



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR) Pegylated bovine granulocyte colony stimulating factor (PEGbG-CSF) (bovine species)

On 8 March 2012 the European Commission adopted a Regulation¹ establishing maximum residue limits for pegylated bovine granulocyte colony stimulating factor (PEGbG-CSF) in bovine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Pegylated bovine granulocyte colony stimulating factor (PEGbG-CSF) is intended for use in bovine species for reducing the incidence of clinical mastitis in periparturient cows at a dose of 20 µg/kg administered by subcutaneous injection approximately 7 days prior to calving and on the day of calving.

Eli Lilly and Company Limited submitted the application for the establishment of maximum residue limits to the European Medicines Agency, on 4 March 2010.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 15 September 2011 the establishment of maximum residue limits for pegylated bovine granulocyte colony stimulating factor (PEGbG-CSF) in bovine species.

Subsequently the Commission recommended on 10 January 2012 that maximum residue limits in bovine species are established. This recommendation was confirmed on 31 January 2012 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 8 March 2012.

¹ Commission Regulation (EU) No 202/2012, O.J. L 71, of 09.03.2012



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Pegylated bovine granulocyte colony stimulating factor
Therapeutic class:	Biological/Immunomodulator
Procedure number:	EU/10/172/ELY
Applicant:	Eli Lilly and Company Limited
Target species:	Bovine
Intended therapeutic indication:	Reduction of clinical mastitis in periparturient cows
Route(s) of administration:	Subcutaneous injection

1. Introduction

Pegylated bovine granulocyte colony stimulating factor (PEGbG-CSF) is a modified form of the naturally occurring immunoregulatory cytokine, bovine granulocyte colony stimulating factor (bG-CSF). The Committee considered that the derogation provided for under Article 1 (2)(a) of Regulation (EC) No 470/2009 for active principles of biological origin intended to produce active or passive immunity or to diagnose a state of immunity, used in immunological veterinary medicinal products, does not apply to PEGbG-CSF, as the substance is a well characterised molecule which acts on specific immune cells namely by increasing the number of granulocytes, which does not represent a direct measure of active immunity. As such the substance is neither an antigen inducing active immunity nor an antibody inducing passive immunity.

Recombinant bovine G-CSF is a water soluble 175 amino acid long protein that shares approximately 81% amino acid sequence identity with recombinant human G-CSF.

Pegylated bG-CSF is identical in primary sequence to the endogenously produced protein with the exception of the addition of an amino terminal methionine and a single amino acid substitution whereby a novel amino acid (p-acetylphenylalanine) is incorporated into the protein (using reconstituting chemically orthogonal directed engineering) to enable site specific covalent attachment of a 20 k dalton polyethylene glycol polymer (PEG) chain to the protein. The protein is obtained through bacterial fermentation of a strain of *Escherichia coli* transformed with a genetically engineered plasmid containing the bovine granulocyte colony stimulating factor gene.

Pegylated bovine granulocyte colony stimulating factor is intended for use in bovines for reducing the incidence of clinical mastitis in periparturient cows at a dose of 20 µg/kg administered by subcutaneous injection approximately 7 days prior to calving and on the day of calving.

2. Scientific risk assessment

Bovine granulocyte colony stimulating factor is a constituent the normal human diet of those that eat meat. The safety for consumers of polyethylene glycols has been assessed before and concluded that no MRLs need to be established. In addition, the rate of absorption decreases with increasing molecular mass of polyethylene glycol polymers.

Substances like bovine granulocyte colony stimulating factor can be expected, also when pegylated, not to be orally bioavailable and to be degraded into their constituent peptides/amino acids through the normal process of digestion in which case no consumer safety concerns would arise. Therefore the risk assessment of PEGbG-CSF was primarily based on data gained from an oral bioavailability study in rats.

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

2.1.1.1 Pharmacodynamic properties including mode of action

The mode of action of PEGbG-CSF is based on an increased production of mature neutrophils from bone marrow stem cells and activation of the functional capabilities of mature circulating neutrophils resulting in a stimulation of the immune system. Restoration of neutrophil mediated microbicidal activity during periods of immunosuppression reduces the susceptibility of cows to new clinical bacterial infections during the periparturient period. Therefore, the proposed indication of the use of PEGbG-CSF is to reduce the incidence of clinical mastitis in periparturient cows.

