

**Public Release Summary
on**

**Evaluation of the new active
Recombinant Urogastrone Epidermal
Growth Factor
in the product**

BIOCLIP

**Biological Wool Harvesting
Injection for Merino Sheep**

**National Registration Authority
for Agricultural and Veterinary Chemicals**

August 1997

**Canberra
Australia**

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ISBN 0 642 26489 9

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Foreword

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent statutory authority with responsibility for assessing and approving agricultural and veterinary chemical products prior to their sale and use in Australia.

In undertaking this task, the NRA works in close cooperation with advisory agencies, including the Department of Health and Family Services (Chemicals and Non-prescription Drug Branch), Environment Australia (Risk Assessment and Policy Section), the National Occupational Health and Safety Commission (Worksafe Australia) and State departments of agriculture and health.

The NRA has a policy of encouraging openness and transparency in its activities and of seeking community involvement in decision making. Part of that process is the publication of public release summaries for all products containing new active ingredients and for all proposed extensions of use for existing products.

The information and technical data required by the NRA to assess the safety of new chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken. Details are outlined in the NRA's publications *Vet Manual: The Requirements Manual for Veterinary Chemicals* and *Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products*.

This Public Release Summary is intended as a brief overview of the assessment that has been completed by the NRA and its advisory agencies. It has been deliberately presented in a manner that is likely to be informative to the widest possible audience thereby encouraging public comment.

More detailed technical assessment reports on all aspects of the evaluation of this chemical can be obtained by completing the order form in the back of this publication and submitting it with payment to the NRA. Alternatively, the reports can be viewed at the NRA Library, Third floor, 10 National Circuit, Barton, ACT.

The NRA welcomes comment on the usefulness of this publication and suggestions for further improvement. Comments should be submitted to the Executive Manager—Registration, National Registration Authority for Agricultural and Veterinary Chemicals, PO Box E240, Kingston ACT 2604.

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Abbreviations and Acronyms

ADI	acceptable daily intake (for humans)
d	day
met-EGF	recombinant mouse epidermal growth factor
EGF	epidermal growth factor
EUP	end use product
Fo	original parent generation
GMAC	Genetic Manipulation Advisory Council
h	hour
HPLC	high performance liquid chromatography
id	intradermal
ip	intraperitoneal
im	intramuscular
iv	intravenous
<i>in vitro</i>	outside the living body and in an artificial environment
<i>in vivo</i>	inside the living body of a plant or animal
kg	kilogram
L	litre
LC50	concentration that kills 50% of the test population of organisms
LD50	dosage of chemical that kills 50% of the test population of organisms.
m	metre
mg	milligram
mL	millilitre
MRL	Maximum Residue Limit (a legal limit)
MSDS	Material Safety Data Sheet
ng	nanogram
NHMRC	National Health and Medical Research Council
NOEC/NOEL	No Observable Effect Concentration/Level
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
po	oral
ppb	parts per billion
ppm	parts per million
s	second
sc	subcutaneous
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGAC	technical grade active constituent
µg	microgram
URO-EGF	recombinant urogastrone epidermal growth factor
u-hEGF	urinary human epidermal growth factor
WHP	withholding period

Introduction

The purpose of this document is to provide a summary of the data reviewed and an outline of regulatory considerations for the proposed clearance and registration of the chemical urogastrone epidermal growth factor (URO-EGF) for use by injection for the biological harvesting of wool in merino sheep. The information provided herein presents only the conclusions reached by the various expert reviewers after consideration of the scientific database. All trial data and methods of assessment presented for evaluation were according to accepted scientific principles and of a standard publishable in reputable refereed journals.

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) has completed an assessment of the data submitted by the applicant in support of this use of URO-EGF and now invites public comment before proceeding to approve this product for use in Australia. The following information is provided for public comment.

Comments should be sent by 2 September 1997 to:

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1. SUMMARY

Applicant details

Biological Wool Harvesting Company Pty Ltd
PO Box 453
Neutral Bay NSW 2089

Purpose of application

This application is for the registration of a new product based on a new active constituent for use by injection in sheep for the biological shearing of sheep. The product contains 7.5mg/mL recombinant urogastrone epidermal growth factor (URO-EGF).

Background

There are two well characterised forms of the bioactive factor known as epidermal growth factor (EGF). One is mouse EGF, extracted from the mouse submaxillary gland. The other is human EGF, extracted from human urine. Both molecules consist of a 53 amino acid peptide folded in a precise manner due to the three disulphide bonds present. Human EGF is functionally related to mouse EGF. Although the molecules are biochemically and antigenically distinct, they elicit identical biological responses in a range of target tissues examined across numerous mammalian species.

Recombinant mouse EGF (Met-EGF) is a structural analogue of mouse EGF produced by a recombinant DNA technique, and was produced in Melbourne Australia by Pitman-Moore Pty. Ltd. with GMAC approval. An evaluation for a product containing met-EGF was previously gazetted. When Biological Wool Harvesting Company Pty. Ltd. took over as the applicant for this product, production ceased at the Melbourne site, and the application for registration of this product was withdrawn. Production of recombinant urogastrone EGF (URO-EGF), a structural analogue of human EGF produced by a recombinant DNA technique, has commenced in Japan. URO-EGF is the molecule to be marketed under the trademark BIOCLIP® as an injectable solution. EGFs stimulate the proliferation of epidermal and epithelial tissues in animals and the proposed use for URO-EGF is as a biological defleecing agent for sheep

Formulation to the injectable End Use Product (EUP) will take place in New Zealand. The end use product will be formulated as a low dose injectable formulation. The packaging of the 50, 250 and 500 mL End Use Formulation in PVC Vaxipaks offers the opportunity to purchase only the correct volume required for the size of the sheep flock to be treated. The soft plastic Vaxipak is packaged in a cardboard box suitable for the size of the pack. A number of packs and their outer cartons are contained in a larger cardboard box to provide protection during handling transport and storage. Transport and storage of formulated product must be in temperature controlled conditions (2- 8 °C. Refrigerate. Do not freeze).

