2.2	14.5 ± 2.1	13.9 ± 2.5
Mean dead implants	0.8 ± 0.8	0.7 ± 0.9	0.6 ± 0.7	0.5 ± 1.0
Mean early embryonic deaths	0.7 ± 0.8	0.7 ± 0.9	0.6 ± 0.7	0.5 ± 1.0
Mean late embryonic deaths	0.05 ± 0.2	0	0	0
Mean foetal deaths	0	0	0	0
Total live male foetuses (%)	144 (51)	157 (50)	143 (43)	175 (57)
Total live female foetuses (%)	140 (49)	154 (50)	190 (57)	131 (43)
Live foetal sex ratio (♂:♀)	1:0.97	1:0.98	1:1.33	1:0.75
Mean total uterus weight (g)	82 ± 9	90 ± 10	88 ± 10	85 ± 14
Mean litter mean foetal weight (g)	3.76 ± 0.15	3.76 ± 0.34	3.81 ± 0.22	3.80 ± 0.25

Means are given ± Standard Deviation

Offspring (Malformations, Variations, etc.)

Polidocanol Developmental Toxicity Study in Rats Group Incidence of Major Fetal Abnormalities

Abnormality	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
	Incidence of Foetuses (Litters)			
Palatines incompletely closed, cervical vertebral arches absent/fused, thoracic vertebra hemicentric, right rib 2 costal cartilage not attached to sternum	0	1(1)	0	0
Thoracic scoliosis, thoracic vertebrae hemicentric, vertebral arches fused, ribs fused/branched, twelve thoracic vertebrae, five sternebrae, five lumbar vertebrae only, umbilical hernia	0	0	0	1(1)
Retro-oesophageal aortic arch	1(1)	0	0	0
Interventricular septal defect	0	1(1)	0	0
Kinked rib(s)	4(3)	2(1)	0	0
Number with major abnormality	5(4)	4(3)	0	1(1)
Total number examined	284(21)	311(21)	333(23)	306(22)

Study title: Polidocanol Developmental Toxicity Study in Rabbits
Study no.: 493265
Study report location: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/28/2002
GLP compliance: yes
QA statement: yes
Drug, lot #, and % purity: Batch No. 82652 98.9% purity

Key Study Findings: The type and distribution of major fetal abnormalities did not indicate an effect of treatment. The incidences of the minor fetal abnormalities and variants did not indicate an effect of treatment. Although the incidence of fetuses at 10 mg/kg/day with complete 13th supernumerary ribs was marginally greater than Control, the difference was considered too small to be attributable to treatment. Considering the ossification parameters together, there was no obvious effect of treatment on the state of skeletal ossification.

Reference ID: 3367720

Methods

Doses:	2.5, 5.0 and 10.0 mg/kg/day
Frequency of dosing:	daily, Days 6-18 inclusive of gestation
Dose volume:	10 mL/kg
Route of administration:	intravenous via surgically implanted catheter
Formulation/Vehicle:	sterile water
Species/Strain:	time-mated female New Zealand White rabbits (Hsd If: NZW strain)
Number/Sex/Group:	20 females/group
Satellite groups:	none
Deviation from study protocol:	minor problems with indwelling catheters did not influence the outcome of the study

Observations and Results

Mortality

One mid-dose animal was prematurely killed for humane reasons. Three abortions were noted (1 control, 1 low-dose and 1 mid-dose.

Clinical Signs

All the animals were examined for reaction to treatment during each day. The nature, onset, and duration and intensity of any signs were recorded. Excreta trays were checked at the start and end of each day, as a minimum, for changes in output or quality of excreta, etc.

In addition to the above, all the animals were checked for viability early in the morning and again as late as possible in each day.

Clinical signs were restricted to the one mid-dose rabbit sacrificed early.

Body Weight

Body weights were recorded on Days 4, 6-19, 22, 26 and 29 of gestation.

There were no intergroup differences in body weight or food consumption that were considered to be related to treatment with Polidocanol.

Feed Consumption

The weight of food consumed by each animal was recorded daily, commencing on Day 4 of gestation

In all groups, including Control, there was a reduction in body weight from Day 4 to Day 6, and in food consumption on Days 5 and 6. These reductions were probably associated with the ear vein cannulation.

Reference ID: 3367720

Toxicokinetics

Toxicokinetic analyses were not performed.

Dosing Solution Analysis

The analyzed concentrations of the dosing formulations indicated that they had been accurately prepared.

Necropsy

Maternal -The contents of the thoracic and abdominal cavities were examined macroscopically. Any findings were recorded and representative samples of abnormal tissues were fixed in neutral buffered 10% formalin, as appropriate.

The reproductive tract was dissected out and weighed intact prior to the maternal carcasses being discarded. The reproductive tract was then opened and the contents examined. The number of corpora lutea graviditatis in each ovary and the number and position of all implantation sites were recorded.

Each implant was classified as being live, or a fetal death (death judged to have occurred during the fetal period, after approximately Day 18 of gestation), or a late embryonic death (embryonic remains visible), or an early embryonic death (only early placental remains or a decidual scar visible).

Fetal - Each live fetus was identified individually within the litter and its weight recorded. These fetuses were killed then examined for externally visible abnormalities, including macroscopic examination of the eyes and cranial bones, following removal of the skin over these regions.

Each fetus was examined by open dissection for abnormalities of the thoracic and abdominal viscera. The cranium was sectioned once, through the coronal suture to allow inspection of the brain in that region. The sex of each fetus was recorded together with any details of any abnormalities found during the dissection procedure. The thoracic and abdominal viscera were then discarded and the eviscerated carcasses fixed in methylated ethyl alcohol.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The incidence and distribution of embryo-fetal deaths did not indicate an association with treatment.

Slight intergroup differences in mean fetal weight did not suggest any effect of treatment.

Reference ID: 3367720

Polidocanol Developmental Toxicity Study in Rabbits Pregnancy Performance and Fetal Weight

	Group/Dose Level (mg.kg^{-1}.day^{-1})			
	1 (0)	2 (2.5)	3 (5)	4 (10)
Number of animals mated	20	20	20a	20
Number pregnant	16	18	19	17
Number of premature decedents	1	1	0	0
Number of Decedents pregnant	1	1	0	0
Number pregnant at Day 29 necropsy	15*	17	19*	17
Pregnancy frequency as %	80	90	95	85
Total corpora lutea graviditatis	146	166	178	161
Total number of implants	132	147	158	148
Pre-implantation loss as %	10	11	11	8
Total live implants (%)	96 (73)	128 (87)	134 (85)	129 (87)
Total dead implants (%)	36 (27)	19 (13)	24 (15)	19 (13)
Total early embryonic deaths (%)	32 (24)	13 (9)	13 (8)	11 (7)
Total late embryonic deaths (%)	2 (2)	2 (1)	0	0
Total foetal deaths (%)	2 (2)	4 (3)	11 (7)	8 (5)
Mean corpora lutea graviditatis	10.4 ± 2.4	9.8 ± 1.2	9.9 ± 1.6	9.5 ± 1.5
Mean implants	9.4 ± 3.3	8.6 ± 2.1	8.8 ± 1.9	8.7 ± 1.5
Mean live implants	6.9 ± 3.8	7.5 ± 2.4	7.4 ± 2.3	7.6 ± 1.3
Mean dead implants	2.6 ± 2.8	1.1 ± 1.4	1.3 ± 2.7	1.1 ± 1.2
Mean early embryonic deaths	2.3 ± 2.8	0.8 ± 1.0	0.7 ± 1.4	0.6 ± 0.7
Mean late embryonic deaths	0.1 ± 0.5	0.1 ± 0.3	0	0
Mean foetal deaths	0.1 ± 0.5	0.2 ± 0.4	0.6 ± 1.5	0.5 ± 0.7
Total live male foteuses (%)	44 (46)	56 (44)	60 (45)	64 (50)
Total live female foetuses (%)	52 (54)	72 (56)	74 (55)	65 (50)
Live foetal sex ratio (male:female)	1:1.18	1:1.29	1:1.23	1:1.02
Mean total uterus weight (g)	446 ± 189	484 ± 119	508 ± 105	508 ± 93
Mean litter mean foetal weight (g)	42.5 ± 6.7	44.0 ± 5.7	42.1 ± 7.4	43.5 ± 4.6

Means are given ± Standard Deviation
Note: Premature decedents and total resorptions excluded below double line
a = Excludes Animal 53 which was replaced by Animal 82 in the early dosing period
* = Includes an animal with total resorption

Offspring (Malformations, Variations, etc.)

