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Veterinary Medicines Division

Bovine casein hydrolysate: 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, biologicals shall be evaluated on a case-by-case basis 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:

Bovine casein hydrolysate (bCNH) is a mixture of polypeptides, oligopeptides and amino acids which is produced by enzymatic hydrolysis of bovine sodium caseinate. It is intended for intramammary use to reduce the risk of new infections in dairy cows at drying off and for treatment of clinical mastitis in lactating cows. The effect is reported to result from a more rapid loss of tight junction integrity followed by drying-off of mammary secretion compared to the physiological processes during mammary involution. The dose is two or three times 1200 mg bCNH per udder quarter at intervals of 12 hours.

Bovine casein is the main structural protein of cow's milk and accounts for approximately 80% of the total milk protein. The manufactured bCNH is closely similar in respect to its qualitative and quantitative composition to bCNH that naturally occurs in bovine milk in low concentrations.

The manufacturing process of casein hydrolysate is based on sodium caseinate from milk collected for human consumption and involves hydrolysation of sodium caseinate by trypsin as well as subsequent heating to inactivate trypsin. The HPLC spectrum of the hydrolysed product was compared to HPLC spectra of functional food and baby foods which consist of or contain bCNH and were not considered significant. The fractions of sodium caseinate present in the bCNH will vary depending on the points of contact of the enzyme with the protein and consequently the spectra will not be identical.

Bovine casein is present in milk together with the endoprotease plasmin and others. It can therefore be assumed that the naturally occurring casein hydrolysate in milk is formed due to hydrolysis by plasmin and further proteases. Unlike plasmin, the digestive enzyme trypsin – used in the

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manufacturing process for bCNH - is not naturally present in cow's milk. However**,** the peptidase activity of plasmin is very similar to that of trypsin and is therefore considered comparable.

Casein components are known food allergens, but the enzymatic hydrolysis is likely to reduce the allergenicity compared to native casein/casein hydrolysate. Potentially immunogenic bCNH polypeptides reactions from bCNH treated animals are not considered to present a hazard different from that of native caseins.

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

Casein naturally accumulates in the mammary gland during the dry period. Its mode of action is based on the plasminogen activator (PA)–plasminogen–plasmin system, which controls the level of the active protease plasmin. Increased plasmin and PA activity in bovine milk are correlated with gradual involution in the declining phase of lactation. Casein hydrolysate has antisecretory and anti-inflammatory-inducing properties, presumably by imitating natural involution phenomena. Within hours after the first treatment, bCNH induces a loss of tight junction integrity resulting in ion shifting between interstitial fluid and milk followed by rapid drying-off of mammary secretion. The reduced time until dry off is most likely a result of the decrease of lactose concentration, since this is the main osmotic component in milk. The process induced by bCNH treatment is more rapid and synchronized among treated animals than the involution induced naturally at drying-off. Consequently, the concentration of casein hydrolysate peptides in the udder is intended to be increased by intramammary treatment with bCNH, to enhance involution in the early phase of the dry period.

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

Bovine casein hydrolysate is naturally present in the bovine mammary gland, acting as part of the internal metabolism process and during evolution/involution processes of the udder. Milk contains several proteases, including kallikrein, cathepsins and plasmins, causing proteolysis of milk proteins within the udder and release of peptide fragments and free amino acids. Endogenous bCNH is present in milk prior to and during involution of the mammary gland. However, the ratio of hydrolysed casein to non-hydrolysed casein is unknown. Based on the available information it can be assumed that bCNH contains no other components than natural bovine casein hydrolysates.

Veterinary medicines containing bCNH manufactured by hydrolysation with trypsin will add to the casein already present in milk. As it is administered into the udder, the metabolic fate can be assumed to be the same as in normal milk.

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

Once ingested, both the hydrolysed casein and native casein undergo the same enzymatic and non-enzymatic degradation processes in the human digestive tract. In principle small peptides (di-and tripeptides) from casein precursors could have pharmacological effects including antihypertensive effects and opioid receptor binding effects. However, since both the hydrolysed casein and casein from the diet undergo the same enzymatic and non-enzymatic degradation in the human digestive tract, the peptide composition can be expected to be similar to that which occurs as a result of ingestion of naturally occurring casein.

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

Based on the available data, it can be assumed that bCNH used in the proposed product does not significantly differ from the hydrolysed casein present in milk for human consumption with regard to its properties, i.e. residues from bCNH resulting from udder treatment are not expected to differ from residues of naturally produced casein already present in milk (and in much lower amounts also in edible tissues) and so will have the same bioavailability and nutritional-physiological characteristics.

In a worst-case exposure assessment for consumers based on a total dose of manufactured bCNH, the treatment-related casein and casein hydrolysate intake would be below 9.2% and 50% of the estimated total daily intake of caseins. Small amounts of casein may also be present in edible tissues from cows with mastitis and thus it cannot be excluded that small amounts of manufactured bCNH enter the systemic circulation in cows with mastitis.

The hydrolysed casein is i) not considered to have other effects than the native casein/casein hydrolysate, ii) most likely more easily digestible compared to non-hydrolysed casein, and iii) considered to be within the normal variation of the human diet as the intended dose results in an additional casein intake of less than 10% of the total casein intake. In addition, consumers may already be exposed to hydrolysed casein through food supplements and infants may be exposed through formula milk for babies. Overall, consumer exposure to hydrolysed casein resulting from intramammary treatment is considered to be without impact on consumer safety.

Having considered that:

- bovine casein hydrolysate is a biological substance,
- the substance presented by the manufacturer is similar to naturally occurring casein/casein hydrolysate and is expected to be degraded in the human digestive tract in the same way as the naturally occurring substance,
- intake of treatment-related bCNH after intramammary administration is not expected to significantly increase the daily casein intake (including naturally occurring casein hydrolysate),
- potentially immunogenic bCNH polypeptides from treated animals are unlikely to present a risk different from native caseins,

the Committee concludes that no further MRL assessment is necessary for the intramammary use of bCNH 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:

- *Bovine casein hydrolysate (bCNH), produced from sodium caseinate hydrolysed with trypsin, heat treated, for intramammary use in cows.*