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Committee for Medicinal Products for Veterinary Use

CVMP assessment report regarding the request for an opinion under Article 30(3) of Regulation (EC) No. 726/2004

For veterinary medicinal products containing gentamicin for parenteral administration to horses

Procedure no: EMEA/V/A/128

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1. Background information on the procedure

1.1. Request for CVMP opinion

On 10 April 2018 the Executive Director of the European Medicines Agency requested the Committee for Medicinal Products for Veterinary Use (CVMP) for a scientific opinion under Article 30(3) of Regulation (EC) 726/2004 concerning veterinary medicinal products containing gentamicin for parenteral administration to horses.

1.2. Steps taken during the referral procedure

- During the April 2018 CVMP meeting, the following was agreed:
  - Mary O'Grady was appointed rapporteur.
  - Wilhelm Schlumbohm was appointed co-rapporteur.
  - The procedure started on 18 April 2018 and lists of questions were adopted.
- On 20 April 2018 letters were sent to the marketing authorisation holders (MAHs) and active pharmaceutical ingredient (API) manufacturers for veterinary medicinal products containing gentamicin for parenteral administration to horses informing them about the start of the procedure including the list of questions and the official notification from the Executive Director of the European Medicines Agency to the CVMP on the procedure under Article 30(3) of Regulation (EC) No 726/2004.
- The deadline for submission of responses by the MAHs and API manufacturers was 1 June 2018.
- The rapporteur's assessment report was circulated to all CVMP members on 26 June 2018.
- The co-rapporteur's critique to the rapporteur's assessment report was circulated to all CVMP members on 2 July 2018.
- On 5 July 2018 the rapporteur's assessment report including the co-rapporteur's critique was forwarded to the MAHs.
- During the July 2018 CVMP meeting, a list of outstanding issues to the API manufacturer was adopted.
- On 19 July 2018 the list of outstanding issues was sent to the API manufacturer.
- The deadline for answers from the API manufacturer was 24 August 2018.
- On 24 September 2018 the revised rapporteur's assessment of the answers to list of outstanding issues was circulated to all CVMP members.
- The co-rapporteur's critique to the revised rapporteur's assessment report was circulated to all CVMP members on 1 October 2018.
- During the October 2018 CVMP meeting, the Committee considered the rapporteur's assessment report including the co-rapporteur's critique and agreed that no outstanding issues remained. The majority of the CVMP members indicated that they would support the (co-)rapporteur's conclusions.

- On 25 October 2018 the updated rapporteur's assessment report including the co-rapporteur's critique was circulated to all CVMP members.
- On 8 November 2018 the CVMP adopted an opinion in accordance with Article 30(3) of Regulation (EC) No 726/2004.

2. Scientific discussion

2.1. Introduction

Veterinary medicinal products containing gentamicin for parenteral administration to horses are currently authorised in a number of EU Member States.

The active pharmaceutical ingredient (API) gentamicin sulfate is manufactured via a standard fermentation process that includes the raw material peptone which may be derived from fish. There are three gentamicin sulfate manufacturers who supply the EU market, two of which are holders of a Certificate of Suitability (CEP) to the monograph of the European Pharmacopeia.

Between 2015 and 2017, at least 154 adverse events (AEs) in horses were reported following the use of gentamicin solution for injection in the European Union, the majority of which described anaphylactic-type reactions, a number of which (at least 9) were associated with death.

In the active substance batches of the gentamicin-containing veterinary medicinal products involved in these reported AEs, residues of histamine were found, which were considered to be the underlying cause of the reactions. All AEs are associated with a single manufacturer of API. The investigation by the concerned API manufacturer revealed the root-cause of the high levels of histamine as being related to inappropriate storage conditions of fish at the premises of one fish peptone supplier, which resulted in histamine residues in the API used to manufacture the affected finished products.

The European Pharmacopoeia does not currently require the control of the level of histamine in the monograph for the API gentamicin sulfate. However, further requirements related to the quality of raw materials have been added to the raw materials section of the monograph on Products of fermentation (1468) with the following texted added:

Special attention must be paid to the levels of free histidine in fish peptones as the presence of free histidine may lead to histamine formation in certain conditions.

The revised monograph has been implemented on 1 April 2018. The revision to the monograph highlights the potential for histamine contamination when fish peptones are used in the manufacturing process, but it does not provide any guidance relating to the requirements that should be applied. Consideration should be given to further revision of the Ph. Eur. monograph 1468 on Products of Fermentation with respect to the requirements on the quality of raw materials.

Three manufacturers of the active substance for the EU market have been identified, two of whom hold CEPs to the monograph of the European Pharmacopeia. The third supplier does not hold a CEP. A limit for histamine of 16 ppm was applied by one of the manufacturers of the active substance as an interim limit pending the results of further investigations.

During meetings of the veterinary incident review group and incident review network (for human medicinal products) held in 2017-2018, it was decided that a further consideration of the issue would be necessary.

In light of the above, it was considered necessary to ask the CVMP to provide an opinion under Article 30(3) of Regulation (EC) No 726/2004 on the need for inclusion of a maximum limit for histamine in API specifications for gentamicin sulfate and/or finished product specifications for gentamicin-containing medicinal products for parenteral administration to horses.

Taking into account that the underlying cause is the same for medicinal products for human and veterinary use a parallel procedure for a CHMP Opinion under Article 5(3) of Regulation (EC) No 726/2004 was initiated.

2.1.1. Information made available to CVMP

Further to the identification of the veterinary medicinal products containing gentamicin for parenteral administration to horses authorised nationally in the EU, the concerned MAHs were invited to provide the following, relating to all batches of finished product produced since 1 January 2013:

1. Where data exist:
    1.1. Information on the level of histamine in batches of **finished product**, along with details of the manufacturer of the active substance and batch numbers of active substances used in these batches and details of the analytical methods used.
    1.2. Information on the level of histamine in batches of **active substance**, along with details of the manufacturer of the active substance and details of the analytical methods used.
2. Where data on histamine levels in batches of finished product do not exist but information on histamine levels in batches of the active substance do exist, an estimate of histamine levels in batches of finished product should be provided, based on the level of histamine known to have been present in the active substance.
3. Information about any hypersensitivity-type adverse events reported in horses following the parenteral use of gentamicin. If known, include the route of administration and the doses used, the batch numbers of the finished product and active substance involved with these reactions and an analysis of the histamine levels in those batches of finished product and the respective batches of active substance.
4. A periodic safety update report (PSUR) for the period 01/01/2013 to 31/12/2017.
5. An analysis of available data, including from the published literature, on levels of histamine known to have been associated with hypersensitivity-type reactions in horses.