The type and distribution of major fetal abnormalities did not indicate an effect of treatment. The incidences of the minor fetal abnormalities and variants did not indicate an effect of treatment either. Although the incidence of fetuses at 10 mg/kg/day with

complete 13th supernumerary ribs was marginally greater than Control, the difference was considered too small to be attributable to treatment.

Considering the ossification parameters together, there was no obvious effect of treatment on the state of skeletal ossification.

Polidocanol Developmental Toxicity Study in Rabbits Group Incidence of Major Fetal Abnormalities

Abnormality	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (2.5)	3 (5)	4 (10)
	Incidence of Foetuses (Litters)			
Hole occipital region; occipital and interparietal absent; hole in right frontal with left frontal incompletely ossified; frontals misshapen; parietals reduced (in size) with discrete unossified/ incompletely ossified areas; occipital condyles reduced (in size); cervical vertebral arch 1 dorsal portion absent with cervical vertebral arch 2 reduced (in size); cervical vertebral arch 2 to 6 open; forelimb flexure.	1(1)	0	0	0
Parietal large hole	0	0	1(1)	0
Nasals, frontals fused and misshapen; protruberance frontal region of skull with orbital sockets and zygomatic arches misshapen; upper jaw shortened; upper incisors absent	0	1(1)	0	0
Eye(s) reduced (in size)	2(1)	0	0	0
Cleft palate with lower jaw shortened; forelimb flexure with hindlimbs malrotated, curvature of the spine	1(1)	0	0	0
Ribs partially fused; thoracic vertebra hemicentric with absent thoracic vertebral arch; absent thoracic centrum with thoracic vertebral arches fused; umbilical hernia	1(1)	0	0	0
Ribs fused, costal cartilages fused; thoracic vertebra hemicentric with thoracic vertebral arches fused	0	0	0	1(1)

(continued) Group Incidence of Major Fetal Abnormalities

Abnormality	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1	2	3	4
	(0)	(2.5)	(5)	(10)
	Incidence of Foetuses (Litters)			
Thoracic hemivertebra with absent rib, with/without thoracic centra fused; thoracic centra misshapen with thoracic vertebral arches asymmetrically aligned	0	1(1)	0	1(1)
Lumbar hemivertebra with lumbar vertebral arches asymmetrically aligned	1(1)	0	0	0
Aortic arch dilated with ductus arteriosus threadlike or narrowed with/without pulmonary artery narrowed	1(1)	0	1(1)	2(2)
Kidney absent	0	1(1)	0	0
Pollex of forepaw present	1(1)	0	0	0
Hindlimbs malrotated	1(1)	0	0	0
Number of major abnormalities	9(8)	3(3)	2(2)	4(4)
Total number examined	96(14)	128(17)	133(18)*	129(17)

* = 134 foetuses were collected at necropsy, but one was lost during processing therefore 133 examined

Polidocanol Developmental Toxicity Study in Rabbits Group Incidence of Minor Fetal Abnormalities and Variants

Abnormality/Variant	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (2.5)	3 (5)	4 (10)
	Incidence of Foetuses (Litters)			
Visceral				
Small foetus	0	0	2(1)	0
Eye(s) corneal/lenticular opacity	2(1)	0	1(1)	1(1)
Eye(s) iridial haemorrhage	1(1)	0	0	1(1)
Eye(s) reddened	0	0	0	1(1)
Incisors not erupted	0	0	0	2(2)
Cervical remnant of thymus	0	1(1)	3(2)	2(2)
Aortic arch misshapen	1(1)	0	0	0
Innominate artery shortened/elongated	1(1)	1(1)	0	0
Common carotid arteries arising separately from aortic arch	3(3)	8(5)	8(5)	6(4)
Variation in origin of arteries arising from aortic arch	16(7)	1(1)	11(7)	6(5)
Lungs unexpanded	1(1)	0	0	0
Liver cyst	0	0	0	1(1)
Gall bladder enlarged	1(1)	0	0	0
Gall bladder reduced (in size)	1(1)	0	0	0
Gall bladder bifurcated/bilobed/misshapen	1(1)	1(1)	2(1)	2(2)
Gall bladder contents clear	4(3)	1(1)	5(4)	1(1)
Stomach distended by gas	0	0	1(1)	0
Spleen reduced (in size)	0	1(1)	0	0
Spleen pale	1(1)	0	1(1)	0
Ovary(ies) cyst	0	1(1)	0	0
Digit(s) of forepaw pointing inwards	1(1)	0	0	0
Number of minor visceral abnormalities	26(11)	14(8)	28(14)	23(15)
Total number examined	96(14)	128(17)	133(18)*	129(17)

* = 134 foetuses were collected at necropsy, but one was lost during processing therefore 133 examined

(continued) Group Incidence of Minor Fetal Abnormalities and Variants

Abnormality/Variant	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (2.5)	3 (5)	4 (10)
	Incidence of Foetuses (Litters)			
Skeletal				
Cranial bone(s) unossified/incompletely ossified area(s)	1(1)	5(4)	6(4)	4(3)
Cranial bone(s) linear ossification irregularity/ies	1(1)	3(3)	14(9)	2(2)
Sutural bone(s) or sutural deviation	3(1)	0	2(2)	0
Central fontanelle enlarged	0	1(1)	0	1(1)
Jugal connected/fused to zygomatic process of maxilla	3(3)	5(4)	2(2)	5(2)
Zygomatic arch misshapen	1(1)	0	0	0
Greater horn of hyoid bent in/outwards	6(6)	5(5)	7(5)	3(3)
Cervical vertebral arch 2 incompletely ossified	1(1)	0	0	0
Cervical/thoracic vertebra hemicentric	2(2)	2(2)	1(1)	0
Additional ossified area ventral to odontoid process	1(1)	0	0	0
Cervical rib(s)	1(1)	4(4)	3(2)	5(3)
Cervical/thoracic centrum(a) misshapen	2(2)	0	0	0
Sternebrae fused/connected	1(1)	2(2)	5(3)	2(2)
Sternebra flattened	1(1)	0	1(1)	0
Sternebra mishapen	0	1(1)	1(1)	2(1)
Sternum additional ossified area(s)	0	3(2)	2(2)	3(2)
Additional sternebra cranial to sternebra 1	0	1(1)	0	0
Rib(s) partially fused	0	0	0	1(1)
Cervical rib costal cartilage fused to thoracic rib costal cartilage	0	1(1)	0	1(1)
Rib(s) thickened area(s)	1(1)	0	0	0
Rib costal cartilage bifurcated	0	1(1)	0	1(1)
Rib costal cartilages asymmetrically aligned at point of attachment to sternum	2(2)	6(5)	6(4)	2(2)
Unilateral/bilateral rib 7 costal cartilage not attached to sternum	2(1)	5(4)	11(6)	5(5)
Xyphoid cartilage bifurcated	0	0	1(1)	0
Bilateral scapula/clavicle incompletely ossified	1(1)	0	0	0
Pollex claw reduced (in size)	0	0	0	1(1)
Sacral centrum connected to sacro-iliac articulation centre	1(1)	0	1(1)	0
Pelvic girdle displaced	29(10)	41(14)	31(13)	43(14)
Astragali incompletely ossified	1(1)	0	1(1)	0

(continued) Group Incidence of Minor Fetal Abnormalities and Variants

Abnormality/Variant	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (2.5)	3 (5)	4 (10)
	Incidence of Foetuses (Litters)			
Number of ribs				
Reduced 12th rib(s) on 12th thoracic vertebra	0	0	1(1)	0
12 complete ribs	41(12)	60(13)	57(18)	47(15)
Vestigial 13th supernumerary rib(s) on 1st lumbar vertebra	9(5)	8(8)	5(4)	7(4)
Reduced 13th supernumerary rib(s) on 1st lumbar vertebra	16(7)	13(10)	24(13)	12(7)
Complete 13th supernumerary rib(s) on 13th thoracic vertebra	30(10)	47(15)	46(17)	63(16)
Number of minor skeletal abnormalities	45(14)	65(17)	61(18)	62(16)
Total number examined	96(14)	128(17)	133(18)*	129(17)

* = 134 foetuses were collected at necropsy, but one was lost during processing therefore 133 examined

9.3 Prenatal and Postnatal Development

Study title: Polidocanol Peri and Post Natal Study in Rats

Study no.: 493134
Study report location: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/21/2002
GLP compliance: yes
QA statement: yes
Drug, lot #, and % purity: Batch No. 82652 98.9% purity

Key Study Findings

Dosage levels of up to 9 mg/kg/day were without effects on the development of the conceptus and offspring to sexual maturity, and effects at 27 mg/kg/day were confined to an equivocal reduction in body weights of F1 males and associated equivocal delay in the age of preputial separation. Although a maternal no-effect level was not established, effects at 3 mg/kg/day were confined to clinical signs of reaction in 3 animals.

