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**PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:**

Methods and compositions for skin necrosis

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This invention is best described in the following statement:

# Methods and compositions for skin necrosis

## Technical Field

The present invention relates to compositions and methods for administering a skin  
5 necrosis composition to an animal. The invention also relates to the administration of a  
composition to an animal for reducing the problem of fly strike.

## Background

A well documented problem experienced by many sheep and wool producers relates  
10 to the occurrence of fly strike in and around the breech area of sheep. Where the breech  
area of the sheep is not appropriately maintained it is subject to soiling by faecal matter  
and urine which is an attractant for insects (such as the gravid fly female) to lay eggs,  
resulting in an enhanced fly strike rate. This is known as breech strike.

Farmers use many methods to control breech strike including chemical application  
15 by jetting and spraying, timing of shearing and crutching to coincide with known times of  
high fly challenge. In addition, the control of diarrhoea by good worm control and  
nutritional management reduces the incidence of breech strike. However, no method is  
permanent and all leave sheep vulnerable to breech strike at certain unpredictable times.

Regular crutching of sheep, that is shearing of wool from the breech region, is a  
20 partially effective preventative method in at least drier localities. However, crutching  
needs to be done reasonably regularly in order to be effective, and is thus laborious and  
adds significantly to the overall cost of wool production. Also, crutching alone, whilst  
assisting with dealing with fly strike, is not a complete solution to the problem because a  
further problem is that sheep generally have loose folds of skin in the breech region, and  
25 moisture urine and faeces caught in the folds of the skin results in dermatitis that creates  
fly attractant sites where fly strike is likely to occur.

Accordingly, methods to tighten the skin and permanently remove wool from the  
breech region of sheep in order to reduce the problem of fly breech have been investigated  
for many years. One such procedure, known as a mulesing operation, was developed by  
30 John Mules during the early 1930s and involves the surgical removal of folds of skin in the  
breech region of the sheep in order to tighten the skin to thereby reduce the folds of skin in  
this region, and increase the area of flat, bare skin on the perineum. In its most basic form,  
the operation involves cutting the fold of skin on either side of the perineal area and then  
the allowing the resulting wound to heal. This operation has been modified over the years

to remove skin on or adjacent the tail region of the sheep and also in the crutch region. More radical procedures involve the removal of skin from the tail, the so called "Radical Mules Operation".

5 Once the wounds caused by the mulesing operation have healed, the skin around the crutch region of the sheep is generally pulled tighter, thereby expanding the naturally occurring bare area of skin around the anus and in the perineum region of the sheep, thereby reducing the problem of soiling in this region, and hence reducing the incidence of breech fly strike.

10 However, the mulesing operation is problematic. One of the main problems is that it is painful and animal welfare considerations prompt a change to this practice. In addition, the wounds resulting from the operation are subject to infection and can be associated with the transmission of arthritis and other infections and are themselves susceptible to fly strike until they are healed. The wounds also take a significant period of time to heal which results in stress to and reduced growth of the lambs.

15 Accordingly, there is a need to find an alternative to the mulesing operation, which is less painful and injurious to the animal, but which will provide at least some of the advantages of the mulesing operation.

### Summary

20 In a first aspect, the invention provides a method of generating skin necrosis in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.

In a second aspect, the invention provides a method for reducing the incidence of fly strike in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.

25 In a third aspect, the invention provides a method of mulesing the breech region of an animal, the method comprising administering a skin necrosis composition subdermally to the animal.

In a fourth aspect, the invention provides a method of inhibiting nutrient supply to a portion of skin of an animal, the method comprising administering a skin necrosis composition subdermally to the portion of skin, wherein the composition forms a plaque isolating the portion of skin from the basal membrane of the dermis.

30 In a fifth aspect, the invention provides a method of inducing scar tissue formation in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.

In a sixth aspect, the invention provides a method of inducing skin tightening in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.

In one embodiment of the first to sixth aspects, the skin necrosis composition asphyxiates a portion of skin. The asphyxiation may result in restriction or termination of nutrient supply to the skin. The asphyxiation may result in the accumulation of waste products in the skin.

In one embodiment of the first to sixth aspects, the skin necrosis composition may comprise at least one polymer glue, at least one polymer precipitant, or a combination thereof.

The polymer glue may be a cyanoacrylate compound.

The skin necrosis composition may further comprise a viscosity imparting agent.

The viscosity imparting agent may be Newtonian, for example glycerol, mannitol, sorbitol, or non-Newtonian, for example a cellulose derivative or polyvinylpyrrolidone.

The skin necrosis composition may further comprise a cyanoacrylate polymerisation inhibitor.

The cyanoacrylate polymerisation inhibitor may be an acid, sulphur dioxide a combination thereof. The acid may be acetic acid or glacial acetic acid. In one embodiment, the cyanoacrylate polymerisation inhibitor is glacial acetic acid.

The skin necrosis composition may be co-administered with a destabilising base.

The destabilising base may be sodium bicarbonate.