Based on the data provided in response to questions 1-5 above, and with a view to mitigating the risk of histamine-related adverse events in horses, the MAHs are requested to:

6. Propose an acceptable upper limit for histamine for inclusion in either the active substance specification for gentamicin sulfate or the finished product specification for veterinary medicinal products for parenteral administration to horses.

In a separate list of questions, the API manufacturers were requested to provide, where data exist, information on the level of histamine in batches of gentamicin sulfate, along with details of the analytical methods used, for all batches of gentamicin sulfate active substance produced since 1 January 2013.

Some MAHs grouped themselves during the procedure (irrespective of company affiliation) in order to provide consolidated answers to the questions raised by CVMP.

By 1 June 2018, the Agency received responses from 13 MAHs and one API manufacturer who is the main manufacturer for the EU market of the active substance and who introduced an interim limit of 16 ppm for histamine on the API specification.

The MAH responses provided answers to the CVMP LoQ together with supporting data as deemed relevant by individual MAHs, including: documentation provided to the MAH from the API manufacturer regarding the histamine content of API batches, details of MAH analyses of affected API batches, documentation regarding the MAH's assessment of the correlation between affected API batches and observed adverse events and copies of articles from literature relating to the effects of histamine in horses.

The data provided by one MAH includes information on the levels of histamine in a large number of active substance (in excess of 200 batches) and a small number finished product batches. The majority of MAHs did not test histamine levels in the finished product but such data has been provided by one MAH. Other MAHs provided information on the levels of histamine in the API and, based on that, estimated the levels of histamine present in the finished product.

The main manufacturer of the active substance for the EU market provided data relating to the histamine levels in 64 batches of API manufactured between January 2013 and October 2017. The API manufacturer has developed a HPLC-MS analytical method (HPLC with Mass Spectroscopy detection) to determine levels of histamine in the active substance. The analytical method and its validation has been provided in response to questions in this procedure. A very brief overview of corrective actions introduced at the manufacturing site once the root cause of the reactions occurring in horses was connected to the levels of histamine in the products was also provided.

No data has been provided by the two other API manufacturers of the active substance. One of those has stated that they currently don't have any data on histamine levels in their batches of gentamicin, but that they will perform testing and will submit it when available.

2.2. Critical evaluation

2.2.1. Levels of histamine in veterinary medicinal products containing gentamicin for parenteral administration to horses

In this procedure, the Committee was to consider the levels of histamine detected during the manufacture of the API and the finished product where available. In instances where testing for histamine was not carried out for the finished product but was carried out for the API then a reasoned estimate of the levels of histamine present in the finished product was to be established.

Discussion

Information provided by the main manufacturer of the active substance for the EU market

Details of the histamine levels in 64 batches of API has been provided. These batches are identified by the manufacturer as 'batches used or potentially used in veterinary medicinal products'. However, based on the information provided by some MAHs, not all relevant batch data has been provided by the API manufacturer. In particular, data for a number of API batches that are associated with AEs in horses have not been provided by the API manufacturer. In the list provided by the active substance manufacturer, 7 batches are identified as being associated with adverse effects. It is noted that no reports have been received from MAHs for 4 of the 7 batches identified by the API manufacturer as being associated with AEs. It is assumed that these batches were used by MAHs who did not reply to the CVMP request for data.

A HPLC-MS method analytical method for the determination of histamine in the API was developed in 2017 and batches were retrospectively tested. All batches manufactured since 1 January 2014 have been tested for histamine content. The method has been validated and has a limit of detection (LOD) of 0.2 ppm and a limit of quantitation (LOQ) of 1 ppm. The analytical validation is deemed to be in accordance with current requirments[1].

Following identification of the root cause of the adverse reactions, the API manufacturer introduced the following corrective actions:

1. Disqualification of the concerned supplier of peptone
2. Testing of histamine levels in incoming peptone with an acceptance limit of 800 ppm (since May 2017).
3. Testing of all API batches for histamine with a limit of 16 ppm.

On receipt at the API manufacturing site, peptone is stored at room temperature and is assigned a retest period which is supported by stability data demonstrating no increase in histamine levels. Further corrective actions detailed by the API manufacturer include development of in-process methods and performance of histamine purge studies with a view to possible process improvements. Small scale studies were conducted to evaluate the capability of the entire extraction process to reduce histamine levels originating from the fish peptone. In two laboratory scale studies, fermentation broth was spiked to ensure a level of 800 ppm, in the third study, the level of histamine in the broth was measured at 637 ppm and not spiked. In each case, the level of histamine in the final API was then measured with results below 9 ppm in all cases.

Having conducted these laboratory scale purge studies, the API manufacturer has concluded that the process results in sufficient reduction in the levels of histamine. The data provided is from a small number of studies and whilst it shows significant reductions in histamine levels during the manufacturing process, it cannot be considered to establish what levels of histamine can be routinely expected in the final active substance when levels in the peptone raw material are limited to 800 ppm. The CVMP therefore recommends that the API manufacturer should continue to optimise the fermentation process to reduce further the histamine content. The results of the ongoing studies should be presented to the relevant competent authorities with a view to revising the API manufacturing process that is registered for gentamicin sulfate utilising fish peptone.

In relation to the possible use of alternative sources of peptone, the API manufacturer has provided data generated from one laboratory study conducted in 2017 when the fish peptone raw material was substituted with soybean meal in quantities to provide the equivalent nitrogen source in the fermentation media. This study is stated to show a reduction in productivity of over 35%. Further studies are stated to be on-going. The CVMP recommends that the API manufacturer should continue to investigate the use of alternative sources of peptone and that results should be presented to the relevant competent authorities with a view to revising the API manufacturing process that is registered for gentamicin sulfate utilising fish peptone.

The API manufacturer proposes to retain the limit of 16 ppm provisionally set after the initial investigation of the issue. The justification for the limit of 16 ppm is based on the following calculation presented by the API manufacturer:

7 µg is stated from literature to be the minimum dose that can induce vasodilatation and increased heart rate. Assuming an average assay content of 622 µg/g for gentamicin sulfate and a single adult

[1] VICH Guideline 2: Validation of analytical procedures: methodology – link

dose of 80 mg gentamicin, this equates to a level of 54 ppm. Given that the 7 batches associated with AEs as noted by the API manufacturer have a minimum level of 48 ppm, the API supplier considers a limit of 16 ppm to encompass a sufficient safety margin.

