26 January 2018
EMA/CVMP/208391/2017
Committee for Medicinal Products for Veterinary Use

# European public MRL assessment report (EPMAR)

## Bromelain (porcine species)

On 14 September 2017 the European Commission adopted a Regulation[1] establishing maximum residue limits for bromelain in porcine 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.

In veterinary medicines, bromelain is intended for use in young pigs for the prophylaxis of diarrhoea due to enterotoxigenic *Escherichia coli.* It is administered as a single oral dose of 16 to 25 mg/kg bw that may be repeated.

Triveritas submitted to the European Medicines Agency an application for the establishment of maximum residue limits on 20 June 2016.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended, on 11 May 2017, the establishment of maximum residue limits for bromelain in porcine species.

Subsequently the Commission recommended, on 28 July 2017, that maximum residue limits in porcine species are established. This recommendation was confirmed on 18 August 2017 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 14 September 2017.

[1] Commission Implementing Regulation (EU) No 2017/1558, O.J. L237 , of 15 September 2017

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# Summary of the scientific discussion for the establishment of MRLs

| | |
|---|---|
| Substance name: | Bromelain |
| Therapeutic class: | Antidiarrhoeal |
| Procedure number: | EMEA/V/MRL/004479/FULL/0001 |
| Applicant: | Triveritas Ltd |
| Target species requested: | Porcine |
| Intended therapeutic indication: | Prophylaxis of diarrhoea |
| Route(s) of administration: | Oral |

# 1. Introduction

Bromelain is a concentrate of proteolytic enzymes from the pineapple plant *Ananas comosus*. Stem bromelain is extracted from the stems but bromelain is found in all parts of the plant. Fruit bromelain is extracted from the fruit. Both are cysteine endopeptidases. Bromelain is a member of the peptidase family C1 of enzymes, as is papain.

In veterinary medicine, bromelain is intended for use in young pigs in the prophylaxis of diarrhoea due to enterotoxigenic *Escherichia coli* as a single oral dose of 16 to 25 mg/kg bw that may be repeated.

Bromelain has a history of use in folk medicine and as a meat tenderizer. A concentrate of proteolytic enzymes enriched in bromelain currently has an approved medicinal use within the EU as a topical medication for the removal of eschars in humans. Oral bromelain is also approved nationally for the treatment of oedema in humans.

# 2. Scientific risk assessment

Bromelain can be characterised as a concentrate of proteolytic enzymes. Although bromelain's primary constituent is a sulfuryl proteolytic fraction, it also contains escharase (a non-proteolytic component in bromelain thought to be important in the action of topical bromelain), peroxidase, acid phosphatase, several protease inhibitors, and organically-bound calcium. Since only water is used in the production process any impurities will only represent natural substances from the plant.

## *2.1. Safety assessment*

### 2.1.1. Overview of pharmacological properties

#### Pharmacodynamic properties including mode of action

Bromelain is intended for use in young pigs in the prophylaxis of diarrhoea due to enterotoxigenic *Escherichia coli*.

The primary pharmacodynamic action is prevention of the attachment of enterotoxigenic *Escherichia coli* that produce the K88 colonisation factor (K88+) to piglet small intestine.

Several pharmacodynamic activities are described in the literature such as anti-inflammatory activity, preventing the formation of fibrin, reducing leukocyte migration, specific inhibition of cyclooxygenase-2

(COX-2) expression, decreases in prostaglandin E2, and immunomodulatory effects. No pharmacological NOELs have been established for these effects.

**Pharmacokinetic properties**

Pharmacokinetic data are available in rats and humans.

Male Sprague-Dawley rats were given single oral doses of [$^{125}$I]bromelain, (3.5 mg/kg bw), as an aqueous solution. Each experimental group consisted of 6 rats and each control group contained 3 animals. At 0, 1, 2, 3, 4, 5, 8 and 12 hours after administration animals were anaesthetised and sacrificed. Blood was collected and analysed for the presence of [$^{125}$I]-proteins and for determination of the molecular weights of proteins detected by polyacrylamide gel electrophoresis. The concentration of total radioactivity in plasma reached a maximum of approximately 3500 ng-equivalent $^{125}$I-bromelain /ml at around 3 hours after administration. A maximum concentration of 270 ng-equivalent $^{125}$I-bromelain /ml in the acid insoluble fraction was found at 1 hour after administration. After 1-3 hours, concentrations of bromelain in plasma steadily declined. Electrophoresis showed at 1 hour after administration a major peak in plasma at 26-32000 Daltons corresponding to the molecular weight of bromelain. Integration of the area under this major peak results in a concentration of about 26 ng bromelain per ml plasma. This study demonstrates that bromelain is absorbed intact from the rat gastrointestinal tract after oral administration but oral bioavailability was very low with plasma concentrations in the ng/ml region.