In another embodiment of the first to sixth aspects, the skin necrosis composition may comprise a polymer precipitate formed in situ by co-administration of a soluble polymer compound and a polyvalent cation.

The soluble polymer compound may be selected from the group comprising an alginate, a polyurethane, a polypropylene, a polycarbonate a polyvinyl compound or a combination thereof.

The alginate may be sodium alginate, potassium alginate or combinations thereof.

The polyvalent cation may be selected from the group comprising calcium chloride, strontium chloride, barium chloride, aluminium chloride, magnesium chloride and zinc chloride or any combination thereof. In one embodiment, the polyvalent cation is calcium chloride.

In another embodiment of the first to sixth aspects, the skin necrosis composition may form a plaque after administration.

In one embodiment of the first to sixth aspects, the skin necrosing composition may be subdermally administered to a skin fold. The skin fold may adhere to itself.

The skin necrosis composition may be administered at a rate of between about 0.1mL and about 2.0 mL per square centimetre of skin.

The necrosis composition may be administered with, or further comprise, one or more anaesthetics.

5 The anaesthetic may be selected from the group consisting of: procaine, prilocaine, mepivacaine, lignocaine, bupivacaine and any combination thereof.

In one embodiment the animal may be selected from the group comprising sheep, goats, and camelids. The camelids may be alpacas, llamas and camels.

10 In one embodiment of the first to sixth aspects, the skin necrosis composition may be administered to the breech region of the animal.

The compositions used in the methods of the first to sixth aspects may be delivered together with a pharmaceutically acceptable carriers, diluents, adjuvants and/or excipients and/or dyes.

15 In the methods of the first to sixth aspects, multiple simultaneous doses of the composition and/or the anaesthetic may be administered.

In one embodiment, the compositions used in the methods of the first to sixth aspects may be delivered by high pressure injection.

20 In a seventh aspect, the invention provides a skin necrosis composition comprising at least one cyanoacrylate compound and optionally at least one of a viscosity imparting agent, cyanoacrylate polymerisation inhibitor, anaesthetic, dye, pharmaceutically acceptable carrier, diluent, adjuvant, excipient, or any combination thereof.

25 In an eighth aspect, the invention provides a skin necrosis composition comprising a polymer precipitate compound and optionally at least one of a viscosity imparting agent, anaesthetic, dye, pharmaceutically acceptable carrier, diluent, adjuvant, excipient, or any combination thereof.

In a ninth aspect, the invention provides a skin necrosis composition comprising a first part including at least one soluble polymer compound and a second part comprising at least one polyvalent cation.

30 The first and/or second parts of the composition of the ninth aspect may optionally further comprise at least one of a viscosity imparting agent, anaesthetic, dye, pharmaceutically acceptable carrier, diluent, adjuvant, excipient, or any combination thereof.

35 In a tenth aspect of the invention, there is provided a method of generating skin necrosis in an animal, the method comprising subdermally administering a composition comprising at least one polymer glue to a fold of skin, wherein a first portion of the fold

adheres to a second portion of the such that the fold of skin substantially isolated from underlying tissue.

In an eleventh aspect of the invention, there is provided a method of generating skin necrosis in an animal, the method comprising subdermally administering a composition comprising at least one polymer compound to a portion of the skin, wherein the polymer compound forms a plaque under the portion of skin, and wherein the plaque substantially isolates the portion skin from underlying issue.

The polymer compound may be a polymer glue, a polymer precipitant or a combination thereof.

The polymer glue may be a cyanoacrylate compound.

The polymer precipitate may be selected from the group comprising calcium alginate, polycarbonate, polyurethane, polypropylene, a polyvinyl compound or a combination thereof.

The calcium alginate may be formed *in situ* after co-administration of sodium alginate with calcium chloride.

### Definitions

The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers, but not the exclusion of any other step or element or integer or group of elements or integers. Thus, in the context of this specification, the term "comprising" means "including principally, but not necessarily solely".

As used herein, the term "skin necrosis composition" refers to a composition which has the effect of causing skin death and/or removing the skin when administered to an animal.

As used herein, the term "skin necrotizing agent" refers to an agent, such as a chemical compound, which has the effect of necrosing the skin.

As used herein, the terms "subdermally" and "subcutaneously" have the same meaning and refer to the zone of the skin beneath the epidermis and dermis but above the musculature. Therefore, the terms specifically exclude any region within the epidermis, dermis or musculature.

As used herein, the term "breech region" refers to the area around the vulva and anus of the animal that may collect urine and/or faeces. It includes the perineum, the backs of the upper part of the hind legs, and also the tail.

5 An "effective amount", as used herein, includes within its meaning a non-toxic but sufficient amount of the particular composition to which it is referring to provide the desired necrotizing effect.

As used herein, the term "multiple simultaneous doses" refers to separate doses of the composition that are delivered at substantially the same time, but not at the exact same location.