Reference ID: 3367720

Methods

Doses:	3, 9 and 27 mg/kg/day
Frequency of dosing:	daily, Day 16 gestation through Day 24 of lactation
Dose volume:	2 mL/kg over 5 min infusion
Route of administration:	intravenous via surgically implanted catheter
Formulation/Vehicle:	sterile water
Species/Strain:	female Sprague-Dawley rats/IGS (Crl: CD®(SD) IGS BR strain)
Number/Sex/Group:	30
Satellite groups:	none
Study design:	
Deviation from study protocol:	no reports

Methods

Viability and Mortality
All the animals were also checked for viability early in the morning and again as late as possible each day of the study.

Clinical Signs
All the animals were examined for signs of reaction to treatment during each day. The onset, intensity and duration of these signs were recorded, particular attention being paid during the dosing and post dosing periods.

Body Weights
F0 body weights were recorded on Days 0, 7 and 13 of gestation, then daily from Day 16 of gestation until termination. For clarity/brevity of reporting, weights have been presented for Days 0,7, 13, 16, 18 and 20 of gestation and Days 1, 7, 14, 21 and 24 of lactation.

Post-weaning F1 animals were weighed weekly, starting on a suitable day within one week of weaning of the majority of litters, until termination for males and until mating commenced for females. Mated F1 females were weighed on Days 0, 7, 14 and 20 of gestation, then on Days 1, 7 and 14 of lactation. F1 females that did not show a positive mating sign continued on a weekly weighing regime. For a female showing an equivocal sign of mating, the weighing regime based on specific days of gestation was implemented.

Food Consumption
The weight of food consumed by each F0 animal was recorded daily from Day 14 of gestation (weighed quantity first offered on Day 13), until parturition, but for clarity only data to Day 20 of gestation have been presented in the report. For females that littered, food consumption was weighed weekly over the period Days 0-7 and 7-14 of lactation. All food consumption data is retained in the raw data.

Reference ID: 3367720

Food consumption was not recorded for F1 animals.

Observations on Females with Litters during Lactation
The females were allowed to litter normally. The day of birth of the litter (day on which parturition commenced) was designated Day a of lactation. The number of live pups born and the number found dead in each litter was recorded as soon as possible after completion of parturition.

The live pups were sexed, counted, examined for the presence of milk in the stomach and for any externally visible abnormalities daily until Day 4 of lactation, then again on Days 7, 14 and 21 of lactation. Where practical, any pups found dead or killed during lactation were sexed and examined as above. Decedent pups on or after Day 14 of lactation were necropsied and gross lesions preserved.

Any deficiencies in maternal care, such as inadequate construction and cleaning of the nest, pups left scattered and cold, physical abuse of pups, or apparently inadequate lactation or feeding were recorded. Detailed information on these observations is retained in the raw data.

For all litters, the live pups were weighed *en* masse (sexes separate) on Days 1, 4, 7 and 14 of lactation. For F0 litters, these animals were then weighed individually on Day 21 of lactation

Assessment of F1 Post-Natal Physical and Functional Development
This was assessed by measuring indices of physical maturation (principally body weight), by assessment of certain sensory and behavioral functions, and by assessment of reproductive function.

Test Profile Prior to Weaning
The indices used, their criteria, and the day of lactation at which observation began are specified below.

Test Day(s) of Lactation	**Test Parameter**
1 - Criterion	Pinna Detachment
7 - Criterion	Upper Incisor Eruption
11	Negative Geotaxis
11 - Criterion	Eye Opening
16	Auditory Function
18	Visual Function

Reference ID: 3367720

Physical Maturation
Each litter was examined daily until all pups in that litter had reached the criterion listed below. For either sex in each litter, the day at which the criterion was reached for all pups and the median day to criterion were used as the bases for the comparison between the treatment groups.

Pinnae Detachment:	Presence of both pinnae unfolded from the head. Observed starting from Day 1.
Incisor Eruption:	Presence of upper incisors that had penetrated the gum. Observed starting from Day 7.
Eye Opening:	Presence of both eyes open. Observed starting from Day 11.

In error, some of the litters were not examined for pinnae detachment until Day 2 of lactation. These deviations were not considered to have affected the integrity of the study

Sensory Function
Each litter was examined for negative geotaxis, auditory function and visual function on a specified day of lactation. For either sex in each litter, the percentage of pups achieving criterion was used as the basis of comparison between treatment groups.

Negative Geotaxis
Each pup was placed in a head-down position on a 25° inclined, rough surfaced board. The ability to make at least a 90° turn was noted, a maximum time of 50 s being allowed. Assessment was conducted on Day 11.

Auditory Function
To prevent untested animals becoming habituated to the stimulus, each litter was removed separately to the testing room for assessment. Each pup to be tested was placed in a container and observed for Preyer's reflex (twitching of the pinnae) in response to a high frequency sound stimulus; the stimulus was repeated if necessary, up to 3 times. Assessment was conducted on Day 16.

Visual Function
The presence of the pupillary reflex was determined by shining a narrow focus light beam into each eye of the pup in a darkened room. The presence of a consensual response in the opposite eye was also checked. Assessment was conducted on Day 18.

Selection and Weaning of *F1 Animals for Post-Weaning Assessment*
On Day 21 of lactation, one male and one female, where available, were selected from each litter. For each litter, the selected pups were those with a body weight closest to the median weight of that sex for that litter.

Reference ID: 3367720

The selected pups were weaned by removal from the dam on Day 24 of lactation. Where the selected pups were judged too small to survive separation, they were not weaned but kept with their mother. These pups were submitted for necropsy with the other unselected pups, and were treated as if they had not been selected.

Test Profile After Weaning (for selected F1 animals)

Age of Animals at Testing	**Test**
Starting from 28 days	Vaginal opening (females)
Starting from 35 days	Balano-prepuital separation (males)
Approximately 5 weeks	Open Field
Approximately 6 weeks	Rota-Rod
Approximately 7 weeks	Multiple Y Water Maze
Approximately 10 weeks	Reproductive Function

The order of animal testing was dependent on animal age and cage order so as to ensure an even distribution between the treatment groups. For each day of testing, the males were tested before the females.

Assessment of *Sexual Maturation*
Commencing from 28 days of age, females were examined daily for vaginal opening. The day when opening was first seen and the body weight of the animal on that day were recorded.

From 35 days of age, males were examined for balano-preputial separation. The day when separation was first seen and the body weight of the animal on that day were recorded. In error, some of the males were examined for balano-preputial separation from 34 days of age. These deviations were not considered to have affected the integrity of the study.

Open Field Test
This test assessed general activity and exploratory behavior in a novel, uniform environment.

The apparatus consisted of a white Plexiglas circular floor of 100cm diameter, surrounded by a dark-colored wall, 30cm high. The floor was marked with fine lines into 19 sectors, including a central sector of 20cm diameter, and was uniformly illuminated.