In a study in healthy male volunteers aged 18-45 years, bromelain was given orally in enteric-coated tablets to 15 volunteers at approximately 4 g/day for 2 days followed by 600 mg on the third day, to yield a total oral dose of 8.6 g (143 mg/kg bw) bromelain. Four volunteers were given placebo.

Plasma bromelain was determined in the period 3 to 51 hours after the start of administration. The presence of undegraded bromelain was assessed using immunoprecipitation and gel electrophoresis and immunodetection. For most volunteers, $C_{max}$ was reached at about 48 hours, with a mean value close to 5000 pg/ml. Immunoprecipitation and the use of anti-bromelain antibodies showed that the material in plasma was undegraded bromelain. The material was associated with $\alpha$2-macroglobulin and $\alpha$1-antichymotrypsin. The estimated plasma half-life was 6-9 hours. This study shows that a small proportion of orally administered bromelain is absorbed from the human gastrointestinal tract.

The above studies in rats and humans indicate that bromelain is absorbed following oral administration and that intact parent substance is present at very low plasma levels (in the order of ng/ml), suggesting very limited bioavailability.

No data on distribution, biotransformation or excretion of bromelain following oral administration are available.

Based on the estimation that the exposure to bromelain via meat from pigs treated with bromelain is expected to be substantially lower than that which occurs via the normal diet (see section 3.3 below) it is agreed that no further data on the pharmacokinetics of bromelain is required.

## 2.1.2. Calculation of pharmacological ADI, if relevant

The available data do not allow establishment of a pharmacological ADI. This can be accepted since the exposure to bromelain residues in pig meat will not be significant compared to that which occurs via the normal diet (see section 3.3).

## 2.1.3. Overview of toxicology

*Single-dose toxicity*

The following $LD_{50}$ values have been reported for bromelain:

- Oral, rat greater than 10 000 mg/kg bw
- Oral, mouse greater than 10 000 mg/kg bw
- Intraperitoneal, rat 85.2 mg/kg bw
- Intraperitoneal, mouse 36.7 mg/kg bw
- Intravenous, mouse 30 mg/kg bw
- Intravenous, rabbit 20 mg/kg bw

A related protease, ananain, has been tested to determine the acute tolerated dose, the highest dose administered in a 24-hour period that does not induce weight loss in an experimental animal in excess of 15% of the original body weight or cause death or severe morbidity at any time during the study period, using female BALB/c mice and the oral, intravenous and intraperitoneal routes of administration. The acute tolerated doses in mice were 40, 30 and 20 mg/kg bw by the oral, intravenous and intraperitoneal routes respectively.

In conclusion bromelain has low acute toxicity when administered orally.

*Repeated dose toxicity*

In a GLP study rats were administered a commercial formulation containing bromelain (for which the composition was not available) at 0, 80, 160 or 330 mg/g feed (equivalent to 0, 5552, 11888 and 24830 mg/kg bw/day in males and 0, 5716, 10349 and 24307 mg/kg bw/day in females, respectively) for 14 days. Rats were observed for body weight, feed intake, histopathological examination and clinical chemistry. Reduction of body weight was significant at day 14 in rats administered bromelain at 330 mg/g feed in both sexes (equivalent to approximately 24500 mg/kg bw/day).

The maximum tolerated dose of ananain was determined in mice based on 15% reduction of body weight. Other parameters included clinical observation and histopathology. Mice were administered 16, 24, 32 or 40 mg/kg bw/day orally for 7 days. The maximum tolerated dose was 32 mg/kg bw/day for the oral route. Mild hepatocellular and myocardial changes were observed at 16 mg/kg bw.

These repeat-dose studies are not considered adequate for the purpose of establishing a NOEL/NOAEL for bromelain: only summary data were available for the rat study and the test substance used in the mouse study was not bromelain and histopathological effects were seen at the lowest dose.

No 90-day or chronic oral toxicity studies are available.

*Reproductive toxicity, genotoxicity and carcinogenicity*

No studies of effects on reproduction, genotoxicity, or carcinogenicity are available.