No data from pharmacodynamic studies in laboratory animals has been provided.

2.1.1.2 Pharmacokinetic properties

Specific pharmacokinetic studies for the intended mode of use were not available.

In order to assess the oral bioavailability of PEGbG-CSF a GLP conforming oral bioavailability study was performed in rats. Groups of 3 male and 3 female rats received PEGbG-CSF as a single dose, either subcutaneously at 250 µg PEGbG-CSF/kg or orally at 2500 µg PEGbG-CSF/kg by gavage. A control group treated subcutaneously with formulation buffer was included. For all dose groups, blood samples were collected prior to dosing and at 0.25, 1, 2, 4, 12, 24, 48, 72, 96 and 120 hours after administration. Absolute neutrophil counts were determined using an automatic cell counter and serum samples were quantitatively analysed for PEGbG-CSF using a validated Electrochemiluminescent (ECL) Immunoassay (LOQ equal to 46.9 ng/ml).

Absolute neutrophil counts at the pre-dose time point were consistently low and normal for all animals regardless of treatment group. Over 120 hours after administration, neutrophil counts in animals treated orally with PEGbG-CSF showed a maximum 2-fold increase over pre-dose levels, which was not statistically significant. In contrast, animals treated with PEGbG-CSF subcutaneously showed statistically significant ($p=0.01$) increases in neutrophil counts starting at about 12 hours after administration. The neutrophil counts in this group continued to rise reaching the highest levels at approximately 24 to 72 hours after administration. From these data, it is apparent that rats responded to the PEG bG-CSF subcutaneous treatment with a nearly 10-fold increase over pre-dose levels in neutrophil count, whereas animals treated orally (and subcutaneously with formulation buffer) did not show a relevant increase.

The quantitative analysis via electrochemiluminescent-immunoassay showed, that PEG bG-CSF levels at the pre-dose time point were consistently below the lower limit of quantification or below 46.9 ng/ml for all animals regardless of treatment group. Over the 120-hour course of the study, PEGbG-CSF levels in animals treated orally with PEGbG-CSF remained below the lower limit of quantification. Rats treated subcutaneously with PEGbG-CSF, however, showed marked increases in PEGbG-CSF levels starting at about 2 to 4 hours after administration. The PEGbG-CSF levels in this group continued to rise, reaching highest levels at 12 to 24 hours after administration. From these data, it is apparent that these rats accumulated up to approximately 20,000 ng/ml PEGbG-CSF in serum following subcutaneous treatment, whereas rats treated with control article and orally with PEG bGCSF showed no measurable levels of PEGbG-CSF in serum.

A comparison of the subcutaneous AUC of about 420000 ng.hour/ml (420 µg.hour/ml) (calculated for 0-72 h) and the worst-case scenario for the area under the curve for the oral dose (i.e. assuming that all values are at the limit of quantification of 46.9 ng/ml) indicated that the relative oral bioavailability

is less than 0.1% (0.08%). From this it can be concluded that the oral bioavailability of PEGbG-CSF is negligible.

2.1.2. Calculation of pharmacological ADI, if relevant

Not relevant.

2.1.3. Overview of toxicology

No toxicology studies were submitted. Since the oral bioavailability of PEGbG-CSF is negligible, meaning that the ingestion of residues of PEGbG-CSF in animal tissues or products would not be a risk for consumers, no studies on toxicology are required.

2.1.4. Calculation of the toxicological ADI or alternative limit

Due to the negligible bioavailability after oral exposure, meaning that ingestion of potential residues of PEGbG-CSF in animal tissues or products from animals treated with the substance would not be a risk for consumers neither studies on toxicology nor the establishment of an ADI are required.