The CVMP notes the calculation of the API manufacturer limit of 16 ppm to be incorrect. For a human the recommended daily dose is 3-6 mg/kg in one dose. Assuming the upper limit of the recommended daily dose, the amount of gentamicin for an adult with a bodyweight of 70 kg is 420 mg and not 80 mg. Considering this, the level of histamine for gentamicin sulfate can be calculated as follows:

$$6\text{mg/kg} * 70\text{kg} = 420\text{mg}$$
$$420\text{mg(Gentamicin)} * 1{,}7 = 714\text{mg(Gentamicin sulfate)}$$
$$714\text{mg (Gentamicin sulfate)} * 16\text{ppm} = 11{,}4\mu\text{g (Histamine)}$$

Therefore, with an assumed limit of histamine of 16 ppm the safe value of 7 µg (0.1 µg/kg) histamine for an adult is exceeded. In addition, the API manufacturer did not correctly identify all API batches associated with adverse events and some of those API batches associated with adverse events contained histamine levels below 48 ppm.

Information provided by MAHs

Of the 13 MAH responses received, several included data on levels of histamine in gentamicin API. The data from 5 of these MAHs was considered significant in the context of this assessment. The remaining MAHs provided limited data for a small number of batches, some of which were not commercialised and/or were not the subject of AEs. Only one MAH provided experimental data on the levels of histamine in finished product, several others provided theoretical calculations of the potential levels based on a limit of 16 ppm in the API and the concentration of gentamicin in the product.

MAH No.1.

The product authorised to MAH No.1 is not yet commercialised and therefore no data is available for it. The MAH provided data on histamine levels for 235 batches of API as provided to them by the API manufacturer. The theoretical maximum concentration of histamine in their product was calculated to be 2.5 ppm. The MAH proposed a limit of 16 ppm in the API in line with the interim limit introduced by the API manufacturer.

MAH No.2 and MAH No.3

MAH No.2 and MAH No.3 have provided a joint response covering the three veterinary medicinal products authorised to them. Histamine levels are provided for 6 batches of active substance. These include results provided by the API manufacturer and results from an independent laboratory, contracted by the MAHs. The method used by the contract laboratory is not stated. The results reported by the API manufacturer are lower than those reported by contract laboratory for all but one batch of active substance. The MAHs used the results from the contract laboratory method to estimate the levels of histamine in each batch of finished product. Results range from 1.03 to 2.3 ppm.

The MAHs reported one adverse event associated with one API batch number. Histamine levels for this API batch are reported as 22.7 ppm using the contract laboratory method and 11 ppm using the API manufacturer method.

MAH No.4

MAH No.4 provided information on 17 batches of API and associated finished product batch numbers. Of these, the histamine levels are only available for 6 API batches and range from 2 – 62 ppm. One

API batch with a level of 62 ppm was associated with several AE reports. Other batches with low levels of histamine (2-6 ppm) were also associated with AEs but only in drug product batches that also included API from another batch number for which histamine levels are not available. There is no data available on levels of histamine in finished product batches and for safety reasons the MAH estimated the levels to be the same as those in the associated API batches. The MAH suggested that the limit of 16 ppm in the API in line with the interim limit introduced by the API manufacturer is appropriate.

MAH No.5

MAH No. 5 provided information on 32 batches of API and associated finished product batch numbers. Data on the levels of histamine is available for 28 API batches as reported by the API manufacturer and range from 2 - 66 ppm. Anaphylactic-type reactions were reported for 13 horses and were associated with several API batches. The range of histamine is between 34 – 66 ppm, but histamine levels are not available for all the relevant API batches. The MAH suggested that the limit of 16 ppm in the API in line with the interim limit introduced by the API manufactureris is appropriate.

MAH No. 6

MAH No. 6 provided data on the levels of histamine in API batches as reported by the API manufacturer and as determined by the MAH using a HPLC-FLD method developed by a contract laboratory (HPLC with fluorescence detection). Validation of the HPLC-FLD method has been provided (LOD of 2 ppm and LOQ of 10 ppm).

Data on the levels of histamine for finished product batches determined using a radioimmunoassay (RIA) has also been provided. Whilst results for the RIA assay can distinguish between batches with high and low levels of histamine, the method is not validated and is not considered reliable to quantify histamine levels. Results reported by this MAH for batches tested using both the API manufacturer and contract laboratory methods show a similar pattern to those reported by MAHs 2 and 3 above, generally results obtained using the the API manufacturer method are lower than those using the contract laboratory method. The MAH has also correlated results of the RIA and HPLC methods and the reported adverse events. Individually both of the HPLC methods show a reasonable correlation with the RIA method albeit that the curve relating to the API manufacturer method is consistently below that for the contract laboratory method.

This MAH uses the terms conspicuous and inconspicuous to distinguish between batches associated with adverse reactions and those that are not. Conspicuous batches are described as ones that led to an increased frequency of AEs for which safety measures were deemed necessary. Inconspicuous batches did not show this marked increase in AEs involving anaphylactic-type reactions and safety measures were not necessary.

Using the levels of histamine in API batches determined with the 3 different test methods the MAH has calculated the concentration of histamine in finished product batches and using the dose rate as proposed for the product the concentration in µg/kg bodyweight. Because the test methods give different quantitative results, this results in a range of theoretical histamine levels in the finished product batches. The ranges of histamine for conspicuous batches are 0.71-1.16, 0.50-0.69, 0.48-0.70 and 0.85-1.13 µg/kg bodyweight. The ranges of histamine for inconspicuous batches are 0.01-0.18 µg/kg bodyweight with the exception of 2 batches which have levels of 0.19-0.30 and 0.04-0.25 µg/kg bodyweight. The vast majority of adverse events reported by this MAH (115 out of 124) are associated with the API batches containing 63 and 74 ppm of histamine. The remaining reports are uncertain/not typical for histamine etc. For details of the assessment of these adverse event reports see section 2.2.2 below.

The MAH had applied an internal limit of 18 ppm based on results from the HPLC-FLD method and saw no reason to apply a limit lower than the 16 ppm limit applied by the API manufacturer.

Other MAHs

The responses from the remaining MAHs contain little relevant information. It is noted that only one MAH utilises a different source of API (i.e. not the main manufacturing site for the EU market).

Summary and conclusions

The analytical method used by the main API manufacturer for the EU market to determine levels of histamine in the API is a validated HPLC-MS method. Results of histamine tested using different analytical method(s) provided by MAHs show, in some cases, different results to those obtained using the API manufacturer's method. However, the number of batches tested using the alternative method(s) is small and it is difficult to base conclusions on that data. Given that the API manufacturer's method has been validated and data generated with it is available for a large number of batches, that data primarily forms the basis for this assessment.

Batch data is available detailing levels of histamine in 283 batches of API. The following observations on the data are made:

- Prior to introduction of a new peptone supplier in June 2014 levels of histamine were typically < 3 – 12 ppm (data from approximately 50 batches);
- When a new peptone supplier was introduced between June 2016 – June 2017 more variation was observed with levels ranging from < 3 ppm – 96 ppm (data from in excess of 200 batches);
- Since 24 March 2017, only 2 out of 100 batches have had levels above 8 ppm (2 batches with a level of 9 ppm).