10 As used herein, the expression "delivered with" in the context of an additional agent such as a local anaesthetic includes the situation where the additional agent is present in the skin necrosis composition that is administered, and also the situation where the additional agent is not present in the skin necrosis composition, but rather administered separately, simultaneously or sequentially with the skin necrosis composition.

15 As used herein, the term "fly strike" means infestation of tissue by larvae or maggots of flies.

As used herein, the term "plaque" means a discrete deposit of material in a bodily tissue or organ.

20

### Detailed Description

The present invention is based on the discovery that the subdermal delivery of a skin necrosis composition, for example in the breech region of an animal, causes the skin to necrose (or die) and eventually detach from the animal. The necrosis of this skin is followed by scar formation and results in a tightening of the remaining skin in the breech region. This removes wrinkles and tightens the skin of the breech region, thereby providing at least some of the advantages of the mulesing operation.

25 The subdermal delivery of skin necrosis compositions facilitates the reduction of the incidence of fly strike in the breech region of the animal and also facilitates the shedding of urine, prevention of dag formation and urine stain on the breech region and the back legs of the animal.

30 The methods of the present invention are useful for the same or similar problems that exist in any animal which suffers from or is prone to flystrike, for example, animals with excessive hair or wool about the breech region which the animal either finds difficult to groom, or neglects to groom. Such animals include sheep, goats, alpacas and other camelids. The animal may be a sheep, for example a lamb, or a weaner or the like.

Without wishing to be bound by theory or mode of action, it is believed that subdermal preferably rapid administration of the skin necrosis composition minimises pain in the animal that occurs in slower and labour intensive delivery methods, such as topical application or multiple non-simultaneous injections. Again, without wishing to be bound  
5 by any particular theory, it is believed that the preferably rapid delivery of the composition beneath the surface of the skin results in the rapid isolation of nerves in the region. As a consequence, the same or similar outcome as the mulesing operation can be achieved with a reduction in pain experienced by the animal. Moreover, an anaesthetic may be included with the composition to further assist in this respect.

10 The compositions used in the methods of the present invention are administered subdermally facilitating rapid necrosis and thus minimising pain and discomfort for the animal. This avoids the considerable pain and discomfort associated with other forms of administration, and in particular the intradermal and intramuscular routes.

#### 15 *Compositions*

The use of cytotoxic, biologically-active, or caustic chemicals to destroy living skin has met with varying success. These categories of chemicals tended to have similar effects at the skin level and shared, in most cases, serious undesirable effects. Consequently as described herein "non-chemically active" injectable substances were used  
20 to destroy and necrose targeted skin. Examples of these "non-chemically active" substances include polymers, polymer glues and glue like compounds (such as cyanoacrylate compounds) and polymer precipitates such as alginate precipitates.

The compositions used in the methods of the invention include one or more skin necrotizing agents.

25 The skin necrosing agents may be polymer glues, polymer precipitates or polymers such as polyurethane, polycarbonate, polypropylene or poly vinyl polymers, or combinations thereof.

The polymer glue may be a cyanoacrylate compound.

The polymer precipitate may be calcium alginate.

30 In one embodiment, the skin necrotizing agent is a cyanoacrylate compound which may be accompanied by a polymerisation inhibitor to stabilise the compound, a viscosity imparting agent or a combination thereof.

In another embodiment, the skin necrosing agent is an alginate precipitant formed in situ by subdermal co-injection of an aqueous sodium alginate solution with a polyvalent  
35 cation.

The compositions used in the methods of the invention may additionally include at least one anaesthetic.

The anaesthetic may be selected from the group comprising procaine, prilocaine, mepivacaine, lignocaine, and bupivacaine. The amount of anaesthetic administered may be between about 1% (w/v) to about 4% (w/v) of the total composition.

In one embodiment, compositions of the invention include a composition comprising a polymer glue and/or polymer precipitate and optionally a viscosity imparting agent.

In another embodiment, compositions of the invention include calcium alginate formed from in situ precipitation of sodium alginate with calcium chloride

In another embodiment, compositions of the invention include at least one cyanoacrylate compound, acetic acid and at least one diluent. In a preferred embodiment, the acetic acid is glacial acetic acid.

#### *Compositions comprising cyanoacrylate compounds*

Cyanoacrylate compounds are advantageous as they are widely used in human and veterinary medicine and dentistry, such as in surgical glues, so they are readily available.

The cyanoacrylate compounds for use in the methods of the present invention may be selected from the group comprising octyl-2-cyanoacrylate, N-butyl-cyanoacrylate, methyl-2-cyanoacrylate, ethyl-2-cyanoacrylate, butyl-2-cyanoacrylate, isobutyl-2-cyanoacrylate, ethoxyethyl-2-cyanoacrylate, allyl-2-cyanoacrylate, methoxyethyl-2-cyanoacrylate, propyl-2-cyanoacrylate, isopropyl-2-cyanoacrylate or any combination thereof.

In one embodiment, the cyanoacrylate compound is octyl-2-cyanoacrylate.