Overseas registration status

The product is not currently registered in overseas countries.

Justification for use

In the early 1980s CSIRO researchers discovered that Epidermal Growth Factor (EGF) could temporarily disrupt fibre growth, allowing the fleeces of sheep to be readily removed by hand. This process is similar to the natural shedding of fleeces from primitive breeds of sheep.

The introduction of this technology will provide the Australian wool producer with unique advantages in the marketplace. The process allows the grazier to produce a superior 'clip' by having the fleece absent of any 'second cuts'. Additional benefits to increasing the quality of the fleece are as follows:

- No shearing cuts to the sheep which are subject to infection.
- No skin pieces to contaminate the clip.
- Significantly less belly wool removed/skirted out, resulting in greater weight of skirted wool.
- Reduced coefficient of variation of staple length across the entire fleece.
- Significantly greater mean length of top fibre.
- Less card waste and less noil.

Part of the BIOCLIP system involves the use of conventional sheep handling equipment to facilitate the injection of the protein and the application and removal of the fleece retention system. The BIOCLIP process significantly reduces stress in the sheep as the process is less vigorous and the animal is handled less than with conventional shearing. The process will also reduce the incidence of back and other workplace injuries currently experienced by shearers. Reducing the incidence of back and other injuries is also likely to extend the working life of the shearer.

Efficacy and safety in target species

Following a full assessment of the data on efficacy and safety in target species submitted by the applicant for the product containing met-EGF, the NRA concluded that it could be approved for use in Australia.

Field trial and blood plasma level study data were submitted for URO-EGF which supported the biological equivalence of the two molecules.

A single subcutaneous injection of BIOCLIP is efficacious for the biological shearing of Merino sheep when used in conjunction with the fleece retention system. EGF is a sequence of amino acids whose function, when injected into the sheep, is to cause a 'break' in the wool growth and a subsequent shedding of the fleece.

Some physiological effects were recorded in merino sheep. One side effect was a temporary inhibition of food intake for up to 48 hours after treatment, quickly followed by a resumption of a normal eating regime. A second side effect was a temporary delay in oestrus in cycling ewes and a temporary reduction in sperm motility in rams following treatment. Reference to the possibility of these side effects is made on the label.

Potential for chemical residues in food

Following an assessment of the residue data submitted by the applicant, URO-EGF and met-EGF have been recommended for inclusion as a Table 5 entry in the *MRL Standard*. This table is for

the listing of substances for which an MRL has not been set because residues do not or should not occur, or where residues are identical to or indistinguishable from natural food components.

Evaluation of toxicology

Urogastrone epidermal growth factor (URO-EGF), the active ingredient in BIOCLIP Biological Wool Harvesting Injection for Merino Sheep, is genetically engineered human epidermal growth factor derived from the bacteria *Bacillus brevis*. Epidermal growth factors are polypeptides which stimulate epithelial cell division in a number of species, including humans, rats and mice. However, their actual physiological role is unknown.

No acute effects were seen when human EGF was given intravenously (IV) or subcutaneously (SC) to rats and mice. Repeated IV administration of EGF to rats caused generalised multiplication of epithelial cells, but did not result in significant treatment related toxic effects. Repeated SC administration of human EGF in monkeys caused similar epithelial cell multiplication, but deaths were seen at doses as low as 0.3 mg/kg/day. The main adverse effects prior to death, and in those monkeys which survived, were gastrointestinal tract disturbances. It would therefore appear that primates are more susceptible to the physiological effects of injected EGF.

While human EGF had minimal embryo-toxic effects in rats, it was toxic to rabbit does and their embryos/foetuses at relatively low doses.

Limited human data from clinical trials with EGF indicate that oral doses up to 600 mg are well tolerated with no reported adverse effects. The highest IV dose reported in these trials was 0.25 µg/kg/h, and 10 µg/ml by dermal application. These doses were potentially in the biological effect range, although they did not appear to produce adverse health effects.

The proposed use for URO-EGF is as an injectable biological defleecing agent. Products which contain URO-EGF will not be available to the general public. Instead they will be used by adequately trained personnel from a company involved with sheep flock management, EGF administration and subsequent wool collection.

The Genetic Manipulation Advisory Committee (GMAC) advised that since the product did not contain live organisms, evaluation and approval by the committee was not required.

Based on an assessment of the product's toxicology, it was considered that provided suitable precautions are taken to avoid accidental self-injection, there should be no adverse effects on human health when this product is used in accordance with label directions.

Occupational health and safety

Worksafe Australia conducted a risk assessment on BIOCLIP containing met-EGF or URO-EGF at 7.5 g/L as an injectable solution for use on Merino sheep. BIOCLIP is classified as a hazardous substance, based on the concentration of EGF in the formulation, and therefore must only be available to trained persons. Users must be provided with information on its toxicity, safe handling and injection techniques.

Environmental safety

Studies on the environmental effects of EGF were not available; however, no environmental hazard is envisaged from the proper use of EGF. Maximum levels exposed to the environment after metabolism and excretion of met-EGF by sheep are in the ppb range from 1–2 hours to 24 hours after injection. After that time only metabolites are excreted. This fact, coupled with EGF's instability in the environment above 8°C, indicates that hazard will be low. Breakdown to the constituent amino acids is fast (<1 day), and these would be absorbed into the environment where they would be mainly utilised as food sources by microorganisms.

It is considered that the low volume of exposure and short life of the protein when exposed to the environment preclude adverse effects. The disposal statement contained on the draft label supplied is suitable for the size and type of packaging .