In contrast, there is very little data available on the levels of histamine present in the finished products. One MAH has provided results from an RIA method for testing of finished product. However, the method has not been validated and is not considered reliable to quantify levels of histamine in finished product. No validated analytical method to determine levels in finished product has been provided and it is likely that development of such a method would be difficult due to the sensitivity that would be required for the method and interference from formulation components. Several MAHs have estimated the levels of histamine in finished product based on levels present in the API or based on the interim limit of 16 ppm introduced by the API manufacturer.

Given the absence of actual data, the absence of a validated method and the different approaches adopted by the MAHs, it is not considered possible to set a limit for histamine in finished product at this time.

Histamine is not a degradation product of the API but a process-related impurity originating from the raw material used in the course of fermentation of the API and hence, provided it is appropriately controlled in the API specification, it will not increase in the finished product over its shelf life. Control of such an impurity on the API specification and not on the finished product is accepted practice and the API specification is considered the appropriate point at which to control it. However, as this would be the only point of regulatory control, the limit applied must be stringent given the significance of failing to comply.

2.2.2. Adverse events in horses to veterinary medicinal products containing gentamicin for parenteral administration

In this procedure, the Committee was to consider the adverse events reported to the MAHs between 1 January 2013 and 31 December 2017, in addition to published research on histamine levels, to ascertain the potential causal association between the reactions that have occurred and the use of gentamicin products containing histamine.

Discussion

Adverse events reported to the MAHs between 01/01/2013 and 31/12/2017

Of the 13 MAH responses provided, four included data on adverse event reports received following use of gentamicin containing products in horses. For the remaining MAHs, relevant products were not marketed, not marketed for use in horses or no suspected adverse events were reported.

Four MAH provided PSURs for the period of 01/01/2013 until 31/12/2017. In the SPC of all products the dosage of a single dose of 6.6 mg/kg body weight given intravenously once daily for 3–5 consecutive days is stated based on a CVMP referral procedure under Article 35 of Directive 2001/82/EC, as amended, for veterinary medicinal products containing gentamicin presented as solutions for injection to be administered to horses[2].

In the PSURs the MAHs used the active substance gentamicin sulfate to estimate the number of animals treated. The MAHs suggested that 1 mg gentamicin equates to 1.7 mg gentamicin sulfate.

MAH No. 2 and MAH No.3

MAH No.2 and MAH No.3 provided a joint response and a PSUR relating to three gentamicin containing products covering authorisations in Germany, Latvia, Lithuania and Denmark. The MAH received one relevant report of an adverse event in a horse following use of one product originating from Germany. The API batch implicated in the batch of finished product associated with the adverse event report was determined by the MAH to have a histamine level of 22.7 ppm; however, it is noted that the MAH also stated that the histamine content declared by the API manufacturer to the MAH for this batch was 11 ppm. It is further noted that there was no reference to this batch included in the response provided by the API manufacturer. The report related to intravenous administration of the product to a mare that presented with restlessness during the injection, therefore the veterinarian withdrew the syringe following administration of a few millilitres of product. The animal recovered without treatment.

The MAH provided results from a literature search conducted in an unspecified database using the search terms ‘histamine’ and ‘horses’. Five publications were deemed relevant by the MAH to the consideration of the potential causal association between the observed reactions and use of gentamicin products containing histamine.

In the articles of Eyre and Lewis (1973), Eyre (1976) and Lanz *et al*. (2017) general symptoms after histamine administration were reported.

Eyre and Lewis (1973) conducted a study to evaluate primary cardiovascular and respiratory responses to histamine and other punitive mediators of acute anaphylaxis in the horse. They observed that an intravenous dose of histamine of 0.1 µg or greater caused a distinct fall in systemic blood pressure. Within the dose range of 0.25 – 0.75 µg histamine/kg bodyweight the hypotension was preceded by a sharp transient rise in blood pressure. Doses of histamine greater than 1.0 µg/kg bodyweight caused a

[2] CVMP referral procedure under Article 35 of Directive 2001/82/EC, as amended, for veterinary medicinal products containing gentamicin presented as solutions for injection to be administered to horses – link

biphasic response in which an initial fall in blood pressure was followed by a transient rise which sometimes preceded further more prolonged hypotension. Ventilation was not markedly changed by doses < 0.25 µg histamine/kg bodyweight, but larger doses of histamine caused a brief period of 'gasping' followed by apnoea and a period of 2-3 minutes of increased respiratory frequency. There was a rise in pulmonary artery pressure accompanying doses of histamine sufficient to affect the systemic pressure. At doses of histamine > 1.0 µg/kg bodyweight, a fall in pulmonary arterial pressure was sometimes observed after the initial rise.

Eyre (1976) stated that biogenic amines such as histamine and serotonin seem to be important in the mediation of the primary carotid depressor phenomenon, presumably by inducing vasodilation.

Lanz *et al*. (2017) showed individual variability in horses to histamine administered by inhalation in a saline solution. The median histamine provocation concentration (PC) when Δ_{flow} values increased by 35% was significantly higher in healthy horses (5.94 [1.11 – 26.33] mg/ml) compared to horses with insect bite hypersensitivity (IBH) (2.95 [0.23 – 10.13] mg/ml) and horses with IBH and equine asthma (2.03 [0.43-10.94] mg/ml; p. < 0.01). The PC_{50} and PC_{75} showed very similar differences between groups. Although there is a significant difference in the mean values for an increase of respiratory volume between healthy horses and horses with an allergic history, no dose of histamine could be derived for which a reaction can be expected, as the individual values varied considerably. Additionally, the values obtained cannot be directly transferred to histamine doses which may cause a reaction following intravenous administration.

Two of the publications referenced by the MAH related to findings in humans, DAZ (2012) and Reese *et al*. (2012), which provided evidence for variable histamine intolerance in individuals following various levels of histamine intake.

MAH No.4

MAH No.4 provided a PSUR covering three gentamicin containing products which are authorised nationally in Germany. There were no reports relating to a fourth product authorised to them and there were no reports in cattle, pigs, dogs or cats which are also authorised target species.

The MAH received 13 reports of adverse events involving anaphylactic-type reactions in 23 reacting horses following use of the 2 products. All reports originated from Germany. All of the adverse event reports involved intravenous administration of the product with doses ranging from 40 ml to 66 ml. It is noted that the authorised dosage in horses for the products is 6.6 mg/kg bodyweight gentamicin which equates to an average dose of 73 ml for a 550 kg horse. Reported clinical signs were typical of anaphylactic-type reactions in horses and the most frequently reported signs included excessive sweating (n=12 reacting animals), tachycardia (n=10), tachypnoea (n=10) and colic/signs associated with colic (n=10). All affected animals recovered typically within 5-10 minutes.