The cyanoacrylate compound in the compositions may be present in concentrations between about 1% and about 95% (w/v) or between about 10% and about 90% (w/v) or between about 40% and about 90% (w/v) or between about 60% and about 90% (w/v) or between about 85% and about 90% (w/v).

In a preferred embodiment, the cyanoacrylate compound in the compositions is present in a concentration of between about 85% (w/v) and about 90% (w/v).

In another preferred embodiment, the cyanoacrylate compound in the compositions comprise cyanoacrylate compound in a concentration of between about 85% (w/v) and about 90% (w/v), and glacial acetic acid in a concentration of between about 10% (v/v) and about 15% (v/v).

The polymerisation inhibitor may be selected from the group comprising acids, sulphur dioxide, or a combination thereof.

The acid may be selected from the group comprising acetic, propionic, butyric, isobutyric, valeric, caproic, heptanoic and hexanoic acids, isoheptanoic, octanoic, 2-ethylhexanoic, nonanoic, decanoic, undecanoic, dodecanoic, neodecanoic, lactic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid or any combinations thereof.

Glacial acetic acid which is a normal ruminant body metabolite and cyanoacrylate polymerisation inhibitor which provides a composition with improved handling properties while dissipating on injection to allow rapid adhesion. Accordingly, in a preferred embodiment the polymerisation inhibitor is glacial acetic acid.

The acid may be present in an amount from about 1% (v/v) to about 50% (v/v) or from about 2.5% (v/v) to about 40% (v/v), or from about 5% (v/v) to about 30% (v/v), or from about 7.5% (v/v) to about 20% (v/v), or from about 10% (v/v) to about 15% (v/v). In a preferred embodiment, the acid is present in an amount from between about 10% (v/v) to about 15% (v/v).

In another embodiment the polymerisation inhibitor may be a sugar. The sugar may be selected from the group comprising mannitol, sorbitol and glycerol.

In one embodiment the compositions containing cyanoacrylates may be co-administered with a destabilising base. The destabilising base may be sodium bicarbonate or sodium carbonate. The sodium bicarbonate may be present in concentrations of between about 0.5% to about 9% (w/v). The pH of the destabilising base should be range in pH from about 7.5 to about 9.5.

The destabilising base can may contain at least one local anaesthetic that may be selected from the sodium channel blocker group, for example procaine, prilocaine, mepivacaine, lignocaine, and bupivacaine. The amount of local anaesthetic administered may be between about 0.1% (v/v) to about 50% (v/v) or about 1% (v/v) to about 4% (v/v) of the total composition. The anaesthetic solution used may have a concentration of about 10 to about 30 mg/ml, or alternatively about 20 mg/ml.

The local anaesthetic acts to even further prevent or reduce any acute and sub-acute pain that may occur in response to the delivery of the skin necrosis composition and resulting necrosis of skin in the area of the animal (e.g. in the breech region).

The concentration of anaesthetic in base solution may be between about 0.1% (w/v) to about to about 80% (w/v) or about 5% (w/v) to about to about 60% (w/v). In one embodiment the concentration of anaesthetic in base solution is about 40% (w/v). In another embodiment the concentration of anaesthetic in base solution is below about 12% (w/v). The concentration of the anaesthetic in base solution may be between about 20mg/ml and 200mg/ml. The base mixture may be applied at a rate of between about 0.1ml/5cm<sup>2</sup> and about 2ml/5cm<sup>2</sup>. In one embodiment the base mixture is applied at a rate of about 1ml/5cm<sup>2</sup> that being about 0.2ml/ cm<sup>2</sup> and anaesthetic concentration being about 10mg/cm<sup>2</sup>.

#### *Compositions comprising polymer compounds*

Many inert polymer compounds such as polyurethane, polycarbonate, polypropylene and poly-vinyl and the like are contemplated for use in the methods of the invention.

The polymer compounds used in the compositions of the invention may be polymer precipitates.

The polymer precipitate in the compositions may be present in concentrations between about 0.1% (w/v) and about 20.0% (w/v) or about 0.1% (w/v) and about 2.0% (w/v) or 0.5% (w/v) and about 1.5% (w/v).

The polymer may be comprised of the seaweed extract and food chemical Sodium Alginate precipitated under the targeted skin with a dilute solution of Calcium Chloride, both harmless body salts. This polymer is extremely biologically safe. The first attempt at application of this mixture produced a satisfactory swelling in the target skin, but then disappointingly dissipated in 2 days. However in about 5 to 6 days time a discolouration in the target skin was noticed not dissimilar the delayed onset of skin destruction effected by temporary clip placement; and probably through the same skin cell self destruction modality triggered by cell insult for the threshold period of about 1 to 2 days.

In one embodiment a polymer precipitate for use in the methods invention is prepared *in situ* by subdermal co-injection of an alginate solution with a polyvalent cationic solution.

The alginate may be sodium alginate.