2. CHEMICAL PROPERTIES OF THE ACTIVE CONSTITUENT

Chemical identity URO-EGF

Approved chemical name: Urogastrone Epidermal Growth Factor (URO-EGF)

Other names: Epidermal Growth Factor (EGF)
Recombinant human Epidermal Growth Factor (Rh-EGF)

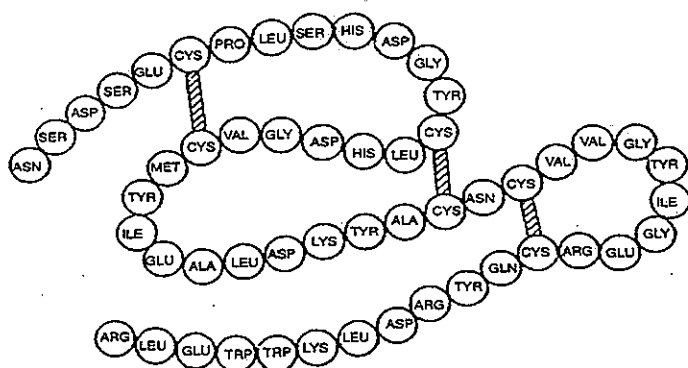
CAS number: for EGF is 62229-50-9

Molecular weight: 6216

Amino acid sequence: NSDSECLSHDGYCLHDGVCMYIEALDK
YACNCVVG YIGERCQYRDLKWWELR

Key:	Alanine	A	Isoleucine	I
	Arginine	R	Leucine	L
	Asparagine	N	Methionine	M
	Aspartic acid	D	Proline	P
	Cysteine	C	Serine	S
	Glutamine	Q	Tryptophan	W
	Glutamic acid	E	Tyrosine	Y
	Glycine	G	Valine	V
	Histidine	H		

Structure:



Purity of TGAC: 80-100% w/w (total protein)

Physico-chemical properties

Appearance: White to off-white amorphous powder

Water Solubility: Miscible in water

Stability: Powder is very heat stable
Aqueous phase decomposes above 8°C

3. EFFICACY AND SAFETY TO TARGET SPECIES

Following a full assessment of the data on efficacy and safety in target species submitted by the applicant for the product containing met-EGF, the NRA concluded that the product could be approved for use in Australia.

Smith et al (1985) conducted a bioassay, inducing eye opening in neonatal mice with a dose response of 0.25-4.0 µg/kg/day of synthetic URO-EGF and mouse EGF. The dose responses showed that the two proteins had precisely the same potency in inducing this biological effect. In addition, both molecules (met-EGF and URO-EGF) bound to the epidermal growth factor receptor with essentially identical affinities (Hollenberg 1978, *Vitamins and Hormones*, 37, 69-110).

A controlled experiment evaluating the clearance rate of URO-EGF in plasma was compared to met-EGF in merino sheep and it was concluded that both molecules had comparable clearance from plasma and that the sheep were defleeced with ease from both treatment groups. Based on the clearance profiles from this experiment it was concluded that a dose of 250 µg URO-EGF/kg body weight was appropriate.

Field trials undertaken confirmed that satisfactory efficacy of URO-EGF was achieved at a dose rate of 250 µg/kg. A summary of trials using met-EGF and URO-EGF are contained in Tables 1 and 2, respectively, and show that reliable and satisfactory efficacy of URO-EGF is achieved at the recommended dose rate of 250 µg/kg.

Table 1. A summary of trials using met-EGF at a dose rate of 250 µg/kg in medium and fine woolled merino sheep

Location	District	No. of sheep treated	Efficacy (%)
Bringelly, NSW	Outer Sydney Metro	33	100
Bringelly, NSW	Outer Sydney Metro	34	100
Goulburn, NSW	South Western slopes	134	99.3
Bringelly, NSW	Outer Sydney Metro	91	100
Yass, NSW	South Western slopes	69	100
Boyup Brook, WA	South West	80	100
Moora, WA	Central West	60	98.5
Horsham, Vic.	Wimmera District	60	100
Casterton, Vic.	Western District	60	100
Albury, NSW	Murray Valley	59	98.3

Table 2. A summary of trials using URO-EGF at a dose rate of 250 µg/kg

Location	District	No. of sheep treated	Efficacy (%)
Badgery's Creek, NSW	Outer Sydney Metro.	70	100
Badgery's Creek, NSW	Outer Sydney Metro.	80	100
Badgery's Creek, NSW	Outer Sydney Metro.	10	100
Hay, NSW	South West	185	96
Stuart Town, NSW	Central West	98	99.9

As with met-EGF, URO-EGF as a wool harvesting agent is expected to be affected by husbandry and management procedures. Sheep on a low plane of nutrition for an extended period tended to show lower efficacy when URO-EGF was administered in comparison to sheep that had an adequate diet. In the trials conducted there appeared to be no difference in response with sheep of different ages (six months of age to adult) and sex treated with BIOCLIP Biological Wool Harvesting Injection for Merino Sheep at a dose of 250 µg/kg.

Resistance

The efficacy of met-EGF and URO-EGF was monitored in trials conducted over four years. Results suggested there is no loss of efficacy following consecutive annual re-treatments of met-EGF or URO-EGF. This evidence also suggests that repeated use of met-EGF or URO-EGF should not elicit an immune reaction nor subsequently reduce their efficacy.

Table 3. Summary of 'keeper trials' conducted in Yass, NSW

Year	Defleecing Active	Efficacy (%)
1993	Met-EGF	100
1994	Met-EGF	100
1995	URO-EGF	97
1996	URO-EGF	100

Safety in target species

The annual treatment of sheep using BIOCLIP (met-EGF and URO-EGF) over four years revealed no deleterious effects on the sheep or its wool. The sheep treated with BIOCLIP showed improved body condition in comparison to sheep which were conventionally shorn. This is attributed to the reduced stress on the animal when removing the fleece retention system in comparison with conventional shearing. The improved body condition of the sheep which had their fleeces harvested using BIOCLIP tended to remain ahead of those sheep which were conventionally shorn.

After four successive annual treatments, sheep treated with BIOCLIP were not any more susceptible to disease than the control sheep. Mortality in the group of sheep treated with BIOCLIP was reduced compared to that of the conventionally shorn flock, with four and eight deaths, respectively.

The quality and quantity of wool removed using BIOCLIP in the fourth successive year (1996) in commercial size trials demonstrated benefits to the wool producer in both terms of quantity and quality of the resultant wool clip. Annual treatment of this flock, referred to as the 'keeper flock' will continue.