2.1.5. Overview of microbiological properties of residues

The direct antimicrobial activity of PEGbG-CSF against a range of Gram positive and Gram negative bacterial pathogens showed no antibacterial activity of PEGbG-CSF using standard MIC assays according to NCCLS guidelines. Direct antibacterial activity was evaluated against multiple isolates of *S. aureus*, *S. uberis*, *E. coli*, *A. pleuropneumoniae*, *M. haemolytica*, *P. multocida*, *S. choleraesuis*, *S. typhimurium* or *H. somni* obtained from veterinary diagnostic laboratories.

Due to the absence of antibacterial activity it is accepted, that no information on further properties was submitted.

2.1.6. Calculation of microbiological ADI

Due to the absence of antibacterial activity, there is not need to establish a microbiological ADI.

2.1.7. Observations in humans

No observations in humans are available regarding (pegylated) bovine granulocyte colony stimulating factor (PEGbG-CSF).

Reports from human patients undergoing myelosuppressive cancer chemotherapy receiving parenteral injections of medicinal products for human use containing either a non-pegylated human G-CSF or a pegylated human G-CSF were available. The most frequently reported adverse event observed after subcutaneous injection was transient bone pain. Extremity pain was reported in some of the treated patients. Further adverse effects reported were e.g. splenic rupture, acute respiratory distress syndrome, allergic reactions including anaphylaxis and severe sickle cell crisis in patients with sickle cell anaemia. These side effects were associated with human patients receiving parenteral injections of the proteins as supportive therapies when they were undergoing myelosuppressive cancer chemotherapy.

In respect to consumer safety regarding the use of PEGbG-CSF in cows as proposed, given the negligible systemic exposure of the consumer, it is highly unlikely consumers would exhibit any adverse events following consumption of edible products from treated cows or their progeny.

2.1.8. Findings of EU or international scientific bodies

No data were available from other EU or international scientific bodies regarding pegylated bovine granulocyte colony stimulating factor (PEGbG-CSF).

Studies with non-pegylated human G-CSF or pegylated human G-CSF have been conducted in the context of marketing authorisations for medicinal products for human use containing these substances in rats and monkeys following injection and assessed by the European Medicines Agency. The results show for a pegylated human G-CSF (pegfilgrastim) a sustained dose-related increase in blood neutrophils effective in restoring neutrophil counts in several mouse chemotherapy models and in a monkey model of myeloablation with reduced survival in mouse and ineffective in monkey. In repeat-dose toxicity studies in rats (weekly dosing for up to 6 months) and cynomolgus monkeys (weekly dosing for 4 weeks) pegfilgrastim produced a range of changes that reflected an exaggerated pharmacological response, or a reaction to the primary response (myeloid hyperplasia in bone marrow). In a two-week rat study where pegfilgrastim was given subcutaneously every other day, plasma concentrations progressively declined. Given the chemical structure and bioreactivity, it was considered inappropriate to undertake genetic toxicity studies, which was considered consistent with ICH Guidelines on products of biotechnological origin. No experimental evaluation of carcinogenic potential was undertaken and this was appropriately justified in view of the limited distribution of cells with appropriate receptors, its cell type-specific mitogenic effects, its limited duration of therapy, data from transgenic models of overexpression of G-CSF and the clinical experience with filgrastim (non-pegylated). The programme of reproductive toxicity tests using subcutaneous administration indicated that the substance was unlikely to impair male or female fertility, not expected to be teratogenic or to affect pup development.

Considering the absence of systemic exposure of the consumer of meat and animal products (milk) from treated animals due to the negligible oral bioavailability, these data are not relevant in the context of the residue risk assessment

Studies with filgrastim (non-pegylated human G-CSF) administered to monkeys, dogs, hamsters, rats, and mice are available in public literature, however these data were not considered relevant in the context of the residue risk assessment.

2.1.9. Overall conclusions on the ADI

Considering the negligible oral bioavailability no further pharmacological or toxicological studies according to Volume 8 of the Rules Governing Medicinal Products in the EU are required. There is consequently also no need to establish an ADI.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

No studies on pharmacokinetics were submitted. Pegylation of the naturally occurring bovine granulocyte colony stimulating factor is expected to enhance the duration of activity for recombinant proteins. The lack of pharmacokinetic studies in target species is of little relevance for the assessment of the substance for consumers safety as the bioavailability of the substance is considered as negligible.