In some embodiments the amount of alginate may be from about 0.1% (w/v) and about 20.0% (w/v) or about 0.1% (w/v) alginate and 2.0% (w/v) sodium alginate. In another embodiment the amount of alginate may be about 1% (w/v) alginate.

In one embodiment the volume of alginate containing composition administered may be between about 1ml per 24 square centimetres and about 6ml per 24 square centimetres. In one embodiment volume of alginate containing composition may be about 3ml/24 square centimetres. That is, in one embodiment the volume of alginate containing composition administered may be between about 0.04ml per square centimetre and about 0.25ml per square centimetre. These volumes being injected with concentration in aqueous solution of between about 0.1% (w/v) to about 20% (w/v) or about 0.1% (w/v) and about 2.0% (w/v) or about 0.1% (w/v) sodium alginate and about 2% (w/v) sodium alginate and preferably about 1% (w/v) sodium alginate.

In one embodiment the polyvalent cation may be in solution. The polyvalent may be calcium chloride administered at volumes of between about 1ml per square centimetre and about 6ml per 24 square centimetres. In one embodiment the polyvalent cation solution may be administered at a volume of about 3ml per 24 square centimetres.

In one embodiment the concentration of the polyvalent cation may be between about 0.02 molar and about 0.2 molar that is between about 2.22g per litre and about 22.2g/litre. In one embodiment the concentration of the polyvalent cation may be about 1.1% (w/v).

In one embodiment the polyvalent cation and polymer compound may be co-injected or injected separately and in various volumes such that anything from 0.222% (w/v) polyvalent cation (e.g. calcium chloride) is injected with 2% (w/v) polymer compound (e.g. sodium alginate). In one embodiment about 1% sodium alginate (w/v) is injected with about 1.1% w/v calcium chloride thus delivering 2.5 mg calcium alginate per square centimetre.

Further it is anticipated that non active or inert chemicals, the core principle of these concepts not only avoid reactions in the target animals metabolism, and with any other drug to be co-injected, but also avoid expensive, time delaying, problems with registration.

Preferably aqueous sodium alginate is used. The polyvalent cationic solution is selected from the group comprising of a solution of calcium chloride ( $\text{CaCl}_2$ ), strontium chloride ( $\text{SrCl}_2$ ), barium chloride ( $\text{BaCl}_2$ ) aluminium chloride ( $\text{AlCl}_3$ ), Magnesium Chloride ( $\text{MgCl}_2$ ) and Zinc Chloride ( $\text{ZnCl}_2$ ). Preferably, the polyvalent cationic solution is calcium chloride.

The amount of the polyvalent cationic solution used in this invention may be between about 0.01% (w/v) and about 50% (w/v), or between about 0.011% (w/v) and

about 11% (w/v) or between about 0.222% (w/v) and about 2.22% (w/v). In one embodiment, the amount of polyvalent cationic solution used is 1.1% (w/v)

In some embodiments *in situ* the polymer gel precipitate is calcium alginate. It will be understood that on precipitation the calcium alginate gel matrix will be substantially aqueous. In an embodiment the gel matrix may include sequestered local anaesthetic may be selected from the sodium channel blocker group, for example procaine, prilocaine, mepivacaine, lignocaine, and bupivacaine. The amount of local anaesthetic administered may be between about 0.1% (v/v) to about 50% (v/v) or about 1% (w/v) to about 4% (w/v) of the total composition. The anaesthetic solution used may have a concentration of about 10 to about 30 mg/ml, or alternatively about 20 mg/ml.

The one or more local anaesthetics act to even further prevent or reduce any acute and sub-acute pain that may occur in response to the delivery of the skin necrosis composition and resulting necrosis of skin in the area of the animal (e.g. in the breech region).

The concentration of the cyanoacrylate compound and the volume given per injection in the skin necrosis composition can be adjusted based on the required total amount of chemical to be administered and the total number of doses delivered subdermally to the animal per unit of skin area.

#### *Viscosity Imparting Agents*

The skin necrosis compositions used in the methods of the invention may be viscous and/or include one or more viscosity imparting agents. The presence of the viscosity imparting agent assists in retaining the composition at the targeted site of administration, thereby minimising diffusion and damage and/or pain to the surrounding areas.

The viscosity imparting agent may be newtonian, for example glycerol, or non-newtonian, for example a cellulose derivative or polyvinylpyrrolidone.

In one embodiment the viscosity imparting agent may include, by way of example, a partial polymer of the alkyl cyanoacrylate, polymethyl methacrylate (PMMA) or other preformed polymers soluble in the alkyl cyanoacrylate, a suspending agent such as fumed silica, and the like.

The amount of the viscosity imparting agent present in the composition may be between about 0.1% (w/v) to about 50% (w/v) or about 0.5% and about 10% (w/v), or between about 0.5% and about 8% (w/v), or between about 1% and about 7% (w/v), or between about 1% and about 6% (w/v), or between about 2% and about 5% (w/v), or between about 2% and about 4% (w/v).