Five API batches were potentially implicated in the finished product batches associated with the adverse event reports. The histamine level of the API in these API batches ranged from 2 - 62 ppm (measured by HPLC-MS). Nine of the 13 AEs were associated with product manufactured with one API batch with a measured histamine content of 62 ppm. However, it is noted that for one of the finished product batches, there were three batches of API associated with its manufacture and for one of these API batches the histamine level was unknown, while for the other two API batches the histamine level was 6 ppm and 3 ppm respectively. Therefore, the finished product involved in the adverse event reports associated with this batch may have been manufactured using an API batch with high histamine level. Additionally it is noted that for one of the reports, it was unclear which of two potential finished product batches was used. One of the batches potentially implicated was manufactured using

a batch of API with histamine level of 2 ppm, however the other finished product batch was the same as that mentioned previously which was manufactured using three batches of API, one of which had an unknown histamine level.

The MAH provided results from a literature search conducted in PubMed using the search string 'histamine AND hypersensitivity AND horse', however none of the results related to histamine levels or associated adverse effects in horses following administration of histamine containing substances.

MAH No. 5

MAH No.5 provided a PSUR covering a gentamicin containing product which is authorised nationally in Spain.

The MAH received 7 reports of adverse events involving 13 reacting horses. All reports are categorised as non-serious, no animal died. All of the adverse event reports involved intravenous administration of the product, in 6 of 7 reports with the recommended dosage of 6.6 mg/kg bodyweight, in one report with unknown dosage. Reported clinical signs were typical of anaphylactic-type reactions in horses and the most frequently reported signs included shivering/trembling (n = 12 reacting animals), excitation (n = 10), tachycardia (n=10), colic (n = 4), excessive sweating (n=3), and anaphylactic-type reaction (n = 3). It is noted that according to the questions and answers document on serious non-fatal adverse events[3] all reports have to be categorised as serious.

Eight API batches were potentially implicated in three finished product batches associated with the adverse event reports. The histamine level of the API in these API batches ranged from 34 ppm to 68 ppm (measured by HPLC-MS). Three of the seven reports of adverse events were associated with product manufactured with three API batches with a measured histamine content of 34-66 ppm. Two of the seven reports of adverse events were associated with product manufactured with three batches of API, one of which has an unknown histamine level. The histamine levels of the two known API batches range from 61-64 ppm.

MAH No. 6

MAH No. 6 received 68 reports of adverse events involving 125 reacting horses following use of one product. All reports originated from Germany.

In its response, the MAH has broken down the data into adverse events observed following use of 'conspicuous' batches and adverse events observed following use of 'inconspicuous' batches. Conspicuous batches are described as ones that led to an increased frequency of adverse events that were recalled and/or for which marketing was ceased. The MAH received 58 reports involving 115 reacting horses for conspicuous batches, nine reports involving nine reacting horses for inconspicuous batches and one report involving one reacting horse for which the batch number was unclear but was deemed to be probably related to a conspicuous batch as the reporting practice had conspicuous batches in use at the time of the reaction.

All reports for both categories of product batches were serious, but no animals died. The most frequently reported clinical signs in the adverse event reports were consistent with anaphylactic-type reactions in horses and included colic (n=46 reacting animals [conspicuous batches], n=3 [inconspicuous batches]), pawing (n=41), restlessness/agitation (n=43 [conspicuous batches], n=5 [inconspicuous batches]), shivering (n=38 [conspicuous batches]) and tachycardia/increased heart

[3] "Questions and answers on serious non-fatal adverse events and reporting rules" (EMA/CVMP/PhVWP/303762/2012-Rev.1) – link

rate (n=48 [conspicuous batches], n=2 [inconspicuous batches]). All of the reports involved intravenous administration of the product at dose rates of 3.3 – 11.1 mg gentamicin/kg bodyweight.

Four batches of finished product which were manufactured using four API batches were deemed to be conspicuous batches, and were associated with a total of 115 reacting horses. It is noted that in two cases finished product batches were associated with respectively two different batches of API. The histamine level of the API in these four API batches ranged from 17 to 74 ppm.

Six batches of finished product which were manufactured using eight API batches were deemed to be inconspicuous batches, and were associated with nine reacting animals.

The other seven API batches potentially implicated in the remaining nine reactions had histamine contents ranging from <3 to 9 ppm according to the API manufacturer's analysis. It is noted that the MAH developed an in-house analytical method for the determination of histamine content in API batches, and for five of these seven API batches, a higher level of histamine content was found in the API batches when analysed using the method employed by the MAH.

It is noted that there is uncertainty surrounding each of these nine reacting horses. For three of the horses it is unknown if the batch number stated was correct, for a further three horses the chronology of the reaction is incompatible with an anaphylactic-type reaction related to histamine content, for one horse the clinical signs (renal failure) were not consistent with an anaphylactic-type reaction, for one horse the observed reaction was mild and followed concomitant use of other products and for the remaining horse a reaction to another gentamicin containing product with unknown histamine content was also noted.

It is noted that the authorised dose rate in horses for the product in question is 6.6 mg gentamicin/kg bodyweight. In relation to the reports that occurred following use of product from batches deemed to be conspicuous, in 31 reports a dose of 6-7 mg/kg bodyweight was used, in 18 reports the dosage was lower, in 9 reports the dosage was higher and in one report was unknown. In relation to the reports that occurred following use of product from batches deemed to be inconspicuous, in two reports a dose of 6-7 mg/kg bodyweight was used, in three reports the dosage was lower and in four reports the dosage was higher. The MAH concluded on the basis of this review that the dosage administered did not play a significant role in the reported adverse events relating to anaphylactic-type reactions.

The MAH provided a literature review conducted using an unspecified database and search parameters. Five publications were discussed by the MAH in the response to the CVMP list of questions.

Derksen *et al*. (1982, 1985) infused histamine phosphate at rates up to 2.2 µg histamine base/kg/min in conscious horses. A large variability in response to histamine was seen in the horses. In these studies, histamine was administered to conscious horses and the description of effects focused on lung function parameters. Furthermore, the authors investigated effects resulting from constant rate infusions of histamine and these may not result in the same effects as bolus application. Derksen *et al.* (1985) described that "ponies became distressed, due to the high degree of pulmonary mechanical dysfunction, which prevented them to use higher histamine infusion rates".