2.2.2. Residue depletion studies

No residue depletion studies in the target animal were provided. Instead, a GLP compliant study was conducted to evaluate oral bioavailability of PEG bG-CSF. The aim of the study was to determine PEG-bG-CSF quantitatively via Electrochemical Luminescence (ECL) Immunoassay in serum of rats after systemic (250 µg/kg bw subcutaneously) and oral (2500 µg/kg bw) treatment, results are described

under 2.1.1.2. "Pharmacokinetic properties". The analytical method (ECL-Immunoassay) based on a sandwich immunoassay employing two capture antibodies (monoclonal IgM anti-PEG antibody and human anti-bG-CSF) and one tracer antibody (sulfo-tagged goat anti-human IgG antibody) which is directed to the "human-anti bG-CSF structure", which in turn is complexed with PEG bG-CSF. The intensity of emitted light from the sulfo-tagged label was proportional to the PEG bG-CSF concentration in the serum samples (quantification via standard curve – concentration range of 49.6 to 6,000 µg/l). The method was validated in relation to accuracy, precision, limit of quantification and stability and provides reliable results. The limit of quantification was 46.9 µg/l.

From the study results it can be concluded that PEG bG-CSF is not bioavailable after oral exposure. Considering the absence of systemic exposure of the consumer of meat and animal products (milk) from treated animals, any potential residues in the target species are of no relevance regarding residue risk assessment for the consumer. Therefore residue studies in the target animal are not required.

2.2.3. Monitoring or exposure data

Not relevant.

2.2.4. Analytical method for monitoring of residues

An analytical method is not required because PEG bG-CSF can be classified according to Article 14(2)(c) of Regulation (EC) No 470/2009 (no MRLs required).

2.2.5. Findings of EU or international scientific bodies

No residue data were available from other EU or international scientific bodies concerning establishment of maximum residues.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing, if relevant

Not relevant.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits, if relevant

Not relevant.

3.3. Elaboration of MRLs

The bioavailability data provided allowed to conclude that the bioavailability of the substance is negligible and therefore the establishment of MRLs for the substance is not required for the protection of the consumer.

3.4. Considerations on possible extrapolation of MRLs

In line with the Article 5 of Regulation No. 470/2009 the Committee considered the possibilities for extrapolating the MRL recommendation in bovine species to other species and foodstuffs with a view to ensuring availability of veterinary medicinal products for conditions affecting food producing animals while ensuring a high level of protection of human health.

In view of the negligible oral bioavailability of the substance, extrapolating to other food producing species would be possible without concerns related to the protection of human health. However, pegylated bovine granulocyte colony stimulating factor is a specific bovine substance developed to stimulate the immune system in bovine. It is very unlikely that species specific substances would exert the same therapeutic activity in other species. Therefore the Committee does not consider appropriate

to extrapolate MRLs to species in which the substance might not have therapeutic activity hence not increasing the availability with regard to treatment in other species.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the bioavailability of PEG-bG-CSF after oral exposure is negligible,
- due to the negligible oral bioavailability of PEGbG-CSF there is no need to establish an ADI,
- since the oral bioavailability of PEGbG-CSF is negligible the ingestion of potential residues of PEGbG-CSF in animal tissues or products does not pose a risk to consumers;

the CVMP recommends the inclusion of pegylated bovine granulocyte colony stimulating factor in table 1 of the Annex to Regulation (EU) No. 37/2010 in accordance with the following table:

Pharmaco- logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Pegylated bovine granulocyte colony stimulating factor	Not applicable	Bovine	No MRL Required	Not applicable	NO ENTRY	Biological/ Immunomodulator

4. Background information on the procedure

Submission of the dossier	4 March 2010
Steps taken for assessment of the substance	
Application validated:	16 March 2010
Clock started:	17 March 2010
List of questions adopted:	14 July 2010
Consolidated response to list of questions submitted:	4 February 2011
Clock re-started:	5 February 2011
CVMP opinion adopted:	5 May 2011
Request for reconsideration from the Commission:	12 July 2011
Revised C VMP opinion adopted:	15 September 2011