Reference ID: 3367720

Each animal was placed in the centre circle and observed for 3 min on one day. The following were recorded:

1 = time in seconds to move from centre circle (latency)
2 = number of sectors entered
3 = number of rearings
4 = number of grooming episodes
5 = number of defecation boluses
6 = Incidence of abnormalities of gait or motor activity

Rota-Rod Test
This test assessed motor co-ordination and balance. The apparatus was an accelerating treadmill consisting of a drum, 5.5cm in diameter. Initially, this treadmill revolved at a constant speed of 4 r.p.m. When the animal(s) to be tested were positioned correctly on the drum the acceleration cycle was started and the drum accelerated up to 40 r.p.m. over a period of 5 min. When an animal fell, it landed on a trip-plate, stopping the timer.

Each animal was given 3 consecutive trials on one day. Failure in anyone trial was recorded if the animal persistently fell from the drum within 10 s. The longest time achieved and the mean time over 3 trials were used as measures of performance.

Multiple Y Water Maze
This test assessed learning and recall of a novel response. The apparatus was a water filled maze of a multiple Y configuration, with 3 successive choice points between the start and the exit ramp. Path length for an error-free escape was approximately 2 m. The water was maintained at a temperature of 18°C ± 1°C

For each trial, the animal was placed in the maze at the entrance and a timer was simultaneously started. The end point of the trail was contact with the escape ramp. The sequence of errors, if they were made, was recorded. A maximum time of 90 s was allowed for each trial and once this had expired, or if the animal was seen to be in undue difficulty, it was guided to the exit or removed, as appropriate. Each animal was given 3 trials on the first day (Day 1), then another 3 trials on Day 3.

An improvement in performance across the first 3 trials was interpreted as an index of learning. Performance in the second trial provided an index of memory.

Assessment of F1 *Reproductive Function*
The animals were paired for mating at approximately 11 weeks old. Pairing was on a one male to one female basis, within the same treatment group. Sibling and other closely related pairings were avoided. Each female was transferred to the cage of an appropriate co-group male near the end of the work day, and remained there until mating was detected or 7 nights had elapsed. Vaginal lavages were taken early each morning commencing on the day of pairing, until mating was detected, and the day of

Reference ID: 3367720

observation of a copulatory plug *in situ* and/or sperm in the lavage was designated Day 1 of gestation.

Each female was left with its first designated male for a maximum of 7 nights. If a positive mating sign was not detected during that time, a 2 night rest period was allowed before the female was placed with a second co-group male that had already successfully mated, for an additional mating period of a maximum of 14 nights. Vaginal lavages continued to be taken and the stage of estrus recorded during the rest period. At the end of the second mating period, if no mating sign was observed, attempts to breed from the female ceased and it was returned to an individual solid bottom cage. The animals were allowed to litter normally. Observations were recorded as previously described for F0 females.

Terminal Studies
F0 females were sacrificed at or shortly after weaning of the litter on Day 24 of lactation. F1 pups not selected for post weaning assessment were sacrificed at the same time. FO females that did not litter were sacrificed after it was apparent that they were not going to litter; FO females that lost their litter were sacrificed after their litter had died.

F1 parents and F2 offspring were sacrificed after most litters had reached Day 14 of lactation; a small number of females and their litters were sacrificed prior to Day 14 of lactation, but not prior to Day 4 of lactation. Animals that were at least 10 days of age were killed by exposure to carbon dioxide followed by exsanguination. Animals less than 10 days of age were killed by injection of sodium pentobarbitone.

F0 and* F1 *Adults
F0 females and F1 post weaning animals were necropsied. This consisted of an external examination followed by macroscopic examination of the tissues and organs of the thoracic and abdominal cavities in situ. For premature decedent FO animals and all F1 animals, examination was extended to the cranial contents. Any gross lesions were described in terms of location, size, shape, color, consistency, number and any other relevant characteristics. Representative samples of abnormal tissues were taken and fixed in neutral buffered 10% formalin. The reproductive tracts of all females were examined for signs of implantation, the number of implantation sites being recorded.

Pups Killed or Found Dead Before Day 14 of Lactation
Where practicable, these animals were sexed, then checked for the presence of milk in the stomach and for the presence of externally visible abnormalities. Externally normal pups found dead prior to Day 13 were discarded.

Pre-Weaning Pups Killed or Found Dead on or after Day 14 of Lactation
These were subjected to a gross necropsy, in which the cranial, thoracic and abdominal contents were examined macroscopically; any findings were recorded and abnormal tissues sampled, as appropriate. The carcasses were then discarded.

F1 Weanlings not Selected for Post Weaning Assessments

Reference ID: 3367720

From each litter of F1 weanlings, 2 male and 2 female pups (where available) were necropsied. This consisted of external examination followed by macroscopic examination of the tissues and organs of the cranial, thoracic and abdominal cavities. Any findings were recorded and representative samples of abnormal tissues were preserved in 10% neutral buffered formalin as appropriate. The carcasses were then discarded.

The remaining F1 weanlings and all F2 pups were killed after external examination; no abnormalities were found and the carcasses were discarded without necropsy.

Results

Analysis of Dosing Formulations
Dosing formulations were sampled on the first day of dosing for each batch. Batch 1 formulations were found to be within ± 10% of the nominal concentration, indicating acceptable accuracy of formulation.

F0 Clinical Observations and Necropsy Findings (Table 1)

At 27 mg/kg/day, all animals showed signs of reaction to treatment which included mastication, a fixed stare, irregular respiration, red cage staining, subdued behavior, chin rubbing and salivation. Twelve animals also had a pale appearance and 10 were lying on back/side/against cage. Similar observations have been noted in previous rat toxicology studies with Polidocanol (28 day study, [redacted] (b) (4) Project No. 660474, developmental toxicity study, [redacted] (b) (4) Project No. 492869 and Female Fertility and Early Embryonic Development to Implantation, [redacted] (b) (4) Project No. 493019). Other clinical signs of reaction to treatment at this level included eyes partially closed, convulsions, staining, gasping, retching motion and unsteady on feet.

Animals 74 and 79 (both at 27 mg/kg/day) were found dead on Days 20 and 17 of gestation, respectively, after showing similar signs, and both deaths were attributed to treatment. Animal 107 at 27 mg/kg/day was found dead on Day 19 of lactation; although this animal also had the typical signs for this level, necropsy findings included an ulcer in the colon, and it was not possible to positively attribute this death to treatment.

At 9 mg/kg/day, many animals had mastication and irregular respiration, 8 had subdued behavior and 7 had a fixed stare. Other clinical signs of reaction to treatment included salivation, a pale appearance, lying flat, fitting, crawling on paws and red cage staining. One animal (Number 106) was killed on Day 4 of lactation due to its condition, similar to the above, which had precluded dosing on some previous days.

At 3 mg/kg/day, three animals had mastication, and there was a single instance of a fixed stare, salivation, piloerection and rolling gait.

F0 Body Weight ***(Table 2)***

Reference ID: 3367720

Slight intergroup differences in body weight performance during gestation were considered too small to be attributed to treatment.

At 27 mg/kg/day, there was a slight reduction in group mean body weight gain in early lactation. At the lower dosage levels, body weight performance during lactation was similar to Control.

F0 Food Consumption ***(Table 3)***
Food consumption during gestation was essentially similar across all groups.

At 27 mg/kg/day, there was a slight reduction in group mean food consumption during lactation.

Duration of *Gestation, Litter Size and Pup Survival* ***(Tables 4, 5)***
There were no obvious effects of treatment on duration of gestation, litter size or pup survival.

F1 *Litter and Pup Weights* ***(Table 6)***
Mean litter and pup weights were similar in all groups

Pre-Weaning Physical and Functional Development ***(Tables 7, 8)***
There were no obvious effects on the pre-weaning physical or functional development of the F1 pups.

Abnormalities Among F1 *Pups*
The type and distribution of abnormalities did not indicate an association with treatment. Other minor findings, such as mild bruising or cold to touch, that are typical for pre-weanlings were observed but have not been reported.

F1 *Clinical Observations and Necropsy Findings* ***(Table 9)***
The clinical observations and necropsy findings among F1 animals did not indicate any association with treatment.