The viscosity imparting agent may result in the skin necrosis composition having a thixotropic property. That is, the composition may form a gel once delivered beneath the surface of the skin.

In one embodiment, the viscosity imparting agent is polyvinylpyrrolidone.

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#### *Local Anaesthetics and Analgesics.*

The skin necrosis composition may also be delivered with one or more local anaesthetics. The one or more local anaesthetics may be present in the skin necrosis composition, or alternatively the local anaesthetics may be administered separately,  
10 simultaneously or sequentially with the skin necrosis composition.

The one or more local anaesthetics act to even further prevent or reduce any acute and sub-acute pain that may occur in response to the delivery of the skin necrosis composition and resulting necrosis of skin in the area of the animal (e.g. in the breech region).

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The local anaesthetics may be selected from the sodium channel blocker group, for example procaine, prilocaine, mepivacaine, lignocaine, and bupivacaine. The use of other components which have local anaesthetic properties is also contemplated in the methods of the invention. For example, opioid analgesics, beta-adrenoreceptor antagonists, and anti-histamines.

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#### *Carriers, Diluents, Adjuvants and Excipients*

Whilst it is possible for the skin necrosis composition to be delivered alone, it may be preferable that the component(s) of the composition be delivered together with one or more pharmaceutically acceptable carriers, diluents, adjuvants, excipients, dyes, activators  
25 or diluent neutralisers.

In general, pharmaceutical compositions may be prepared according to methods which are known to those of ordinary skill in the art.

Each carrier, diluent, adjuvant and/or excipient must be pharmaceutically acceptable such that it is compatible with the components of the skin necrosis composition  
30 and does not cause adverse side effects to the animal.

Examples of pharmaceutically acceptable carriers or diluents are demineralised or distilled water, saline solution, polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysiloxane; volatile silicones, cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium  
35 carboxymethylcellulose or hydroxypropylmethylcellulose, lower alkanols, for example ethanol or iso-propanol; lower aralkanols, lower polyalkylene glycols or lower alkylene

glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerine, polyvinylpyrrolidone, agar, carrageenan; gum tragacanth or gum acacia, and petroleum jelly. The carrier or carriers may form from between 10% to 99.9% by weight of the compositions. In one embodiment the carrier or carriers may form about 80% by weight of the compositions.

For administration as an injectable solution or suspension, non-toxic diluents or carriers can include Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2-propylene glycol.

Suitable adjuvants typically include emollients, emulsifiers, viscosity imparting agents, preservatives, bactericides and buffering agents.

It is contemplated that the pharmaceutically acceptable carrier may include a water-soluble substance or a water-insoluble substance.

The pH of the skin necrosis composition may be between about 6.5 and about 8.2, or between about 6.7 and about 8.0, or between about 6.8 and about 7.9, or between 6.9 and 7.9 or between 7.0 and 7.8.

The compositions used in the methods of the invention may also include dyes to allow visualisation of treated areas of the skin so as to avoid double treatment. Examples of suitable dyes include but are not limited to, brilliant blue FCF, methylene blue or combinations thereof.

#### *Methods of administration*

In one embodiment the invention provides methods for using a composition comprising at least one cyanoacrylate compound to glue a skin fold to produce a thin line of pathology. This results in an uncomplicated short healing time, very little initial discomfort and no residual discernable pain. Typically the skin fold would be drawn up and the sides compressed together before subdermal injection of a composition comprising a cyanoacrylate compound at one or more sites. This produces an adherence between areas of skin surrounding the fold such that the skin of the fold is substantially isolated from the body and remaining skin of the animal by the cyanoacrylate adhesion. Necrosis of the skin follows injection of the cyanoacrylate compound and a normal healing process, including scar formation and preferably skin tightening ensues.

In some cases skin segments surrounding the fold may not adhere but subdermal injection of the cyanoacrylate compound results in a plaque of polymerised cyanoacrylate under the skin which induces necrosis of the overlying skin followed by a normal healing process including scar formation and preferably skin tightening .

In one embodiment the invention provides methods for using a composition comprising at least one polymer precipitate (for example, calcium alginate, polyurethane, polycarbonate, polypropylene or a polyvinyl compound) for subdermal injection of the polymer precipitate to form a plaque of polymer precipitate under the skin which induces necrosis of the overlying skin followed by a normal healing process including scar formation and preferably skin tightening.

The fold of skin targeted is typically one that would otherwise be cut in traditional mulesing practice. However, it is contemplated that the methods of the invention are applicable to any area of skin which it may be desirable to remove.

Where the animal to which the skin necrosis composition is to be administered is a sheep, it is envisaged that the composition may be administered to young sheep at or around the age when a mulesing operation would traditionally be conducted on the lamb. Treatment of older sheep is now also possible as welfare objections to treating older sheep no longer apply.

The skin necrosis composition may also be subdermally administered beneath the surface of an area of the skin more than once at various time intervals, for example prime administration followed by a boost administration.