In the study reported by Robinson and Scott (1981), 3.3 µg/kg/min histamine base was administered to anaesthetised horses over a time period of 60 minutes. Histamine infusion caused cyanosis, hyperpnoea and sweating in all ponies. Also a significant increase of the hematocrit was seen, together with an increased cardiac output and decreased total peripheral and pulmonary vascular resistance. The authors report that: "When histamine infusion began, there was a period of apnoea with intermittent gasping, and then the respiratory rate increased from baseline of 8.5 ± 1.4 breaths/minute to 22.1 ± 4.7 breaths/minute." Also: "Approximately 1 minute after histamine infusion

was started, there was an initial transient systemic hypotension (lasting less than 1 minute) followed by an increase in blood pressure above base line. Pulmonary arterial pressure increased transiently to 37 ± 2 mm of Hg within 1 or 2 minutes of starting the infusion. The pulmonary hypertension coincided with systemic hypotension and was of similar duration."

The remaining publication discussed was the study by Eyre and Lewis (1973), the findings of which are already listed above as presented by MAH No. 2 and 3. MAH No. 6 additionally commented in relation to this study that the article does not present individual data of all ponies and does not address individual deviations from the postulated categorisations, whereas other publications mention that a variability in response to histamine between individuals exists (Pollock *et al*., 1981 and Derksen *et al*., 1982). The MAH concluded that this variability in response between individuals documented in the published literature was in line with the experience gained from adverse events reported following use of affected batches with elevated histamine levels, as not all treated animals experienced adverse reactions and in the reacting horses the nature of the reaction varied.

Based on the measured concentrations of histamine in the API, this MAH estimated the amount of histamine that horses may have been exposed to when administered finish product (manufactured using API with high histamine content) at the recommended treatment dose. As measured histamine quantities vary according to the analytical method, the histamine in µg/kg was presented as a range (basis for calculation lowest and highest analytical value).

Ranges for 'conspicuous' batches are:

0.71-1.16 µg/kg bodyweight

0.50-0.69 µg/kg bodyweight

0.48-0.70 µg/kg bodyweight

0.85-1.13 µg/kg bodyweight

Based on the results of Eyre and Lewis (1973), it is known that doses higher than 0.25 µg/kg bodyweight are associated with clinical effects on cardio-respiratory function.

When estimating the amount of histamine that horses may have been exposed to when administered 'inconspicuous' batches of finish product, the MAH calculated a range between 0.01- 0.18 µg/kg bodyweight except for two batches:

Batch 1: 0.19 – 0.30 µg/kg bodyweight: No adverse event reports were reported for this batch of finished product.

Batch 2: 0.04 - 0.25 µg/kg bodyweight: Three adverse event reports were reported for this batch of finished product (one uncertain if batch correct, one unusual pattern for histamine, one horse also reacted to a product containing 50 mg/ml gentamicin with unknown histamine content).

These two batches have slightly higher histamine levels than the other inconspicuous batches, but are categorised to be inconspicuous due to quantity and quality of adverse event reports.

The vast majority of adverse event reported by this MAH (115 out of 124) are associated with the API batches containing 63 and 74 ppm.

The MAH stated that based on the results of Eyre and Lewis (1973), it is known that doses higher than 0.25 µg/kg bodyweight are associated with clinical effects on cardio-respiratory function. However, based on the work of Eyre and Lewis (1973), it appears that a total dose of about 0.1 µg/kg

bodyweight or less of intravenous histamine produces no or only minor general symptoms (sighing, short term gasping or apnoea).

All these calculation are based on API gentamicin sulfate based on a dosage for horses of 6.6 mg gentamicin (not gentamicin sulfate as other MAHs). In the PSUR the MAH calculated the animals treated by the dosage of 6.6 mg/kg bodyweight gentamicin sulfate. Therefore differences between calculated content of histamine in the finished products could occur.

Other pharmacovigilance data

In addition to the MAH responses received to the CVMP LoQ, some additional data was available to the Committee via the CVMP Pharmacovigilance Working Party (PhVWP-V) relating to another product containing gentamicin. The product in question was authorised in Belgium, Denmark, Iceland, Spain and the United Kingdom. A total of 41 adverse event reports involving 53 reacting horses, of which four died, were received in the UK. Five reports were also received from Belgium. Two of the UK reports involving two horses related to injection site reactions, and the remaining 39 reports involving 51 horses involved anaphylactic-type reactions. Clinical signs in these reports included increased respiratory rate, sweating, weakness, recumbency, shaking and colic.

In relation to the four reports involving death:

- One horse was euthanised following anaesthetic complications shortly after developing anaphylactoid signs post administration of the gentamicin product.
- One horse recovered from the initial signs following administration of the gentamicin product but was then administered penicillin and was found dead 1.5 hours later, necropsy results associated with this report were inconclusive.
- One horse received the gentamicin product and procaine penicillin, went into hyperexcitation, jumped a fence and broke its leg and was euthanised.
- One horse received the gentamicin product, flunixin, procaine penicillin and sedation and suffered collapse, arrhythmia, dyspnoea and death.

Two batches of the gentamicin product were involved in the reports from the UK and Belgium. Three batches of API were associated with the manufacture of one finished product batch, while two batches of API were associated with the manufacture of the second finished product batch. The histamine content in these API batches was 74, 91, 83, 18 and 17 ppm respectively.

Summary and conclusions

On the basis of the literature reviews relating to the effects of histamine in the horse the following can be concluded:

- Systemic histamine administration results in dose-dependent effects on some respiratory and cardiovascular parameters.
- It appears that the occurrence of general clinical signs after an intravenous histamine dose is dependent on the total dose and the speed of administration.
- While no deaths were reported in any of the experimental studies related to the administration of histamine, high total doses (196 µg/kg bodyweight) produce clear clinical signs, including cyanosis, hyperpnoea and sweating.

- Based on the work of Eyre and Lewis (1973), it appears that a total dose of about 0.1 µg/kg bodyweight or less of intravenous histamine produces no or only minor general symptoms (sighing, short term gasping or apnoea).
- However, the response of individual animals to parenteral administration is likely to vary considerably such that it may not be possible to determine with absolute certainty a threshold for histamine dose below which AEs will not be detected in the event of parenteral (intravenous) administration to horses.

A specification limit of not more than 16 ppm has been proposed as an interim limit for histamine content in the API.

On the basis of the MAH responses provided to the LoQ, there were a total of 89 adverse event reports received involving 162 reacting horses, none of which resulted in fatalities. Including the additional data from the PhVWP-V, there were a total of 135 reports involving 220 reacting horses, of which 4 died.