F1 *Body Weight Performance* ***(Tables 10-12)***
At 27 mg/kg/day, mean weight of males in the early part of the post weaning period was marginally lower than Control, although a similar finding was not observed prior to weaning (see Table 6) nor for females, and therefore the post weaning finding may have been incidental.

Body weights of females during the pre-mating period and throughout gestation and lactation were comparable with Control.

F1 *Assessment* of *Sexual Maturation* ***(Table 13)***
The mean age of vaginal opening was essentially similar in all groups.

Reference ID: 3367720

At 27 mg/kg/day, the mean age of preputial separation was marginally increased, possibly reflecting the slightly lower weights of these animals. At 3 and 9 mg/kg/day, the age of preputial separation was similar to Control.

F1 ***Post Weaning Functional Development (Tables 14 – 16)***
There were no obvious intergroup differences in the performances on the Open Field, Rota-rod or V-maze tests.

F1 ***Reproductive Performance (Tables 17 – 20)***
Mating performance, duration of gestation, litter size, weight and survival were similar in all groups.

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 1 F0 Generation - Group Incidence of Clinical Observations and Necropsy Findings

Observation/Finding	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Clinical Observations				
No. of animals examined	29	26	27	26
Mastication	0	3	18	25
Fixed stare	0	1	7	24
Laboured/irregular respiration	0	0	13	25
Red cage staining	0	0	1	21
Subdued behaviour	0	0	8	23
Salivation	0	1	2	17
Lying on side/back/against cage	0	0	2	10
Pale appearance	0	0	3	12
Chin rubbing	0	0	0	17
Infusion site (tail): - shavings in cuff/sparse hair/wet lesions/ulcers/blood in line/swollen	10	9	13	11
Sparse hair	2	6	1	3
Soft faeces	1	0	0	0
Low water consumption	1	0	0	1
Scratches on leg, tail and feet	1	0	0	0
No teats	0	1	0	0
Piloerection	0	1	2	0
Rolling gait	0	1	0	0
Fitting during dosing	0	0	1	0
Crawling on front paws	0	0	1	0
Eyes partially closed	0	0	0	2
Convulsions	0	0	0	1
Staining	0	0	0	2
Gasping motion	0	0	0	3
Unsteady on feet	0	0	0	2
Retching motion	0	0	0	1
Animal found dead	0	0	0	3
Animal killed prematurely	0	0	1	0
Animal killed due to infusion line problem	2	0	1	0

Table 1 (continued) ***F0 Generation - Group Incidence of Clinical Observations and Necropsy Findings***

Observation/Finding	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Necropsy Findings				
Infusion site – Raw area/scab/crusting/lesion	9	10	11	14
Staining on body	2	1	1	2
Ileum reddened	1	0	0	0
Lumbar lymph nodes enlarged	1	1	0	2
Uterus – Horn dark	0	1	0	0
Uterus – Horns dilated with fluid	0	0	1	0
Cervix enlarged	0	1	0	0
Staining axillary lymph node	0	1	0	0
Ovarian cyst(s)	0	1	0	0
Hairloss	0	1	0	0
Tail – Active dermatitis, abscess formation, myosotis, osteomyelitis and crusting	0	2	1	0
Kidney – Vascular mineralisation costicomedullary junction	0	1	0	0
Liver - Prominent lobulation on all lobes	0	0	1	2
Liver – All lobes pale	0	0	0	1
Thymic atropy. Lung - focal aveolar macrophage accumulation (minimal)	0	0	1	0
Thymus small	0	0	0	1
Lung – All lobes spongy	0	0	0	1
Ulcer in colon	0	0	0	1
Spontaneous cardiomyopathy, severe vasculitis vena cava, thymic atrophy	0	0	0	1

Polidocanol
Peri and Post Natal Study in Rats
Table 2 F0 Generation: Group Mean Body Weights (g) During Gestation and Lactation ± Standard Deviation

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Day of Gestation[a]				
0	267 ± 20	265 ± 22	262 ± 19	260 ± 24
7	304 ± 22	302 ± 27	294 ± 22	293 ± 27
13	339 ± 24	335 ± 31	328 ± 25	326 ± 32
16	360 ± 26	358 ± 34	347 ± 30	347 ± 37
18	386 ± 29	385 ± 36	376 ± 31	373 ± 38
20	404 ± 32	403 ± 35	397 ± 31	395 ± 42
Weight Gain Days 16-20	44	45	50	48
% of Control	-	102	111	109
Day of Lactation[b]				
1	293 ± 24	288 ± 28	286 ± 27	286 ± 31
7	344 ± 24	337 ± 34	334 ± 29	325 ± 36
14	369 ± 24	367 ± 34	356 ± 32	351 ± 39
21	366 ± 28	360 ± 27	353 ± 29	350 ± 29
24	353 ± 26	343 ± 33	340 ± 24	337 ± 28

a = Pregnant animals only
b = Animals rearing young to Day 21 only

Polidocanol
Peri and Post Natal Study in Rats
Table 3 F0 Generation: Group Mean Food Consumption (g) During Gestation and Lactation

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Day of Gestation[a]				
14	28	29	29	29
15	30	29	29	29
16	32	31	30	30
17	32	31	31	32
18	32	31	31	29
19	28	30	30	28
20	20	21	24	22
Day of Lactation[b]				
0-7	284	283	277	261
7-14	484	495	499	461

a = Pregnant animals only
b = Animals rearing young to Day 21 only

Polidocanol
Peri and Post Natal Study in Rats
Table 4 F0 Generation: Duration of Gestation and Overall Litter Performance

	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (3)	3 (9)	4 (27)
Number Pregnant	25	23	24[b]	23[c]
Duration of Gestation (Days)				
20	1	0	0	0
21	10	12	10	8
22	13	11	14	14
23	1	0	0	1
Mean Duration	21.6	21.5	21.6	21.7
Number of females producing a live litter	25	23	24	23
Gestation index as %	100	100	100	100
Mean number of implant sites[a] per pregnancy ± standard deviation	15.0 ± 2.9	15.1 ± 1.7	15.2 ± 2.1	14.2 ± 2.3
Mean total number of pups[a] born	13.6 ± 3.0	13.9 ± 1.2	14.0 ± 2.0	13.3 ± 2.2
Mean number of live pups[a] per litter ± standard deviation:				
Day 0 of lactation	13.3 ± 2.9	13.7 ± 1.4	13.6 ± 2.0	13.1 ± 2.2
Day 1 of lactation	12.5 ± 2.5	12.4 ± 1.8	13.1 ± 1.8	12.4 ± 2.3
Day 4 of lactation	12.3 ± 2.5	11.9 ± 2.2	12.7 ± 1.7	12.2 ± 2.3
Day 7 of lactation	12.3 ± 2.5	11.9 ± 2.2	12.7 ± 1.7	12.2 ± 2.3
Day 14 of lactation	12.3 ± 2.5	11.9 ± 2.2	12.7 ± 1.7	12.2 ± 2.3
Day 21 of lactation	12.3 ± 2.5	11.9 ± 2.2	12.7 ± 1.7	12.1 ± 2.3

[a] = Excludes litters where all pups died
[b] = Excludes Animal 70 where infusion line became detached
[c] = Excludes premature decedents prior to parturition

Polidocanol
Peri and Post Natal Study in Rats
Table 5 F1 Generation: Survival Indices

		Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
		1	2	3	4
		(0)	(3)	(9)	(27)
Birth Index	Mean Litter Index (%)	92	93	92	94
	Number Losing >2 pups	6	5	4	2
	Number of Litters	25	23	23	22
Live Birth Index	Mean Litter Index (%)	95	98	97	99
	Number Losing >1 pup	0	0	0	0
	Number of Litters	25	23	23	22
Viability Index Days 0-4	Mean Litter Index (%)	90	83	94	93
	Number Losing >3 pups	2	0	0	0
	Number of Litters	25	23	23	22
Lactation Index Days 4-21	Mean Litter Index (%)	100	100	100	100
	Number Losing >1 pup	0	0	0	0
	Number of Litters	24	22	23	22
Overall Survival Index Birth-21	Mean Litter Index (%)	91	85	91	92
	Number Losing >4 pups	0	0	0	0
	Number of Litters	24	22	23	22