CSIRO Division of Animal Production trials demonstrated a consistent yield advantage from wool which was harvested using BIOCLIP, principally due to the absence of what are termed 'second cuts'. Second cuts are short pieces of wool left by the manipulation and severance of the wool by the shearers handpiece. The absence of second cuts results in less card waste and noil at processing and significant benefits to the producer.

Because met-EGF and URO-EGF bind to the same receptor with similar affinity and therefore induce identical biological responses, it is appropriate for URO-EGF to have the identical reproductive effects in rams and ewes as met-EGF.

The sexual activity and fertility of rams was not influenced by EGF treatment. There was no statistical difference in the percentage of morphological normal or of live spermatozoa in the ejaculates of control or EGF treated rams during a ten-week period after treatment. The motility of spermatozoa, although initially depressed following treatment, had returned to normal by weeks 9-10.

Non-pregnant ewes treated with EGF may experience a temporary disruption to cyclicity, presumably by delaying the onset of the ensuing oestrus. Ewes should not be treated with BIOCLIP within 28 days of joining, nor should pregnant ewes.

Birth weight of lambs born to ewes treated with EGF showed no evidence of abnormality or reduced ability to survive beyond birth.

CSIRO conducted many controlled experiments with EGF and noted some of the physiological effects in merino sheep. EGF was found to temporarily inhibit food intake for up to 48 hours after treatment and then quickly resume to a normal eating regime.

4. POTENTIAL FOR CHEMICAL RESIDUES IN FOOD

Background

Urogastrone epidermal growth factor (URO-EGF), the active constituent in BIOCLIP Biological Wool Harvesting Injection for Merino Sheep, is produced by a recombinant process involving *Bacillus brevis* HPD31. The amino acid sequence of URO-EGF demonstrates 70% homology with that of methionine-EGF (met-EGF). EGFs stimulate the proliferation of epidermal and epithelial tissues in animals. URO- and met-EGF molecules bind to the epidermal growth factor receptor with essentially identical affinities and elicit identical biological responses.

Met-EGF has been assessed previously for use as a biological defleecing agent for merino sheep. The applicant now proposes using URO-EGF for this purpose and for the additional purpose of fellmongering. The applicant justifies the change from met-EGF to URO-EGF on the basis that higher yields of recombinant protein are attainable and the expressed protein will not be susceptible to degradation from extracellular proteases. Compared with met-EGF, the production costs will be lower and the purity of the expressed protein higher for URO-EGF.

Biological Wool Harvesting Company Pty Ltd has applied for the registration of URO-EGF to be used in conjunction with the BIOCLIP Fleece Retention System. It is proposed that the product be administered by subcutaneous injection into the inguinal region of sheep at a dose rate of 0.25 mg/kg bodyweight. Because of the similarities between met-EGF and URO-EGF described above and because the proposed use pattern is unlikely to result in detectable tissue residues, the applicant has proposed that, from a residues perspective, URO-EGF and met-EGF be treated the same. The applicant has also proposed a slaughter WHP of 7 days for URO-EGF and a reduction of the slaughter WHP from 14 days to 7 days for met-EGF to facilitate the extension of the use pattern to fellmongering.

The applicant has provided the appropriate residue and metabolism studies, in accordance with the *Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products*, for met-EGF to support the proposed use of URO-EGF on merino sheep in Australia.

Residues in food commodities

The applicant has provided plasma profiles to demonstrate the similarity between met-EGF and URO-EGF after a single subcutaneous injection of each at the recommended label rate. Because of the similarity in the comparative plasma concentration versus time profiles of URO-EGF and met-EGF, the applicant argued that the residue data for met-EGF are also applicable to URO-EGF. As a consequence, no residue data were submitted for URO-EGF.

In the blood plasma studies, two treatment groups of six wethers were injected subcutaneously with either met-EGF or URO-EGF at approximately the recommended label rate. Blood samples were taken at different times from zero up to 48 hours after treatment and plasma was assayed using the radioimmunoassay method.¹ The mean plasma

¹ Panaretto, B.A., 1982, *Journal of Endocrinology*, 94, 191-202.

concentration versus time profiles for URO-EGF and met-EGF demonstrated that URO-EGF depleted at least as quickly as the met-EGF. Importantly, residue levels were undetectable in plasma at 48 hours following treatment with URO- or met-EGF. It appears that the acidified formulation of met-EGF is more sustained in blood plasma than the neutral formulation of URO-EGF and the blood plasma profiles of the two formulations are comparable.

In reference to the residue studies for met-EGF, tissue residue levels were found to be negligible by the final sampling point at 48 hours. In commercial practice, it is unlikely that wool-producing sheep will be sent for slaughter within 48 hours after treatment, and it is impossible to harvest wool until 7-10 days following treatment. The use pattern therefore has a 'built-in' slaughter restraint period of at least 7 days for wool harvesting and tissue residues persisting to slaughter are most unlikely.

MRL Standard

The establishment of an NOEL/ADI has been considered unnecessary as residues of URO-EGF are unlikely to occur under the proposed use pattern. The Advisory Committee on Pesticides and Health has previously recommended that met-EGF treated sheep be withheld from slaughter for two weeks post-treatment as suggested from the previous use pattern. In view of the proposed extension of the use pattern to incorporate fellmongering and the unlikelihood of tissue residues to persist beyond 48 hours following treatment, the applicant's proposal of a slaughter withholding period (WHP) of 7 days is considered appropriate. Accordingly, the Chemistry and Residue Evaluation Section of the NRA has recommended a single combined Table 5 entry for URO-EGF and met-EGF and a slaughter withholding period of 7 days for both. The following amendments the *MRL Standard* are proposed:

Table 5

<i>Compound</i>	<i>Use</i>
DELETE:	
Methionine epidermal growth factor	As subcutaneous injection for biological shearing of sheep
ADD:	
Urogastrone- and methionine-epidermal growth factor	As subcutaneous injection for biological shearing of sheep

5. PUBLIC HEALTH AND SAFETY

Evaluation of Toxicology

The toxicological data for URO-EGF consisted of published journal articles. In interpreting the data, it should be noted that toxicity tests generally use doses which are high compared to likely human exposures. The use of high doses increases the likelihood that potentially significant toxic effects will be identified. Toxicity tests should also indicate dose levels at which the specific toxic effects are unlikely to occur.