In one embodiment, multiple simultaneous doses are administered by an apparatus capable of parallel delivery. This allows separate chemicals to be injected, such that they only meet and mix in the target skin; after injection. This is particularly useful in applying a calcium alginate precipitate as a necrosis inducing plaque by for example injecting sodium alginate and calcium chloride, such that they mix in the skin to form a calcium alginate precipitate.

Multiple simultaneous doses of the compositions may be administered to an area of the skin of an animal, for example in the breech area. Multiple simultaneous doses may be administered between about 0.5 and 2.0 cm apart in a line or grid pattern on the area of the skin.

For example, a total of 16 doses may be delivered in a line or grid-like fashion about 1 cm apart from each other to allow for 0.5cm diffusion of the compound once delivered beneath the surface of the skin simultaneously in a 3cm x 3cm grid. This results in treatments of a 16cm<sup>2</sup> skin area (allowing for diffusion)

The skin necrosis composition may be a liquid that is administered in a volume of between about 0.01 ml to 1 ml, or between about 0.1 ml and 0.5ml. In one embodiment, the volume of the composition delivered may be about 0.1ml.

The skin necrosis composition may be at physiological pH, i.e. between about pH 7.0 to about 7.8

A number of means of subdermal delivery of the composition under the surface of the skin are contemplated.

For example the composition may be delivered under the surface of the skin by use of a mechanical injection means, i.e., either needle or a needle-less means. A needle means may include multiple needles arranged in a predetermined manner to inject the composition at spaced apart sites. Needle-less means may include multiple means arranged in a predetermined manner for injecting at high pressure the skin necrosis composition at a sufficient velocity to rapidly penetrate the epidermis under the dermal region directly, without piercing the layer of muscle. The composition may be delivered by a needle-less means so as to avoid issues that would otherwise arise with the use of a needle mechanical injection means, such as disposal of needles. Examples of suitable needle-less means include the MIT Agro jet and the dermaject.

In some embodiments the skin necrosing agent may be formed *in situ* at the injection site. In such cases the necrosing agent may be for example calcium alginate formed *in situ* by co-injection, or sequential injection at or near the same site of two parts (for example aqueous sodium alginate as one part and a solution of calcium chloride as another part) which form the calcium alginate after injection.

It is also contemplated that the compositions of the invention may be delivered under the surface of the skin by use of a non-mechanical means that allows for the rapid delivery of the composition beneath the surface of the skin.

Delivery of the composition as already described may also provide that relatively lower concentrations are required as most (if not all) of the composition is delivered subdermally. This also provides safer handling by the operator, and minimises toxicity to the animal.

Although these compositions are contemplated to be typically administered at the rear or breech region of the animal in order to reduce the incidence of fly strike, the area of skin where the compositions is delivered is not limited to its breech region. It is contemplated that it may be necessary to deliver such a composition beneath the surface of the skin in other regions. Furthermore, the compositions may be delivered beneath the surface of the skin for the purpose of treating other conditions which require an area of the skin to be necrotized, e.g., removal of skin cancers, branding, pizzle ringing, wiggling and jowling.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text. All of these different combinations constitute various alternative aspects of the invention.

## Examples

The invention will now be described in more detail, by way of illustration only, with respect to the following examples. The examples are intended to serve to illustrate this invention and should not be construed as limiting the generality of the disclosure of the description throughout this specification.

### Example 1 – Skin Necrosis Compositions

Examples of skin necrosis compositions that may be administered in the methods of the invention include the following:

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#### *1.1 Cyanoacrylate composition*

Methyl-2-cyanoacrylate:	85-90% (v/v)
Glacial acetic acid:	10-15% (v/v)

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#### *1.2 Alginate composition:*

Part 1: sodium alginate:	1-2%
Part 2: calcium chloride:	0.02 – 0.2 M

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**The claims defining the invention are as follows:**

1. A method of generating skin necrosis in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.
- 5 2. A method for reducing the incidence of fly strike in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.
3. A method of mulesing the breech region of an animal, the method comprising administering a skin necrosis composition subdermally to the animal.
4. A method of inhibiting nutrient supply to a portion of skin of an  
10 animal, the method comprising administering a skin necrosis composition subdermally to the portion of skin, wherein the composition forms a plaque isolating the portion of skin from the basal membrane of the dermis.
5. A method of inducing scar tissue formation in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.
- 15 6. A method of inducing skin tightening in an animal, the method comprising administering a skin necrosis composition subdermally to the animal.
7. The method according to any one of claims 1 to 6, wherein the skin necrosis composition asphyxiates at least a portion of the skin.
8. The method according to any one of claims 1 to 7, wherein the skin  
20 necrosis composition comprises at least one polymer glue, at least one polymer precipitant, or a combination thereof.
9. The method according to claim 8, wherein the polymer glue is a cyanoacrylate compound.
10. The method according to any one of claims 1 to 9, wherein the skin  
25 necrosis composition further comprises a viscosity imparting agent.
11. The method according to claim 10, wherein the viscosity imparting agent is a Newtonian viscosity imparting agent.
12. The method according to claim 11, wherein the Newtonian viscosity  
30 imparting agent is selected from the group consisting of glycerol, mannitol, sorbitol, or combinations thereof.
13. The method according to claim 11, wherein the viscosity imparting agent is a non-Newtonian viscosity imparting agent.
14. The method according to claim 13, wherein the non-Newtonian viscosity imparting agent is a cellulose derivative or polyvinylpyrrolidone.