*Studies of other effects including immunotoxicity and neurotoxicity*

No studies are available in animals. However hypersensitivity has been described in humans after oral exposure (see section 2.1.7 below).

### 2.1.4. Calculation of the toxicological ADI or alternative limit

The available data do not allow the establishment of a toxicological ADI for bromelain. This can be accepted since the exposure to bromelain via meat from pigs treated with bromelain stem is expected to be substantially lower than that which occurs via the normal diet (see section 3.2).

### 2.1.5. Overview of microbiological properties of residues

There are no data to suggest that bromelain possesses microbiological activity. Bromelain, as a proteolytic enzyme, is unlikely to have any significant microbiological effects.

### 2.1.6. Calculation of microbiological ADI

As bromelain is not expected to possess antimicrobial activity the establishment of a microbiological ADI is not relevant.

### 2.1.7. Observations in humans

Allergic asthma has been reported following pineapple ingestion and studies have confirmed the implication of bromelain in the induction of respiratory sensitisation and the involvement of IgE antibodies.

The safety of bromelain in humans has been studied using a double-blind, randomised, multiple dose, and placebo controlled methodology. The study was conducted in compliance with Good Clinical Practice.

Healthy volunteers (6 per group) were given oral doses of bromelain equivalent to 0, 26.7 or 53.4 mg/kg bw/day for 28 days. No adverse effects attributable to bromelain were seen in this study.

No bromelain was detected in the plasma of any of the bromelain-treated subjects. However, a bromelain-specific immunoglobulin G response was noted in the high dose group but not in the low dose or placebo groups after 14 days of treatment. By day 28 there was a significant dose-related bromelain-specific immunoglobulin G response that was not observed in placebo controls.

A study of the safety of orally administered bromelain was conducted in children aged under 11 years. This study comprised children aged 0-2 years (n = 3), 3-5 years (n = 11) and 6-11 years (n = 48) made up of 28 male and 34 female subjects all of Caucasian ethnicity and given bromelain as a monotherapy, primarily for the treatment of sinusitis. A further group of 34 subjects was given bromelain and conventional therapy while a third group of 20 children treated with conventional therapies only served as controls. The duration of administration was between 4-5 days. The only side effect seen in the study was a mild self-limiting allergic reaction in the bromelain only group in a subject known to have an allergy to pineapples.

In conclusion, even in the absence of bromelain plasma detection, oral administration of bromelain to humans induced an immunological reaction, as shown by bromelain-specific immunoglobulin G response. Moreover allergic reactions to bromelain and pineapple have been reported. A systematic review on prevalence of plant food allergies indicates that on the basis of skin prick tests, the prevalence of allergic reactions to pineapples was very low (0.3%) and lower than for apples and kiwis. There is no information on what exposure can sensitize the consumer or trigger an allergic reaction in already sensitized individuals and no safe level can therefore be established on the basis of the available data. However, bromelain residues are not considered to constitute a higher risk for allergy for the consumer than other components of pig feed.

### 2.1.8. Findings of EU or international scientific bodies

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) assigned the ADI as 'not limited' for bromelain. The basis for this classification was not available.

Bromelain has a GRAS (generally recognised as safe) status in the United States for direct addition to human food.

### 2.1.9. Overall conclusions on the ADI

A microbiological ADI is not considered necessary as the substance is not expected to possess antimicrobial activity. The available data do not allow establishment of a pharmacological or toxicological ADI for bromelain and consequently no overall ADI can be established.

This can be accepted since the exposure to bromelain via meat from pigs treated with bromelain is expected to be substantially lower than that which occurs via the normal diet (see section 3.3).

## *2.2. Residues assessment*

### 2.2.1. Pharmacokinetics in target species

No pharmacokinetic data are available in the target species pig.

### 2.2.2. Residue depletion studies

No residue depletion data are available in the target species, pig. This can be accepted since the exposure to bromelain via meat from pigs treated with bromelain is expected to be substantially lower than that which occurs via the normal diet (see section 3.2).

**Selection of marker residue and the ratio of marker to total residues**

A "No MRL required" classification is intended and therefore identification of a marker residue or the ratio of marker to total residues is not necessary.

### 2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report were available.

### 2.2.4. Analytical method for monitoring of residues

No analytical method was provided. For a "No MRL required" classification an analytical method is not needed.

### 2.2.5. Findings of EU or international scientific bodies

No relevant reports were available.

# 3. Risk management considerations

## *3.1. Potential effects on the microorganisms used for industrial food processing*

Bromelain, as a proteolytic enzyme, is unlikely to have relevant microbiological effects. Therefore potential effects in dairy products or other food products were not investigated and are not necessary.