Acute studies

No clinical effects, or treatment related autopsy changes, were seen in rats and mice when treated with a single IV or SC dose of urinary human EGF (u-hEGF). Similarly, a single intramuscular dose of mouse EGF given to angora rabbits had no adverse clinical effects.

Short-term studies

Groups of rats or monkeys received doses of 0, 0.3, 0.9 or 3 mg of u-hEGF/kg/day for 4 weeks by the IV or SC route, respectively. An additional two groups of rats, which had received the control and highest dose, were kept for 2 weeks after stopping treatment as a recovery group.

Rats

A minor increase in body weight associated with an increase in food consumption was noted in all treated and recovery groups. Anaemia was noted in males and females at all doses, and at the conclusion of the 2 week recovery phase these changes remained in males given 3 mg/kg/day. A dose related increase in total serum protein and cholesterol was seen in male rats. In both sexes, an increase in serum albumin, and a decrease in the globulin fraction were noted at all treatment doses. Liver weight was increased in males and females at doses of 0.9 or 3 mg/kg/day, in addition to the spleen and uterus weights in all treated female groups, and the ovaries at doses of 0.9 or 3 mg/kg/day. Evidence of increased cell numbers was noted in various tissues in the body at all doses, which, at the conclusion of the recovery period showed some evidence of resolution.

Monkeys

Treatment of monkeys with 0.9 or 3 mg of u-hEGF/kg/day resulted in death, or the animals were killed for humane reasons, after 7–23 days. At 0.3 mg/kg/day, the animals died, or were killed for humane reasons, within 14–25 days of commencement of treatment. The main clinical signs prior to death were related to gastrointestinal tract disturbances *viz.* vomiting, diarrhoea and inappetence as well as respiratory distress, sedation, weakness, loss of body weight and shedding of skin. Clinical biochemistry changes indicated the animals had developed renal failure at all doses. Relative liver, kidney and adrenal weights were increased. Histology showed a slight to severe increase in cell numbers in various organs at all doses. In addition, decreased size of ovarian follicles and uterus lining disruption were noted in all, or some of the treated females.

Doses of 0 or 40 g of recombinant EGF/kg were injected into the amniotic fluid, and into the peritoneal cavity, of foetal *in utero* monkeys on gestation days 121, 123, 125 and 127. Caesarean sections were performed on gestation day 128 (78% of gestation). The percentage of lung air spaces and the size of the individual air spaces were greater in EGF treated animals, and the time course of respiratory distress induced by premature delivery was shorter.

Teratogenicity studies

Groups of rats were given 0, 0.3, 0.9 or 3 mg of u-hEGF/kg/day by IV injection from day 6 to day 15 of pregnancy. Adult rats showed slight increases in body weight, as did the foetuses. No adverse treatment-related embryo/foetal effects were seen.

Groups of rabbits were given 0, 0.3, 0.9 or 3 mg of u-hEGF/kg/day by IV or SC injection from day 6 to day 18 of pregnancy. A dose related increase in death rate, and clinical illness were noted. At autopsy, the does showed congestion of the heart, liver, lungs and kidneys. Most treated does showed evidence of foetuses which died.

Genotoxicity

Human urinary EGF did not exhibit mutagenicity in *S. typhimurium*. Furthermore, it did not induce genetic damage as indicated by negative results for chromosome aberrations in Chinese hamster bone marrow and human lymphocytes. Furthermore, u-hEGF failed to cause gene damage in HeLa cells.

Human exposure

To date, no human therapeutic agents utilising EGF have been developed. However, a number of clinically applicable uses of injected EGF have been proposed, and limited trials have been conducted to investigate the efficacy and safety of injected EGF.

Adult, male human subjects were infused (IV) with either 0.125, 0.25 or 0.5 g of purified human EGF/kg/hour for 1 hour. No significant alterations in clinical condition, haematology or clinical biochemistry were seen. At the infusion rate of 0.5 g/kg/hour, headaches were reported 20-30 minutes after initiation of the infusion, which persisted for up to 3 hours after the infusion was stopped. EGF inhibited gastric acid secretion stimulated by pentagastrin, histamine and insulin.

Enhanced rates of GIT ulcer healing were demonstrated when EGF was administered to patients at a dose of 6 µg IV twice a week for 8 weeks. Similarly, orally administered EGF (450 or 600 mg of recombinant EGF/day for a period of up to 6 weeks) has been shown to accelerate healing of duodenal ulcers without producing adverse clinical effects. In addition, injected EGF shows some promise in the treatment of necrotising enterocolitis, Zollinger-Ellison syndrome (ZES) and congenital microvillus atrophy. It has been reported that a patient who was treated for necrotising enteritis with a continuous infusion (100 ng/kg/h) of human recombinant EGF for 6 days showed a noticeable improvement in the condition. Four patients with ZES who were treated with 0.25 µg/kg/h of human EGF (urogastrone) showed an inhibition of gastric acid hypersecretion.

Skin, in addition to GIT ulceration, may benefit from EGF. Following a clinical trial, which examined the effects of 10 µg/mL of EGF applied topically twice daily to venous ulcers, it

was concluded that the treatment was safe, but did not enhance ulcer re-epithelialisation, although ulcer size was reduced and there were a greater number of healed ulcers.

In clinical trials, results indicated that EGF may protect the oral epithelium from cytotoxic damage, encourage re-epithelialisation in herpetic corneal ulcers, and superficial corneal injuries. The rate of chronic wound, and partial thickness skin wound healing was increased following topical application of 10 µg/g, or 10 µg/mL, respectively, of EGF.