Overall, a range of 2 - 91 ppm histamine level for batches of API was associated with the reported adverse events. It is noted, however, that for reports involving levels below the proposed API limit of 16 ppm, the data is inconclusive for the following reasons:

- In relation to two adverse event reports involving one of the finished product batches (MAH No. 4), there were three batches of API associated with its manufacture and for one of these API batches the histamine level was unknown, while for the other two API batches the histamine level was 6 ppm and 3 ppm, respectively. Therefore the finished product involved in the adverse event reports associated with this batch may have been manufactured using an API batch with high histamine level.
- In relation to one adverse event report involving another finished product (MAH No. 4), it was unclear which of two potential finished product batches was used. One of the batches potentially implicated was manufactured using a batch of API with histamine level of 2 ppm; however, the other batch was manufactured using three batches of API, one of which had an unknown histamine level.
- In relation to the adverse event report involving a product authorised to MAH No. 2, the API batch used to manufacture the batch of finished product associated with the report was determined by the MAH to have a histamine level of 22.7 ppm; however, it is noted that the MAH also stated that the histamine content declared by the API manufacturer to the MAH for this batch was 11 ppm. It is further noted that this batch was not included in the response provided by the API manufacturer.
- In relation to five adverse event reports involving nine reacting horses relating to product authorised to MAH No. 6, a number of sources of uncertainty were noted:
  - For three of the horses it was unknown if the batch number stated was correct;
  - In a further three reacting horses the chronology of the reaction was incompatible with an anaphylactic-type reaction related to histamine content;
  - For one horse the clinical signs (renal failure) were not consistent with an anaphylactic-typereaction;
  - For one horse the observed reaction was mild and followed concomitant use of other products;

- For the remaining horse a reaction to another gentamicin containing product with unknown histamine content was also noted.

A total of 13 of the 89 adverse event reports involved overdose of the product, therefore this was not considered to be a significant factor in the development of adverse clinical signs.

On the basis of the literature reviews provided and supported by evidence from the observed adverse event reports, there is considerable inter-individual variability in treated horses with regard to susceptibility to histamine induced physiological changes and/or adverse effects. Additionally there does not appear to be any conclusive evidence for a definitive level or dose of histamine at which adverse events could be expected. It is noted that the API batches listed in the responses which had the highest histamine levels at 93 ppm and 96 ppm, were not associated with any adverse event reports. This may be due to missing data however, or the batch may not have been used in the manufacture of products used for parenteral administration to horses.

Based on the information available at this time, it is not possible to state with absolute certainty that finished product manufactured with API batches containing histamine at the interim limit of 16 ppm will not be associated with AEs in the event of parenteral (intravenous) administration to horses. Therefore, the CVMP suggests that the limit be reduced further.

2.2.3. Recommendations for histamine levels in gentamicin products for parenteral administration to horses

In this procedure, and drawing from the data provided on the two points above, the Committee was to propose an acceptable level of histamine in the API and/or the finished product of gentamicin containing products used parenterally in horses.

Discussion and Summary

Establishing a limit for histamine in finished product is hampered by the fact that there is effectively no actual data on histamine levels in batches of finished product, no validated method and the MAHs adopted different approaches in estimating levels of histamine in finished product batches. It is therefore not considered possible to recommend a limit for histamine in finished product.

Histamine is not considered a degradation product of the API but a process related impurity originating from the raw material used in the course of fermentation of the API and hence, it is not expected to increase in the finished product over its shelf life. Control of such an impurity on the API specification and not on the finished product is accepted practice. The API specification is therefore considered the appropriate point at which to control this impurity. However, as this would be the only point of regulatory control, the limit applied must be stringent given the significance of failing to comply.

Batch data is available detailing levels of histamine in 283 batches of API. Prior to introduction of a new peptone supplier in June 2014 levels of histamine in the API were typically <3 – 12 ppm (data from approximately 50 batches). When a new peptone supplier was introduced between June 2016 – June 2017 more variation was observed in histamine content with levels ranging between < 3 ppm – 96 ppm (data from in excess of 200 batches). Following investigation of the root cause of adverse events, additional controls were introduced by the peptone supplier and API manufacturer and levels of histamine in the peptone raw material and the final API dropped significantly. Since 24 March 2017, only 2 out of 100 batches have levels above 8 ppm (2 batches with a level of 9 ppm).

It is clear that following the introduction of corrective actions at the API and peptone manufacturing sites, the API manufacturing process and controls have improved with respect to controlling the levels of histamine in the API, resulting in lower levels being present in the API.

The data provided demonstrated some correlation between histamine levels in the API and adverse events when gentamicin injection is administered intravenously in horses. High levels of histamine in API batches are associated with the vast majority of AEs reported in response to the CVMP list of questions. Whilst AEs are not reported for all batches of API that contain high levels of histamine, it is assumed that the batches not associated with AEs in horses were not used in the manufacture of finished product indicated for horses and/or they are associated with finished product batches authorised to MAHs who did not respond to the CVMP list of questions. It is noted that the API batches listed in the responses which had the highest histamine levels at 93 ppm and 96 ppm were not associated with any adverse event reports. There are also AEs associated with API batches with low levels of histamine. Overall, a range of 2 ppm to 74 ppm histamine level for batches of API was associated with the reported adverse events. It is noted however that for reports involving levels below 16 ppm, the data is inconclusive for various reasons as detailed above (Summary and conclusions of section 2.2.2).

On the question of what is an appropriate limit for histamine in the API, the API manufacturer proposes to retain the limit of 16 ppm provisionally set after the initial investigation of the issue. This limit is also supported by all MAHs that responded to this point. The 16 ppm limit was an interim limit based on limited data. Now that additional data is available, the CVMP considered it appropriate to review the limit.

On the basis of the literature reviews provided and supported by evidence from the observed adverse event reports, there is considerable inter-individual variability in treated horses with regard to susceptibility to histamine induced physiological changes and/or adverse effects. Additionally there does not appear to be any conclusive evidence for a definitive level or dose of histamine at which adverse events could be expected or below which the risk of AEs is eliminated.

Based on the information available at this time, it is not possible to state with absolute certainty that finished product manufactured with API batches containing histamine at the interim limit of 16 ppm will not be associated with AEs in the event of parenteral (intravenous) administration to horses. Therefore, the CVMP recommends that the limit be reduced further.

It is recommended that a limit of not more than 8 ppm in the active substance specification is applied. This limit is proposed with reference to the fact that:

- The gentamicin containing finished products range in concentration from 40 – 100 mg/ml gentamicin. Taking the highest and the lowest concentration products, with dose rates of 0.4 ml and 1.65 ml of product per 10 kg bodyweight respectively, we can estimate the levels of histamine in finished product batches if limited to 8 ppm in the active substance. The concentration of histamine is calculated at 0.05 – 0.085 µg/kg bodyweight[4]. With reference to the data provided by one MAH on 'conspicuous' and 'non-conspicuous' batches, this level is well below the levels observed in conspicuous batches (0.54 – 1.16 µg/kg). Only one batch classified as non-conspicuous for which adverse event reports were received had levels within this range. For this finished product batch levels were estimated at 0.04 - 0.25 µg/kg, and 3 adverse event reports were received - one uncertain if batch was correct, one unusual pattern for histamine, one horse also reacted to a finished product with unknown histamine content.