## *3.2. Other relevant risk management considerations for the establishment of maximum residue limits*

Bromelain is naturally present in pineapple and so in human food. It is also marketed in the EU as a dietary supplement.

Bromelain is also used for tenderizing meat. Enzymes are generally heat-labile and denatured in the cooking process, further reducing the risk when eating pig meat if residues are present. For bromelain it has been reported that incubation at 80°C for 8 min caused almost complete activity loss.

No other relevant factors were identified for consideration of the risk management recommendations.

## *3.3. Elaboration of MRLs*

Studies monitoring bromelain residues in meat from treated animals have not been provided. However, a worst case consumer exposure estimate has been performed and compared with the exposure that is likely to arise from ingesting a portion of pineapple.

Using the proposed dose of 125 mg bromelain for a 6 kg weaner pig and a worst case oral absorption figure of 4% results in an estimated total systemically available dose of 5 mg, or approximately 0.85 mg/kg bw. Assuming uniform distribution and no elimination, a consumer ingesting 500 g of meat from a treated animal would therefore be exposed to 0.425 mg bromelain. This is acknowledged to be a worst case estimate as the oral absorption figure of 4% is based on total radioactivity seen in plasma following an oral dose of radiolabelled bromelain in rats and does not take account of fact that a substantial portion of this radioactivity is likely to be due to inactive break-down products and not to parent bromelain.

Based on literature data it is estimated that the bromelain content of a 250 g portion of pineapple will be at least 1.6 mg (the efficiency of the extraction process used to calculate this figure is not known and consequently the actual level of bromelain present in pineapple may be considerably greater).

The above calculations indicate that a consumer ingesting a standard 500 g portion of meat from a treated pig would be exposed to approximately 4 times less bromelain than a consumer ingesting a 250 g portion of pineapple. It should be noted that the assumptions made in arriving at these figures are likely to overestimate the amount of bromelain in pork and underestimate the amount of bromelain in pineapple. Further reassurance is provided by the fact that enzymes are generally heat labile and denatured during the cooking process, further reducing the amount of active bromelain likely to be present in ingested pork.

Overall, considering that bromelain is obtained from the pineapple plant, a source of human food, and that exposure to bromelain in pig meat is expected to be substantially lower than that which occurs via the normal diet, the establishment of maximum residue limits for bromelain is not considered necessary for the protection of human health.

### *3.4. Considerations on possible extrapolation of MRLs*

In line with Article 5 of Regulation (EC) No 470/2009, the CVMP considered the possibility of extrapolating the maximum residue limits recommended for pigs to other food producing species and commodities.

The recommendation for a "No MRL required" classification for bromelain in pigs is largely based on the fact that consumer exposure to bromelain resulting from ingestion of meat from treated pigs is expected to be substantially lower than that which occurs via the normal diet. Consumer exposure to residues will, to some extent, be dependent on the dosing regimen employed in target species and on consumption figures for the commodities (including milk) produced by that species. In the absence of any information on the dosing regimens that might be employed in other target species it is not possible to conclude that the estimates performed for pigs are applicable in these other species. Consequently no extrapolation can be recommended.

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

Having considered that:

- no ADI can be established for bromelain as appropriate data are not available.
- bromelain is a concentrate of proteolytic enzymes from the pineapple plant, a source of human food,
- the consumer exposure to bromelain resulting from ingestion of meat from treated pigs is expected to be substantially lower than that which occurs via the normal diet,

the Committee recommends the inclusion of bromelain in table 1 of the Annex to Regulation (EU) No. 37/2010 as follows:

| **Pharmaco-logically active substance** | **Marker residue** | **Animal species** | **MRLs** | **Target tissues** | **Other provisions** | **Therapeutic classification** |
|---|---|---|---|---|---|---|
| Bromelain | NOT APPLICABLE | Porcine | No MRL required | NOT APPLICABLE | NO ENTRY | Antidiarrhoeal agents |

# 4. Background information on the procedure

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| Submission of the dossier | 20 June 2016 |
| Steps taken for assessment of the substance | |
| Application validated: | 13 July 2016 |
| Clock started: | 14 July 2016 |
| List of questions adopted: | 10 November 2016 |
| Consolidated response to list of questions submitted: | 3 February 2017 |
| Clock restarted: | 13 February 2017 |
| CVMP opinion adopted: | 11 May 2017 |