These data indicate that oral doses of EGF up to 600 mg appear to be well tolerated in humans with no reported adverse effects. The highest dose rates administered in clinical trials were 0.25 µg/kg/h by the IV route, and 10 µg/ml by dermal application. These doses were potentially in the biological effect range, although they did not appear to produce adverse health effects.

Public Health Standards

Poisons scheduling

The National Drugs and Poisons Schedule Committee (NDPSC) considered the toxicity of the product and its active ingredients and assessed the necessary controls to be implemented under States' poisons regulations to prevent the occurrence of poisoning.

The NDPSC recommended that URO-EGF be listed in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). In addition to a Schedule 7 listing, NDPSC recommended that URO-EGF is "not to be available except to authorised or licensed persons". There are provisions for appropriate warning statements on the label, and a training manual has been produced to educate authorised or licensed persons who will be administering URO-EGF.

No Observed Effect Level/Acceptable Daily Intake

The establishment of a No Observed Effect Level/Acceptable Daily Intake is considered unnecessary, as residues of URO-EGF in treated animals slaughtered following wool shedding would be indistinguishable from naturally occurring growth factors.

6. OCCUPATIONAL HEALTH AND SAFETY

BIOCLIP Biological Wool Harvesting Injection for Merino Sheep (BIOCLIP) is an injectable solution containing epidermal growth factor (EGF), which includes met-EGF and URO-EGF at 7.5 mg/mL.

BIOCLIP will be used in conjunction with the 'Bioclip Fleece Retention System' which is a coat designed to retain biologically defleeced wool for 6 weeks after EGF treatment.

EGF in the form of URO-EGF will be imported from Higeta Shoyu Co Ltd in Tokyo, Japan. BIOCLIP will be formulated at Fernz Health and Science in Auckland, New Zealand.

URO-EGF is classified as a hazardous substance according to NOHSC criteria.

Manufacture, formulation, transport, storage and retailing

Manufacture of EGF and formulation of BIOCLIP occur outside Australia. Transport workers, store persons and retailers can only be exposed to BIOCLIP if packaging is breached and spillage occurs.

End use

The product is injected using a standard auto-fill injector which is connected to the top of the Vaxipak. The sheep is prepared for the Bioclip Fleece Retention System by being crutched and having wool removed from around the pizzle and the ears. The animal is restrained on its back, and 3 mL with an average of 2 mL of product/sheep is injected in the inguinal area. Treated sheep are then fitted with the appropriate sized coat by slipping each leg into the corresponding leg of the coat and fastening the coat along the backline.

Exposure to the product is possible via accidental self-injection, needle-stick injury, drips from needle changes, or breakage of product packaging.

EGF is a highly toxic substance which must be handled with care. Users must be provided with information on its toxicity, safe handling and injection techniques. Therefore, use must be restricted to trained operators.

Recommendations for safe use

- BIOCLIP should only be available to trained harvesting teams.
- The vaccinator and sheep restraining device should be used.
- Operator fatigue should be avoided by observing the standard work rates which includes maximum injection rates, and rotation to enable rest breaks.

- A Summary Incident Report on any self-injection and needle-stick injuries is to be submitted to the NRA on an annual basis, and in the case of a serious adverse effect, an incident report must be lodged immediately.
- There are no safety directions for this product; however, operators should refer to the precaution statement on the label and MSDS when using BIOCLIP.
- A training manual is required which includes detailed information on EGF, by including the Public Release Summary and Technical Report for this product.

7. ENVIRONMENTAL SAFETY

Environmental exposure

The application of URO-EGF by subcutaneous injection to the sheep at a dose rate of 0.25 mg/kg body weight is the first step in a system of wool harvesting. Also involved is the use of a fleece retention system (coat) that retains the wool in contact with the sheep up to six weeks to allow a sufficient growth of replacement wool. The coat and wool are then removed and the sheep released to the paddock with the short covering of new wool to protect it from the elements.

Packaging is in 50, 250 and 500 mL ready to use Vaxipaks contained in a cardboard box outer. Refrigerated storage (2–8°C) is required until time of use. The Vaxipaks are connected to an automatic dosing system and the injection is applied subcutaneously. They empty by gravity flow and are designed to contain little to no residue when completely empty. Disposal to landfill via the household waste stream is suggested for the empty Vaxipaks and protective outer boxes.

Environmental chemistry and fate

No specific studies were submitted in this area but supported argument as to the environmental fate is provided.

In an animal's body system met-EGF is transported by the bloodstream to the site of action at the primary wool follicles in the skin. The material is rapidly metabolised and excreted by the sheep.

In testing on sheep by 24-hour intravenous infusion at 120 $\mu\text{g.kg}^{-1}$ it was found that met-EGF present in the urine had fallen from 5 ppb 1 hour after injection to below 1 ppb 24 hours after treatment². They also found that the main removal pathway is in the excreta, with over a 3 day period radio label expression being proportioned in the urine 55% and faeces 10%. After the first day only the metabolites were being expressed and this continued for up to 8 days.

In the same test met-EGF administered as a subcutaneous single injection at the recommended rate of 250 $\mu\text{g.kg}^{-1}$ and 2X rate of 500 $\mu\text{g.kg}^{-1}$ had been metabolised or transported from the injection site within 48 hours. Levels in the urine immediately after injection would be expected to be higher than above but these were not measured.

Field exposure of met-EGF causes denaturing of the protein chain when the temperature rises above 8°C. The breakdown to the constituent amino acids is fast (<1 day) and these would be absorbed into the environment where they would be mainly utilised as food sources by microorganisms.

The main pathway for removal of EGF is metabolism by the sheep after injection. On excretion, exposure to temperatures above 8°C causes rapid denaturation of the protein. The

² O'Keef J H, Sharry L F and Panaretto BA 1988, *The fate of titrated rm-epidermal growth factor in the sheep: Validation of the labelling procedure and rate of tissue clearance*. Aust. J. Biol. Sci. 41, 539-52

protein is specific needing injection or infusion for efficacy. If ingested orally met-EGF is broken down in the digestive tract.