[4] Calculation based on histamine results reported by the main API supplier for the EU market for batches of active substance used in relevant finished product batches

- The gentamicin containing finished products range in concentration from 40 – 100 mg/ml gentamicin. The approved dosage regimen for all products is a single dose of 6.6 mg/kg body weight gentamicin given intravenously once daily for 3–5 consecutive days based on a CVMP referral procedure under Article 35 of Directive 2001/82/EC, as amended, for Veterinary medicinal products containing gentamicin presented as solutions for injection to be administered to horses.
- The MAHs proposed the limit of histamine in the API of 16 ppm in line with the interim limit. By dosing 6.6 mg/kg bodyweight gentamicin which is equivalent to 11.22 mg/kg bodyweight gentamicin sulfate (factor 1.7), a horse (550 kg) would receive 0.18 µg histamine/kg bodyweight. Based on the work of Eyre and Lewis (1973), it appears that a total dose of about 0.1 µg histamine/kg bodyweight or less applied intravenously produces no or only minor general symptoms (sighing, short term gasping or apnoea). So by a limit of histamine in the API of 16 ppm a horse would receive more than 0.1 µg/kg bodyweight histamine. Applying a limit of 8 ppm for histamine in the API, a 550 kg horse would receive a potential maximum of 0.09 µg/kg bodyweight histamine by a recommended dose of a gentamicin containing product. This would be lower than the dose of 0.1 µg/kg bodyweight histamine which is considered to be associated with no or only very mild general clinical effects according to the work of Eyre and Lewis (1973).
- It is above the LOQ of the validated HPLC-MS method.
- Since the interim limit of 16 ppm was applied in October 2017 there have been no reported AEs. The limit of not more than 8 ppm represents an additional safety factor with respect to that interim limit.
- Corrective actions at the API and peptone manufacturing sites have resulted in lower levels of histamine present in the final API, but a risk cannot be totally eliminated. Batch data from 2017 indicate levels of 3-13 ppm are routinely achieved with the majority of batches having levels between 3-8 ppm. Based on this recent batch data, a limit of not more than 8 ppm is realistic and readily achievable on a routine basis. Approximately 93 % of batches manufactured in 2017 for which batch data has been provided would comply with this limit. A lower limit could result in rejection of a large number of batches and potential shortage of API in the marketplace.
- Prior to the introduction of the new peptone supplier in June 2014 levels of histamine were typically <3 – 12 ppm. The histamine limit of not more than 8 ppm now proposed by the CVMP is below that which was previously assumed to be acceptable based on the absence of a signal for histamine-related adverse effects.

In addition to this lower limit, the CVMP also recommends the following further measures to minimise the potential risk of histamine contamination of veterinary medicinal products:

- The EDQM should consider further revision of the Ph. Eur. monograph 1468 on 'Products of Fermentation' with respect to the requirements on the quality of raw materials. The following text is proposed as a possible alternative to the current text in the raw materials section of the monograph:

  'Special attention must be paid to minimising the public and animal health risks of histamine and other biogenic amines from fish and fishery products. Where fish-based raw materials are used in the synthesis of the drug substance the manufacturer should carry out a risk assessment and propose suitable limits for histamine levels in the raw material based on experimental purge studies. Additionally, the manufacturing process should include at least one validated step to remove residual histamine and the drug substance specification should include a qualified limit for histamine levels'.

- The API manufacturer should continue to investigate the use of alternative sources of peptone in order to minimise the presence of histamine in gentamicin sulfate. Results should be presented to the relevant competent authorities with a view to revising the API manufacturing process that is registered for the gentamicin sulfate utilising fish peptone.
- The API manufacturer should continue to optimise the gentamicin sulfate fermentation and purification processes to reduce further the histamine levels in the final API. Results should be presented to the relevant competent authorities with a view to revising the API manufacturing process that is registered for the gentamicin sulfate utilising fish peptone.
- MAHs should continue to monitor any new adverse event reports and to notify regulatory authorisities in accordance with pharmacovigilance reporting requirements. Any adverse event reports should include details of the levels of histamine in both the relevant API and the finished product batches.

3. Overall summary of the scientific evaluation

The Committee, having considered the matter, reviewed data from published literature, data received from the active substance manufacturer and marketing authorisation holders, and consulted with the Committee for Medicinal Products for Human Use, came to the following conclusions:

Based on the information available at this time, it is not possible to state with absolute certainty that finished product manufactured with API batches containing histamine at the interim limit of 16 ppm will not be associated with AEs in the event of parenteral (intravenous) administration to horses. Therefore, the CVMP recommends that the limit be reduced further. Additional measures are also proposed by CVMP to minimise the potential risk of histamine contamination of veterinary medicinal products.

The CVMP recommends the following measures:

1. The interim limit for histamine in gentamicin sulfate which utilises fish peptone as a raw material should be reduced to as low as reasonably practicable in line with safety and pharmacovigilance data, manufacturing capability and batch data. An API limit of 8 ppm is below the limit of 0.1 µg/kg bodyweight histamine, which is considered to be associated with no or only very mild general clinical effects according to the work of Eyre and Lewis (1973), is considered to be within the current manufacturing capability of the API manufacturer/supplier based on current batch data and is within the validated range of the analytical method.
2. The EDQM should consider further revision of the Ph. Eur. monograph 1468 on 'Products of Fermentation' with respect to the requirements on the quality of raw materials. The following text is proposed as a possible alternative to the current text in the raw materials section of the monograph:

   'Special attention must be paid to minimising the public and animal health risks of histamine and other biogenic amines from fish and fishery products. Where fish-based raw materials are used in the synthesis of the drug substance the manufacturer should carry out a risk assessment and propose suitable limits for histamine levels in the raw material based on experimental purge studies. Additionally, the manufacturing process should include at least one validated step to remove residual histamine and the drug substance specification should include a qualified limit for histamine levels'.

3. The API manufacturer should continue to investigate the use of alternative sources of peptone in order to minimise the presence of histamine in gentamicin sulfate. Results should be presented to the relevant competent authorities with a view to revising the API manufacturing process that is registered for gentamicin sulfate utilising fish peptone.

4. The API manufacturer should continue to optimise the gentamicin sulfate fermentation and purification processes to reduce further the histamine levels in the final API. Results should be presented to the relevant competent authorities with a view to revising the API manufacturing process that is registered for gentamicin sulfate utilising fish peptone.

5. MAHs should continue to monitor any new adverse event reports and to notify regulatory authorities in accordance with pharmacovigilance reporting requirements. Any adverse event reports should include details of the levels of histamine in both the relevant API and the finished product batches.

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