15. The method according to any one of claims 1 to 14, wherein the skin necrosis composition further comprises a cyanoacrylate polymerisation inhibitor.

16. The method according to claim 15, wherein the cyanoacrylate polymerisation inhibitor is glacial acetic acid.

5 17. The method according to any one of claims 1 to 16, wherein the skin necrosis composition is co-administered with a destabilising base.

18. The method according to claim 17, wherein the destabilising base is sodium bicarbonate.

10 19. The method according to any one of claims 1 to 7, wherein the skin necrosis composition comprises a polymer precipitate formed in situ by co-administration of a soluble polymer compound and a polyvalent cation.

20. The method according to claim 19, wherein the soluble polymer compound is selected from the group consisting of an alginate, a polyurethane, a polypropylene, a polycarbonate, a polyvinyl compound, or a combination thereof.

15 21. The method according to claim 20, wherein the alginate is sodium alginate, potassium alginate, or a combination thereof.

22. The method according to any one of claims 19 to 21, wherein the polyvalent cation is calcium chloride.

20 23. The method according to any one of claims 1 to 22, wherein the skin necrosis composition forms a plaque after administration.

24. The method according to any one of claims 1 to 22, wherein the skin necrosing composition is subdermally administered to a skin fold.

25 25. The method according to any one of claims 1 to 24, wherein the skin necrosis composition is administered at a rate of between about 0.1mL and about 2.0 mL per square centimetre of skin.

26. The method according to any one of claims 1 to 25, wherein the skin necrosis composition is administered with, or further comprises one or more anaesthetics.

30 27. The method according to claim 26, wherein the anaesthetic is selected from the group consisting of procaine, prilocaine, mepivacaine, lignocaine, bupivacaine, and any combination thereof.

28. The method according to any one of claims 1 to 27, wherein the animal is selected from the group comprising sheep, goats, and camelids.

29. The method according to any one of claims 1 to 27, wherein the skin necrosis composition is administered to the breech region of the animal.

35 30. The method according to any one of claims 1 to 27, wherein the skin necrosis composition is administered to the tail of the animal.

31. The method according to any one of claims 1 to 30, wherein the skin necrosis composition is administered together with a pharmaceutically acceptable carrier, diluent, adjuvant, excipients, dye or combination thereof.

32. The method according to any one of claims 26 to 30, wherein multiple  
5 simultaneous doses of the skin necrosis composition, anaesthetic or combination thereof are administered.

33. The method according to any one of claims 1 to 30, wherein the skin necrosis composition is delivered by high pressure injection.

34. A skin necrosis composition comprising at least one cyanoacrylate  
10 compound and optionally at least one of a viscosity imparting agent, cyanoacrylate polymerisation inhibitor, anaesthetic, dye, pharmaceutically acceptable carrier, diluent, adjuvant, excipient, or any combination thereof.

35. A skin necrosis composition comprising a polymer precipitate compound and optionally at least one of a viscosity imparting agent, anaesthetic, dye,  
15 pharmaceutically acceptable carrier, diluent, adjuvant, excipient, or any combination thereof.

36. A skin necrosis composition comprising a first part including at least one soluble polymer compound and a second part comprising at least one polyvalent cation.

37. The skin necrosis composition according to claim 36, wherein either or  
20 both of the first and second parts further comprise at least one of a viscosity imparting agent, anaesthetic, dye, pharmaceutically acceptable carrier, diluent, adjuvant, excipient, or any combination thereof.

38. A method of generating skin necrosis in an animal, the method comprising subdermally administering a composition comprising at least one polymer glue  
25 to a fold of skin, wherein a first portion of the fold adheres a second portion of the fold such that the fold of skin is substantially isolated from underlying tissue.

39. A method of generating skin necrosis in an animal, the method comprising subdermally administering a composition comprising at least one polymer compound to a portion of the skin, wherein the polymer compound forms a plaque under  
30 the portion of skin, and wherein the plaque substantially isolates the portion skin from underlying issue.

40. The method according to claim 39, wherein the polymer compound is a polymer glue, a polymer precipitant, or a combination thereof.

41. The method according to claim 40, wherein the polymer glue is a  
35 cyanoacrylate compound.

42. The method according to claim 40 or 41, wherein the polymer precipitate is selected from the group comprising calcium alginate, polycarbonate, polyurethane, polypropylene, a polyvinyl compound, or a combination thereof.

43. The method according to claim 42, wherein the calcium alginate is  
5 formed *in situ* after co-administration of sodium alginate with calcium chloride.

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