The biochemical structure of met-EGF and URO-EGF are very similar and the biological activity and therefore the environmental fate is considered to be the same.

Environmental effects

No results for the environmental effects of EGF are available. It is considered that the low volume of exposure and short life of the protein when exposed to the environment preclude adverse effects.

Prediction of environmental hazard

Hazard arising from use

No environmental hazard is envisaged from the proper use of EGF. Maximum levels exposed to the environment after metabolism and excretion by the sheep are in the ppb range from 1-2 hours to 24 hours after injection. After that time only metabolites are excreted. This factor, coupled with EGF's instability in the environment above 8°C, indicates that hazard will be low.

Hazard arising from disposal

The disposal statement contained on the draft label supplied is suitable for the size and type of packaging.

Conclusions and recommendations

EGF is a compound of low persistence and is unlikely to present a hazard to the environment when used as directed.

8. IMPLICATIONS FOR TRADE

The export commodities associated with BIOCLIP use are sheep meats and offals.

URO-EGF is recommended for listing in Table 5 of the *MRL Standard*. This table is for listing of substances where an MRL has not been set because residues do not or should not occur, or where residues are identical to or indistinguishable from natural food components.

No trade issues were identified in the EPA report.

An AQIS import permit has been granted to the applicant.

BIOCLIP has been discussed with two wool peak industry groups (the International Wool Secretariat and Wool International), the Meat Industry Council and the Australian Trade Commission. All groups support the use of the product. Consistent with registrations for other similar products, an ESI has not been proposed.

DANGEROUS POISON

**KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING
FOR ANIMAL TREATMENT ONLY**

BIOCLIP®

Biological Wool Harvesting Injection for Merino Sheep

Active Constituent: 7.5 mg/mL Urogastrone epidermal growth factor (URO-EGF)

Not to be available except to authorised or licensed persons

Read the accompanying leaflet before using this product

Refer to the Material Safety Data Sheet (MSDS) before using this product

**For biological shearing of Merino sheep when used in conjunction with the
Bioclip® Fleece Retention System**

50 mL
[250 mL]
[500 mL]

**BIOLOGICAL WOOL HARVESTING COMPANY PTY LIMITED
ACN 065 027 054
41 Military Road, Neutral Bay, NSW 2089, Australia**

DIRECTIONS FOR USE:**Read Accompanying Leaflet Before Using This Product**

Restraints: DO NOT USE in pregnant or lactating ewes
DO NOT USE in ewes within 28 days prior to joining
DO NOT USE in rams within 70 days prior to joining
DO NOT INJECT animals other than Merino sheep
DO NOT USE in nutritionally compromised sheep

Prior to treatment sheep should be crutched, have a ring of wool removed from around the pizzle and a heavy wig with a band of wool removed from behind the ears. Treated sheep are fitted with the appropriate size of Bioclip® Fleece Retention System.

Dose: Subcutaneously into the inguinal region

Up to 30 kg	1 mL	61-75 kg	2.5 mL
31 - 45 kg	1.5 mL	Above 75 kg	3 mL
46 - 60 kg	2 mL		

CAUTION: Casting has been observed in trials at a very low incidence. Regular observation of netted sheep for signs of casting is therefore recommended.

Not to be used for any purpose or in any manner contrary to this label unless authorised under appropriate legislation

WITHHOLDING PERIOD: MEAT - 7 DAYS

WARNING: While this product is well tolerated by sheep, there is a risk of severe injury to humans associated with accidental self-injection. This may include reproductive effects. Care should be taken to avoid needle-stick injury when injecting this product. Refer to the Procedures manual for further safety instructions.

FIRST AID:	Injection:	In case of self injection get to a doctor or hospital quickly.
	Needle stick or scratch:	Stop work, monitor and seek medical advice if symptoms develop.
	Spillage:	Wash with water immediately.

No safe level of EGF exposure has been demonstrated in primate studies and EGF is a known tumour promotor.

STORAGE AND DISPOSAL:

Store at 2°C to 8°C (REFRIGERATE, DO NOT FREEZE). Protect from light. Do not expose directly to heat and light during use. The use of an insulated container is recommended during the day to protect Bioclip®.

Used vaccine packs, including any unused product, should be wrapped up and disposed of with regular garbage.

NRA No. 49100/01

Batch Number:

Expiry:

BIOLOGICAL WOOL HARVESTING COMPANY PTY LIMITED

ACN 065 027 054

41 Military Road, Neutral Bay, NSW 2089, Australia

Top Panel

BIOCLIP® Biological Wool Harvesting Injection for Merino Sheep**Conditions of Sale:**

"The Biological Wool Harvesting Company Pty Ltd ("BWHC") shall be liable for any loss, injury, damage or death whether consequential or otherwise whatsoever or howsoever arising whether through negligence or otherwise in connection with the sale, supply, use of application of this product.

The supply of this product is on the express condition that the purchaser does not rely on BWHC's skill or judgement in purchasing or using the same and every person dealing with this product does so at his own risk absolutely. No representative of the BWHC has any authority to add to or alter these conditions."

Bottom Panel

BIOCLIP® Biological Wool Harvesting Injection for Merino Sheep

<p>In case of emergency Phone 1800 033 498 Ask for Shift Supervisor. Toll free 24 hours.</p>

® Biological Wool Harvesting Company Pty Ltd Trademark

End Panel

BIOCLIP® Biological Wool Harvesting Injection for Merino Sheep

DANGEROUS POISON

**KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE USING
FOR ANIMAL TREATMENT ONLY**

BIOCLIP®

Biological Wool Harvesting Injection for Merino Sheep

Active Constituent: 7.5 mg/mL URO-EGF

**For biological shearing of Merino sheep when used in conjunction with
the Bioclip® Fleece Retention System**

Not to be available except to authorised or licensed persons

Read the accompanying leaflet before using this product

Refer to the Material Safety Data Sheet (MSDS) before using this product

WITHHOLDING PERIOD: MEAT - 7 DAYS

WARNING: While this product is well tolerated by sheep there is a risk of severe injury to humans associated with accidental self-injection. This may include reproductive effects. Care should be taken to avoid needle-stick injury when injecting this product. Refer to the Procedures manual for further safety instructions.