Polidocanol
Peri and Post Natal Study in Rats
Table 6 F0 Generation: Group Mean Litter and Pup Weight (g) ± Standard Deviation

Day of Lactation	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
LITTER				
Day 1	76 ± 11	75 ± 14	79 ± 8	77 ± 15
Day 4	111 ± 13	108 ± 21	115 ± 11	111 ± 20
Day 7	162 ± 21	162 ± 30	167 ± 19	159 ± 28
Day 14	320 ± 43	324 ± 53	330 ± 31	309 ± 54
Day 21	529 ± 73	531 ± 85	545 ± 60	519 ± 91
Mean of Litter Mean Pup Weight				
MALES				
Day 1	6.5 ± 1.1	6.2 ± 0.7	6.2 ± 0.6	6.4 ± 0.5
Day 4	9.6 ± 1.8	9.5 ± 1.0	9.4 ± 1.1	9.4 ± 1.0
Day 7	14.0 ± 2.5	14.2 ± 1.7	13.6 ± 1.9	13.5 ± 1.6
Day 14	27.4 ± 3.5	28.4 ± 3.0	27.1 ± 3.2	26.2 ± 3.0
Day 21	45.3 ± 6.5	46.7 ± 5.2	44.4 ± 5.9	44.0 ± 5.6
FEMALES				
Day 1	6.0 ± 0.9	5.9 ± 0.6	6.0 ± 0.7	6.1 ± 0.5
Day 4	9.1 ± 1.8	8.9 ± 0.9	8.9 ± 1.3	8.9 ± 0.9
Day 7	13.2 ± 2.4	13.3 ± 1.5	12.9 ± 1.9	12.9 ± 1.5
Day 14	26.0 ± 4.1	26.8 ± 2.9	25.5 ± 3.1	25.0 ± 2.8
Day 21	42.9 ± 7.2	44.0 ± 4.8	42.2 ± 5.3	42.7 ± 5.6

Means exclude litters where all pups died

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 7 F1 Generation: Assessment of Post-Natal Physical Development: Group Mean Day ± Standard Deviation

	Group/Dose Level (mg Polidocanolkg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
MALES				
All Pups Reach Criterion				
Pinna Detachment	3.5 ± 0.8	3.6 ± 0.7	3.7 ± 0.7	3.2 ± 0.7
Upper Incisor Eruption	12.3 ± 1.1	12.6 ± 0.9	12.3 ± 1.2	12.9 ± 1.2
Open Eyes	14.9 ± 1.0	15.4 ± 0.9	15.4 ± 0.9	15.3 ± 0.8
Median Day to Criterion				
Pinna Detachment	3.1 ± 0.8	3.1 ± 0.7	3.0 ± 0.8	2.8 ± 0.7
Upper Incisor Eruption	11.4 ± 0.9	11.9 ± 0.6	11.6 ± 0.9	11.8 ± 0.8
Open Eyes	14.4 ± 1.1	14.9 ± 0.8	14.9 ± 0.9	14.9 ± 0.8
FEMALES				
All Pups Reach Criterion				
Pinna Detachment	3.8 ± 0.8	4.0 ± 0.7	3.6 ± 0.6	3.3 ± 0.8
Upper Incisor Eruption	12.7 ± 1.0	12.9 ± 0.9	12.4 ± 1.0	12.7 ± 1.5
Open Eyes	15.0 ± 0.9	15.2 ± 0.8	15.3 ± 0.9	15.3 ± 1.0
Median Day to Criterion				
Pinna Detachment	3.3 ± 0.6	3.3 ± 0.9	3.0 ± 0.7	2.8 ± 0.7
Upper Incisor Eruption	11.7 ± 1.0	12.0 ± 0.7	11.6 ± 1.1	11.5 ± 1.1
Open Eyes	14.5 ± 0.9	14.8 ± 0.7	14.9 ± 0.9	14.8 ± 0.8

Reference ID: 3367720

Table 8 ***Polidocanol***
Peri and Post Natal Study in Rats
F1 Generation: Assessment of Post-Natal Functional Development – Group Mean Day ± Standard Deviation

Parameter	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
MALES				
Negative Geotaxis	100	100	100	100
Auditory Function	100	100	100	100
Visual Function	100	100	99	100
FEMALES				
Negative Geotaxis	99	100	99	100
Auditory Function	100	100	100	100
Visual Function	100	100	100	100

Polidocanol
Peri and Post Natal Study in Rats
Table 9 F1 Generation: Group Incidence of Clinical Observations and Necropsy Findings – Males and Females

Observation/Finding	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Clinical Observations				
Total No. of animals examined	47	43	46	44
Staining	5	4	2	1
Lesion/scab on mouth	1	0	0	0
Tail damaged	1	1	0	0
Wheezing	0	0	0	1
Red liquid around vagina	1	0	0	0
No teats evident	1	0	0	0
Teat(s) red/swollen	1	1	0	0
Encrustations around muzzle	1	0	0	0
Diarrhoea	1	0	0	0
Litter loss	2	0	0	0
Sparse hair/bald areas	1	3	2	0
Scab(s)	0	0	1	0
Animal pale	0	0	0	0
Necropsy Findings				
Kidney – Pelvic dilation	0	1	0	0
Thymus enlarged	1	0	0	0
Pancreas – Red foci with firm white centres	0	1	0	0
Spleen, liver and pancreatic lymph nodes infiltrated by lymphoblastic lymphoma cells	0	1	0	0

Polidocanol
Peri and Post Natal Study in Rats
Table 10 F1 Generation: Group Mean Body Weight (g) ± Standard Deviation – Males

Nominal Age (Weeks)	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
4	103 ± 17	111 ± 15	108 ± 15	104 ± 17
5	165 ± 16	167 ± 20	165 ± 20	158 ± 24
6	225 ± 19	222 ± 27	227 ± 23	216 ± 31
7	289 ± 22	288 ± 29	287 ± 26	273 ± 32
8	343 ± 25	335 ± 31	338 ± 25	325 ± 33
9	386 ± 29	379 ± 36	381 ± 28	368 ± 36
10	420 ± 33	412 ± 39	413 ± 31	398 ± 38
11	443 ± 35	435 ± 40	440 ± 38	422 ± 39
12	470 ± 38	458 ± 42	464 ± 37	448 ± 40
13	492 ± 42	477 ± 46	483 ± 41	446 ± 46
14	507 ± 46	488 ± 50	495 ± 43	482 ± 51
15	529 ± 50	507 ± 51	514 ± 46	502 ± 53
16	546 ± 54	522 ± 55	531 ± 46	524 ± 55

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 11 F1 Generation: Group Mean Body Weight Prior to Mating (g) ± Standard Deviation

Nominal Age (Weeks)	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
4	96 ± 14	99 ± 15	97 ± 11	93 ± 11
5	134 ± 17	137 ± 15	134 ± 14	132 ± 13
6	166 ± 18	167 ± 17	165 ± 14	166 ± 14
7	192 ± 19	194 ± 16	190 ± 16	193 ± 15
8	214 ± 22	215 ± 20	211 ± 18	216 ± 18
9	233 ± 25	235 ± 23	230 ± 17	234 ± 21
10	244 ± 36	249 ± 24	245 ± 23	250 ± 24

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 12 F1 Generation: Group Mean Body Weight (g) During Gestation and Lactation ± Standard Deviation

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Day of Gestation[a]				
0	255 ± 36	252 ± 23	249 ± 20	256 ± 21
7	293 ± 37	291 ± 25	285 ± 22	293 ± 23
14	338 ± 39	333 ± 24	329 ± 22	337 ± 27
20	421 ± 47	413 ± 30	409 ± 30	423 ± 35
Weight Gain Weeks 0-20	166	161	160	167
% of Control	-	97	96	101
Day of Lactation[b]				
1	292 ± 35	291 ± 27	293 ± 23	298 ± 30
7	338 ± 36	338 ± 30	336 ± 24	346 ± 27
14	363 ± 27	359 ± 28	354 ± 20	364 ± 25

a = Pregnant animals only
b = Excludes litters where all pups died prior to Day 14

