EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Recombinant bovine IL-8: summary of assessment undertaken to determine whether the substance may be entered into the list of biological substances considered as not requiring an MRL evaluation

In accordance with Commission Regulation (EU) 2018/782, biological substances other than those identified in Article 1(2)(a) of Regulation (EC) No 470/2009 shall be evaluated on a case-by-case basis where the biological substance is chemical-unlike, and a report describing the scientific basis for the request on whether a full MRL evaluation is required or not shall contain information as set forth in the following sections A to E.

A. *Nature of the biological substance (e.g. cell, tissue, live or killed organism) and a comparison with similar biological substances to which consumers are known to be routinely exposed*

Recombinant bovine interleukin-8 (rbIL-8) is a protein which is produced in *E. coli* strain One Shot BL21 Star (DE3) containing the pET28b plasmid. rbIL-8 consists of a 79 amino acid variant from the gene sequence of bovine IL-8 (bIL-8) as well as a 40 amino acid peptide leader sequence, which includes a 6-histidine tag utilized for purification purposes. Hence, only part of the peptide is similar to the naturally occurring molecule. No information was provided on the natural bovine IL-8 levels in meat and offal, hence the normal exposure of the consumer to bovine IL-8 is not known. In bovine milk, normal bIL-8 concentrations are in the range of 0–6.17 ng/ml. The concentration of IL-8 in human breast milk (hIL-8) is up to 13 ng/ml.

Based on comparison of the sequence of rbIL-8 to known allergen databases, rbIL-8 is not likely to exhibit allergenic cross-reactivity in comparison to known allergens and the risk for immunogenicity is similar to that from consuming bovine milk not containing rbIL-8.

B. *Description of the mechanism of action underlying the substance's therapeutic effect and, if available, information on its potency*

Interleukin-8 is a chemoattractant that can bind to high-affinity receptors present on the surface of neutrophilic granulocytes. rbIL-8 attracts neutrophils to the reproductive tract in Holstein cows and

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intrauterine treatment with rbIL-8 is reported to significantly reduce the incidence of puerperal metritis in multiparous cows. The biological effect of rbIL-8 is not expected to be higher than that of bIL-8.

C. Fate of the substance in the treated animal (i.e. is it bioavailable, are residues expected in food commodities)

After intrauterine treatment with 500 µg/animal, rbIL-8 was detected in the milk of 1 out of 24 cows (LOQ = 3.13 ng/ml). Therefore, it cannot be excluded that residues of rbIL-8 will occur in edible tissues or milk.

However, from studies in rats, it can be concluded that oral bioavailability of rbIL-8 is low.

D. Any activity that the substance may have in the human gut (are the residues inactive or do they produce local effects)

rbIL-8 is extensively degraded in the human digestive tract in adults and it is therefore unlikely to have relevant local effects in adults.

Local effects of rbIL-8 in the gut of human infants are considered possible based on literature reports of *in vitro* studies and decreased digestion in infants as compared to adults due to a higher gastric pH value. Nevertheless, considering the exposure of infants to hIL-8 through human breast milk and the lower activity of rbIL-8 on human CXCR1/2 receptors (hCXCR1/2) when compared to recombinant hIL-8 (rhIL-8) or bIL-8, the risk for infants from consuming rbIL-8 can be considered low.

E. Systemic availability of residues following ingestion of residues by consumers, along with a worst-case consumer exposure estimate

From a pharmacokinetic study in rats, it can be concluded that bioavailability of rbIL-8 is low. rbIL-8 is effectively digested in the stomach and intestine, and residues possibly available for absorption are estimated to be < 1% of human endogenous IL-8 levels.

Conclusions

Having considered that:

- rbIL-8 does not naturally occur in bovine milk and tissues,
- the potency of rbIL-8 to attract human neutrophils is similar to that of rhIL-8 and bIL-8,
- rbIL-8 activates hCXCR1/2 receptors to a lesser degree than rhIL-8 or bIL-8,
- the peptide presented is effectively degraded/inactivated in the human digestive tract in adults,
- the peptide is likely to be degraded to a lesser degree in the digestive tract of infants, but potential local effects in the gut are expected to be no different to those produced by natural hIL-8 present in breast milk,
- rbIL-8 is not likely to exhibit allergenic cross-reactivity in comparison to known allergens and the risk for immunogenicity is similar to that from consuming milk not containing rbIL-8,
- a worst-case estimation of possible combined exposure (rbIL8 + bIL8) shows that there is no increased risk for the consumer following intrauterine treatment of cows with rbIL-8 up to a dose of 1,000 µg,

the Committee concludes that no further MRL assessment is necessary for recombinant bovine IL-8 and that the substance can be included in the list of biological substances considered as not requiring an MRL evaluation as per Regulation (EU) No 2018/782 with the following entry:

Recombinant bovine IL-8 (His-tag) for intrauterine use in cattle at a dose of up to 1,000 µg per animal