FIRST AID: In case of self injection get to a doctor or hospital quickly.

PROTECT FROM LIGHT
Store at 2°C to 8°C (REFRIGERATE, DO NOT FREEZE)

Batch Number:

Expires:

50 mL
250 mL
500 mL

NRA Number: 49100/01

BIOLOGICAL WOOL HARVESTING COMPANY PTY LIMITED

DANGEROUS POISON

KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING
FOR ANIMAL TREATMENT ONLY

BIOCLIP®

Biological Wool Harvesting Injection for Merino Sheep

Description:

BIOCLIP® is a clear, straw colour, sterile solutions for subcutaneous injection into the inguinal (groin) region of Merino sheep.

Pack Sizes:

50 mL
250 mL
500 mL

Composition:

Each mL contains 7.5 mg of URO-epidermal growth factor (URO-EGF).

Clinical Pharmacology:

The wool follicles of sheep given depilatory doses of URO-EGF (250 - 320 µg/kg) undergo regression involving inhibition of bulb cell proliferation, decline in fibre formation and deletion of cell. This results in a complete break in the fleece within 7 - 14 days.

Indications:

For biological shearing of Merino sheep when used in conjunction with the Bioclip® Fleece Retention System.

Not to be available except to authorised or licensed persons.

Refer to the Material Safety Data Sheet (MSDS) before using this product.

DIRECTIONS FOR USE

Restraints: DO NOT USE in pregnant or lactating ewes
DO NOT USE in ewes within 28 days prior to joining
DO NOT USE in rams within 70 days prior to joining
DO NOT INJECT animals other than Merino sheep
DO NOT USE in nutritionally compromised sheep

Prior to treatment sheep should be crutched, have a ring of wool removed from around the pizzle and a heavy wig with a band of wool removed from behind the ears. Treated sheep are fitted with the appropriate size of Bioclip® Fleece Retention System.

Dosage and Administration:**PICTURE OF INJECTION SITE**

Following is the dosage table for administration of Bioclip®

Up to 30 kg	1 mL
31 - 45 kg	1.5 mL
46 - 60 kg	2 mL
61-75 kg	2.5 mL
Above 75 kg	3 mL

The injection procedure

CAUTION: Avoid self injection - Avoid carcass damage

1. Sterilise all injection apparatus by boiling for at least 10 minutes before use. Avoid use of strong disinfectants on apparatus.
2. Maintain maximum cleanliness at all times.
3. Keep needles sharp and clean. Change needles frequently (every 20-30 animals). Use short needles. Recommended maximum size is 15mm x 18 gauge for sheep.
4. Check that the equipment is functioning properly and that it is delivering the correct dose.
5. As far as possible avoid injection of animals during wet weather or under dusty conditions.
6. Inject only under the skin and not into the muscle.
7. Sheep should be injected at the inguinal (groin) region.

The fleece retention system:

Prior to treatment sheep should be crutched, have a ring of wool removed from around the pizzle and a heavy wig with a band of wool removed from behind the ears. Treated sheep are fitted with the appropriate size of Bioclip® Fleece Retention System (Coat); viz

Up to 45 kg	Small Coat
45 - 55 kg	Medium Coat
Above 55 kg	Large Coat

Fitting of a coat consists of slipping the appropriate leg of the sheep into the corresponding leg of the coat and then fastening along the backline. When the sheep is coated it can be temporarily held in a holding yard until later release back to the paddock.

When satisfactory regrowth has occurred (e.g. 5-7mm), usually at approximately 3-4 weeks after treatment, sheep should be mustered so that coats can be removed. This is accomplished by opening the coat along the backline. The wool is also opened up so that the coat and fleece are removed from the sides of the sheep and off its legs. The coat, with fleece, can then be hung from a rack or hanger and the fleece brushed off the coat onto the floor. Use of a sheep handler or suitable conveyor is recommended.

CAUTION: Casting has been observed in trials at a very low incidence. Regular observation of netted sheep for signs of casting is therefore recommended.

Not to be used for any purpose or in any manner contrary to this label unless authorised under appropriate legislation

WITHHOLDING PERIOD: DO NOT ADMINISTER later than 7 days before slaughter for human consumption.

WARNING: While this product is tolerated by sheep, there is a risk of severe injury to humans associated with accidental self-injection. This may include reproductive effects. Care should be taken to avoid needle-stick injury when injecting this product. Refer to the Procedures manual for further safety instructions.

FIRST AID:	Injection:	In case of self injection get to a doctor or hospital quickly.
	Needle stick or scratch:	Stop work, monitor and seek medical advice if symptoms develop.
	Spillage:	Wash with water immediately.

No safe level of EGF exposure has been demonstrated in primate studies and EGF is a known tumour promotor.

STORAGE AND DISPOSAL:

Store at 2°C to 8°C (REFRIGERATE, DO NOT FREEZE) Protect from light. Do not expose directly to heat and light during use. The use of an insulated container is recommended during the day to protect Bioclip®.

Discarded needles should be immediately placed in a designated and appropriately labelled "sharps" container. The container should be of a type to reduce the possibility of injury to handlers during collection and disposal. Used vaccine packs, including any unused product, should be wrapped up and disposed of with regular garbage.

CONDITIONS OF SALE:

"The Biological Wool Harvesting Company Pty Ltd ("BWHC") shall be liable for any loss, injury, damage or death whether consequential or otherwise whatsoever or howsoever arising whether through negligence or otherwise in connection with the sale, supply, use of application of this product.

The supply of this product is on the express condition that the purchaser does not rely on BWHC's skill or judgement in purchasing or using the same and every person dealing with this product does so at his own risk absolutely. No representative of the BWHC has any authority to add to or alter these conditions."

**In case of emergency
Phone 1800 033 498
Ask for Shift Supervisor. Toll free 24 hours.**

NRA Number: 49100/01

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