Polidocanol
Peri and Post Natal Study in Rats
Table 13 Assessment of Sexual Maturation

Day of Gestation	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Females				
Age (days) at vaginal opening	34.8 ± 3.1	34.7 ± 2.7	34.4 ± 2.1	35.5 ± 2.2
Weight (g) at vaginal opening	117 ± 19	116 ± 13	113 ± 13	117 ± 15
Males				
Age (days) at preputial separation	43.9 ± 2.3	44.1 ± 2.4	44.3 ± 2.7	45.8 ± 3.3
Weight (g) at preputial Separation	211 ± 22	210 ± 16	215 ± 22	216 ± 18

All values given ± Standard deviation

Polidocanol
Peri and Post Natal Study in Rats
Table 14 F1 Generation: Open Field – Group Mean Values ± Standard Deviation

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
MALES				
Time (s) to move from central sector	4.6 ± 5.5	3.1 ± 4.1	3.0 ± 3.6	2.2 ± 1.1
Number of sectors traversed	54.0 ± 25.1	66.4 ± 23.9	60.6 ± 21.0	64.3 ± 21.1
Number of rearings	12.2 ±.6.1	12.8 ± 7.9	12.0 ± 7.1	11.9 ± 5.9
Number of grooming episodes	1.1 ± 1.6	1.0 ± 1.4	0.6 ± 0.7	1.4 ± 1.7
Number of defecation boluses	1.2 ± 1.9	1.9 ± 2.3	1.3 ± 1.7	0.8 ± 1.2
Incidence of abnormalities of gait or general motor activity	0	0	0	0
FEMALES				
Time (s) to move from central sector	2.5 ± 2.3	3.0 ± 1.9	3.0 ± 3.4	2.5 ± 2.8
Number of sectors traversed	76.4 ± 25.7	67.5 ± 21.0	72.6 ± 20.0	73.0 ± 24.6
Number of rearings	18.3 ± 9.2	16.7 ± 8.7	15.5 ± 8.7	13.9 ± 7.4
Number of grooming episodes	1.1 ± 1.3	1.7 ± 1.3	1.3 ± 1.4	0.8 ± 0.8
Number of defecation boluses	1.0 ± 2.1	0.2 ± 0.8	0.5 ± 1.2	0.2 ± 0.9
Incidence of abnormalities of gait or general motor activity	0	0	0	0

Polidocanol
Peri and Post Natal Study in Rats
Table 15 F1 Generation: Rota-Rod Test – Group Mean Time(s) ± Standard Deviation

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
MALES				
Maximum[a]	54.4 ± 33.9	50.5 ± 23.0	59.8 ± 31.5	52.9 ± 27.6
Mean[b]	33.5 ± 15.7	32.1 ± 14.3	38.9 ± 21.2	37.4 ± 18.7
FEMALES				
Maximum[a]	64.0 ± 39.1	66.1 ± 30.6	66.2 ± 38.9	67.8 ± 36.2
Mean[b]	40.9 ± 25.5	44.6 ± 23.8	40.1 ± 24.1	44.5 ± 25.0

a = Mean of the longest time achieved over the 3 trials by each animal
b = Mean of the mean length of time achieved over the 3 trials by each animal

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 16 F1 Generation: Males and Females – Multiple Y Maze Test Group
Mean Values ± Standard Deviation

Males

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
TIME (s)				
Day 1, Trial 1	64.4 ± 25.2	66.6 ± 23.6	70.0 ± 23.0	61.6 ± 23.2
Day 1, Trial 2	47.3 ± 33.2	37.6 ± 26.1	31.0 ± 26.3	43.2 ± 26.8
Day 1, Trial 3	19.3 ± 18.8	18.4 ± 14.3	23.2 ± 20.1	21.0 ± 17.4
Day 3, Trial 1	27.7 ± 24.2	22.3 ± 13.9	28.5 ± 18.6	19.7 ± 13.6
Day 3, Trial 2	20.3 ± 14.8	23.4 ± 18.3	20.3 ± 16.0	19.3 ± 14.5
Day 3, Trial 3	17.2 ± 11.8	15.2 ± 10.7	18.9 ± 17.4	22.3 ± 12.2
ERRORS				
Day 1, Trial 1	4.3 ± 3.0	4.0 ± 2.8	4.7 ± 2.6	4.2 ± 3.2
Day 1, Trial 2	3.7 ± 4.1	2.2 ± 2.7	2.0 ± 3.3	2.7 ± 2.5
Day 1, Trial 3	0.9 ± 1.5	1.0 ± 2.3	1.4 ± 2.5	1.0 ± 2.0
Day 3, Trial 1	2.0 ± 3.1	1.4 ± 2.0	1.3 ± 2.0	0.8 ± 1.4
Day 3, Trial 2	1.2 ± 1.8	1.3 ± 2.0	0.9 ± 1.4	0.9 ± 1.3
Day 3, Trial 3	0.9 ± 1.3	0.6 ± 1.8	0.9 ± 1.4	1.2 ± 1.7

Note: A time of 90 s has been assumed for animals that had to be guided out of the maze

Females

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
TIME (s)				
Day 1, Trial 1	60.2 ± 28.5	64.0 ± 27.4	65.5 ± 23.5	59.4 ± 26.8
Day 1, Trial 2	30.5 ± 22.7	28.0 ± 18.7	28.3 ± 20.9	40.1 ± 28.0
Day 1, Trial 3	15.8 ± 10.8	21.7 ± 16.4	20.3 ± 19.7	27.7 ± 26.3
Day 3, Trial 1	26.6 ± 22.3	18.8 ± 18.0	18.0 ± 16.3	21.1 ± 22.6
Day 3, Trial 2	12.9 ± 10.1	21.8 ± 17.1	12.0 ± 7.0	16.8 ± 13.5
Day 3, Trial 3	22.0 ± 19.0	13.7 ± 11.5	10.6 ± 5.2	14.5 ± 11.8
ERRORS				
Day 1, Trial 1	4.8 ± 3.7	4.2 ± 3.1	5.1 ± 3.6	3.4 ± 2.7
Day 1, Trial 2	1.8 ± 2.6	1.8 ± 2.2	2.5 ± 3.0	2.3 ± 3.0
Day 1, Trial 3	0.9 ± 1.7	1.8 ± 2.9	1.1 ± 2.2	1.9 ± 3.0
Day 3, Trial 1	2.3 ± 3.0	1.4 ± 2.6	1.1 ± 2.1	2.0 ± 3.7
Day 3, Trial 2	0.6 ± 1.1	1.9 ± 2.7	0.8 ± 1.2	0.9 ± 1.0
Day 3, Trial 3	1.8 ± 2.3	0.9 ± 1.7	0.4 ± 0.8	1.2 ± 2.0

Note: A time of 90 s has been assumed for animals that had to be guided out of the maze

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 17 F1 Generation: Mating Performance and Fertility Indices

Number of Nights to Positive Mating Sign	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
	Number of Animals (All became pregnant)			
1	8	3	5	2
2	5	7	9	4
3	0	6	3	8
4	10	6	4	6
7	0	0	1	0
8-14	0	0	1	1
Over 14	1	0	0	0
No clear indication of mating	0	0	0	1
Median number of nights to positive mating sign	2	3	2	3
Number passing one oestrus	0	0	1	0
Number passing two oestruses	0	0	1	0
Number of males paired	23	21	23	22
Number of siring males	22	21	22	21
Male Fertility Index (%)	96	100	96	95
Number of females paired	24	22	23	22
Number pregnant	24	22	23	22
Female Fertility Index (%)	100	100	100	100

Polidocanol
Peri and Post Natal Study in Rats
Table 18 F1 Generation: Duration of Gestation and Overall Litter Performance

	Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
	1 (0)	2 (3)	3 (9)	4 (27)
Number Pregnant	24	22	23	22
Duration of Gestation (Days)				
21	9	7	8	5
22	15	14	14	16
23	0	1	1	0
No clear indication of mating	0	0	0	1
Mean Duration	21.6	21.7	21.7	21.8
Number of females producing a live litter	24	22	23	22
Gestation index as %	100	100	100	100
Mean number of implant sites[a] per pregnancy ± standard deviation	15.4 ± 2.1	15.9 ± 1.7	15.1 ± 1.8	15.9 ± 1.8
Mean total number of pups[a] born	14.5 ± 2.1	14.8 ± 1.8	14.1 ± 2.2	14.9 ± 1.9
Mean number of live pups[a] per litter ± standard deviation:				
Day 0 of lactation	14.5 ± 2.0	14.6 ± 1.7	13.9 ± 2.4	14.9 ± 1.8
Day 1 of lactation	14.2 ± 2.2	14.4 ± 1.6	13.6 ± 2.8	14.9 ± 1.8
Day 4 of lactation	14.1 ± 2.3	14.2 ± 1.5	13.7 ± 2.3	14.7 ± 1.9
Day 7 of lactation	14.1 ± 2.3	14.2 ± 1.5	13.4 ± 2.4	14.7 ± 1.9
Day 14 of lactation	13.9 ± 2.1	14.1 ± 1.6	13.3 ± 2.5	14.6 ± 1.9

[a] = Excludes litters where all pups died

Reference ID: 3367720

Polidocanol
Peri and Post Natal Study in Rats
Table 19 F2 Generation: Survival Indices

		Group/Dose Level (mg Polidocanol.kg^{-1}.day^{-1})			
		1	2	3	4
		(0)	(3)	(9)	(27)
Birth Index	Mean Litter Index (%)	95	93	93	94
	Number Losing >2 pups	3	2	2	2
	Number of Litters	24	22	23	22
Live Birth Index	Mean Litter Index (%)	100	99	99	100
	Number Losing >1 pup	0	0	0	0
	Number of Litters	24	22	23	22
Viability Index Days 0-4	Mean Litter Index (%)	90	97	97	99
	Number Losing >3 pups	2	0	1	0
	Number of Litters	24	22	23	22

Polidocanol
Peri and Post Natal Study in Rats
Table 20 F2 Generation: Group Mean Litter and Pup Weight (g) ± Standard Deviation

Day of Lactation	Group/Dose Level ($mg.kg^{-1}.day^{-1}$)			
	1 (0)	2 (3)	3 (9)	4 (27)
LITTER				
Day 1	92 ± 13	94 ± 12	91 ± 16	101 ± 11
Day 4	136 ± 18	140 ± 21	137 ± 21	149 ± 18
Day 7	197 ± 26	208 ± 30	202 ± 35	216 ± 26
Day 14	375 ± 92	407 ± 45	377 ± 98	415 ± 32
Mean of Litter Mean Pup Weight				
MALES				
Day 1	6.7 ± 0.4	6.8 ± 0.8	7.0 ± 0.8	7.0 ± 0.5
Day 4	9.9 ± 0.7	10.2 ± 1.3	10.4 ± 1.4	10.4 ± 1.0
Day 7	14.2 ± 1.6	15.1 ± 2.1	15.4 ± 1.9	15.1 ± 1.7
Day 14	29.0 ± 3.1	29.6 ± 3.2	30.5 ± 3.0	29.5 ± 2.9
FEMALES				
Day 1	6.3 ± 0.5	6.3 ± 0.7	6.6 ± 0.6	6.7 ± 0.6
Day 4	9.5 ± 0.8	9.6 ± 1.3	9.9 ± 1.2	9.9 ± 1.1
Day 7	14.0 ± 1.5	14.2 ± 1.9	14.8 ± 1.7	14.6 ± 1.8
Day 14	28.2 ± 3.2	28.2 ± 3.2	29.5 ± 2.8	28.1 ± 3.1

Means exclude litters where all pups died

10 Special Toxicology Studies

Study Title: Polidocanol Antigenicity Studies Active Systemic Anaphylactic (ASA) Reaction and Passive Cutaneous Anaphylactic (PCA) Reaction in the Albino Guinea Pig

Key study findings: There was no antigenic potential detected in the ASA and PCA test after treatment with at 1 % stock solution of Polidocanol. Polidocanol used in the described conditions is considered not to be a functional antigen. The clinical signs

Reference ID: 3367720

noted after intravenous treatment with Polidocanol are regarded as toxic responses and not as immunological responses.

Study no: 833398
Volume #, and page #: eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: 9/28/20001
GLP compliance: yes
QA reports: yes
Drug, lot #, radiolabel, and % purity: Batch No. 82652; Purity 100 %
Formulation/vehicle: sterile water

Methods/Results:
An antigenicity study, which consisted of an active systemic anaphylaxis (ASA) and a passive cutaneous anaphylaxis (PCA) test in albino GOHI guinea pigs, was conducted to evaluate the antigenic potential of Polidocanol. Xenogeneic ovalbumin was used as reference test item and was applied in the same manner to confirm the allergenic potential of Ovalbumin in the test method.

The ASA phase comprised 4 groups each of 5 guinea pigs. The first group was sensitized with NaCl 0.9 %, the second and third group with a 1 % stock solution of Polidocanol at 5 mg/kg and 30 mg/kg, respectively. The last group was used as reference group using Ovalbumin at 5 mg/kg. The animals were sensitized once weekly by intraperitoneal injections using a dose volume of 1 (group 1 and 4), 0.5 (group 2) or 3 ml/kg body weight (group 3). The interval between the three sensitization exposures was one week. Two weeks after the last sensitization, the same procedure as in the induction was repeated by using another route of administration: the same application volumes described above were injected into the penis vein.

No deaths were noted in any of the animals challenged with Polidocanol. Four out of 5 guinea pigs previously sensitized with the 1 % stock solution of Polidocanol at 30 mg/kg and intravenously challenged at the same dose showed convulsions with muscular hypertonus during the challenge treatment. They recovered approximately 45 minutes after application. Signs of anaphylaxis and death occurred in all animals challenged with Ovalbumin.

The PCA study comprised 4 groups each of 5 guinea pigs which were sensitized by once weekly intraperitoneal injections. The sensitization phase was performed in the same way as in the induction procedure of the ASA phase by using NaCl 0.9 %, 1 % stock solution of Polidocanol administered at 5 and 30 mg/kg and the reference item Ovalbumin. Spontaneous death occurred in one guinea pig treated at 30 mg/kg 6 days after the last intraperitoneal induction. Blood sampling from the retro-orbital plexus was collected 13 days after the last PCA sensitization in all animals. After blood sampling, the blood was used to determine the total leukocyte count and the serum obtained from the remaining blood was intradermally injected to recipient guinea pigs.

Reference ID: 3367720

In antisera obtained from guinea pigs sensitized with the 1 % stock solution of Polidocanol at 5 and 30 mg/kg, the results of PCA were negative following a 1 ml intravenous or intraperitoneal application/animal with Polidocanol at 0.01 g/ml. However, immediately after the intravenous challenge, convulsions with muscular hypertonus were observed in three out of 9 recipient animals. They recovered approximately 15 minutes later. The skin of the anti-OA-injected guinea pigs (reference group) showed positive reactions in all five animals, defined as the formation of a blue spot more than 5 mm in diameter after intravenous injection of OA at 5 mg/ml in 1 % Evans Blue.

Conclusions:
In conclusion, there was no antigenic potential detected in the ASA and PCA test after treatment with at 1 % stock solution of Polidocanol. Polidocanol used per the described conditions is considered not to be a functional antigen. The clinical signs noted after intravenous treatment with Polidocanol are regarded as toxic responses and not as immunological responses.

Ovalbumin confirmed its antigenic potency throughout the ASA and PCA test.

Reference ID: 3367720

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WILLIAM T LINK
09/04/2013

ALBERT F DEFELICE
09/05/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: **NDA 205098** **Applicant: Provensis Ltd** **Stamp Date: 2/4/13**

Drug Name: VARITHENA (polidocanol) **NDA/BLA Type:** Type 1- New Molecular Entity

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			n/a
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	abuse is unlikely
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

APPEARS THIS WAY ON ORIGINAL

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WILLIAM T LINK
03/13/2013

ALBERT F DEFELICE
04/01/2013