EFSA’s assistance for the 2015 Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) in relation to rBST

European Food Safety Authority

Abstract

The use of recombinant bovine somatotropin (rBST) as a milk production enhancer in dairy cattle is authorised in some countries but is banned in others, including the Member States of the European Union. At the request of the Codex Alimentarius Commission, the Joint Expert Committee on Food Additives (JECFA) evaluated in three different occasions several aspects related to the safety of rBST and concluded that there was no evidence to suggest that the use of rBST would result in a higher risk to human health due to the possible increased use of antimicrobials to treat mastitis. JECFA assessments consider human health concerns related to the exposure to residues of antimicrobials in milk. They do not consider other aspects related to antimicrobial resistance (AMR), such as the possible development of AMR in dairy cattle, and dairy cattle farms following the treatment with antimicrobials and the possible development of AMR in humans due to the exposure to pathogenic and non-pathogenic resistant bacteria originating from the cattle reservoir. Assuming that treatment with rBST can lead to increased incidence of mastitis in dairy cattle, that cases of mastitis are usually treated with antimicrobials, that the use of antimicrobials can lead to the development of AMR in dairy cattle (and in dairy cattle farms), and that AMR in humans may derive from both the exposure to AMR bacteriaigenes of cattle origin and residues of antimicrobials, it is concluded that an increase of AMR in humans following to the use of rBST in dairy cattle is plausible. Current knowledge does not allow quantifying these four steps. Recommendations for additional studies to investigate these four steps and quantify them are formulated.

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Key words: recombinant bovine somatotropin (rBST), somatotropin, mastitis, antimicrobial, antimicrobial resistance (AMR), animal health, public health

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Summary

The use of recombinant bovine somatotropin (rBST) as a milk production enhancer in lactating dairy cows is authorised in the USA and some other countries but it is prohibited in several countries, among others Canada, Australia, New Zealand, Japan, Israel and the Member States of the European Union (EU), following animal welfare concerns connected to the use of the substance, in particular vis-à-vis increased mastitis in dairy cows.

At the request of the Codex Alimentarius Commission, the Joint Expert Committee on Food Additives (JECFA) evaluated in three different occasions several aspects related to the safety of rBST. JECFA concluded that ‘there was no evidence to suggest that the use of rBST would result in a higher risk to human health due to the possible increased use of antimicrobials to treat mastitis’.

The European Commission asked EFSA to assess the above conclusions of the 78th meeting of JECFA, and in particular if these justify to support the earlier conclusions of the 50th meeting of JEFCA, i.e. ‘that the use of rBSTs would not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use’. EFSA was also asked to advise on what, if any, type of studies/data could potentially be useful to exclude a possible link between the use of rBST and the development of antimicrobial resistance (AMR).

With regard to the systematic review conducted by JECFA, it is not clear what search terms were used, and it is concluded that, without a clear definition of the search terms used and further details on the paper selection process, the literature search is not repeatable and a full assessment of the accuracy of the search strategy is not possible. In addition, the search strategy may not allow identifying papers related to all AMR-related concerns, since aspects other than antimicrobials residue levels in milk and meat from rBST treated animals are not covered, and since the search terms defined are not specific to AMR-related concerns.

With regard to the conclusions reached by JECFA, assuming that the appropriate withdrawal times for antimicrobial treatments are respected, JECFA conclusions can be supported in relation to the presence of residues of antimicrobials in bulk milk, i.e. ‘that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use’.

JECFA interprets the absence of evidence for the association between the treatment with rBST and the development of resistant mastitis pathogens as sufficient to support the previous JECFA-50 conclusions. In the absence of such evidence, the link between the use of rBST and AMR in dairy cattle should be further investigated before being able to provide definite conclusions.

The assessments by JECFA only consider human health concerns related to the exposure to residues of antimicrobials in milk. They do not consider other AMR-related aspects, such as the possible development of AMR in dairy cattle and dairy cattle farms following to the treatment with antimicrobials and the possible development of AMR in humans due to the exposure to pathogenic and non-pathogenic resistant bacteria originating from the cattle reservoir. Overall, it is concluded that AMR-related concerns are only partially considered by JECFA, and therefore the conclusions from JECFA-50, unchanged by JECFA-78, i.e. that ‘the use of rBST would not result in a higher risk to human health due to the use of antibiotics to treat mastitis’ cannot be considered valid in relation to all human AMR-related concerns.

It would be difficult to design and execute single experiments investigating the direct link between the use of rBST in dairy cattle and AMR in humans. Rather, an indirect link should be investigated by considering and studying all the intermediate steps that may incur between the use of rBST in dairy cattle and AMR in humans. Assuming that treatment with rBST can lead to an increased incidence of mastitis in dairy cattle, that cases of mastitis are usually treated with antimicrobials, that the use of antimicrobials can lead to the development of AMR in dairy cattle (and in dairy cattle farms), and that AMR in humans may derive from both the exposure to AMR bacteria/genes of cattle origin and residues of antimicrobials, it is concluded that an increase of AMR in humans following to the use of rBST in dairy cattle is plausible. Current knowledge does not allow quantifying these four steps.
Recommendations for additional studies to investigate these four steps and quantify them are formulated.
Use of rBST in dairy cattle and AMR in humans

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

EFSA's assistance is needed by the European Commission in respect of recombinant bovine somatotropin (rBST), a substance which is banned at the EU level following animal welfare concerns connected to the use of the substance, in particular vis-à-vis increased mastitis in cows. The decision to ban the use of rBST was taken on the basis of the report of the Scientific Committee on Animal Health and Animal Welfare (SCAWAH) adopted on 10 March 1999.

At the request of the 35th session of the Codex Alimentarius Commission, the 78th Joint Expert Committee on Food Additives (JECFA) re-evaluated rBST. It did so considering all new data related to BST, including information related to the possible increased use of antimicrobials to treat mastitis in cows and aspects of human antimicrobial resistance (AMR) associated with the use of rBST.

Following its re-evaluation, JECFA concluded that there was no evidence to suggest that the use of rBST would result in a higher risk to human health due to the possible increased use of antimicrobials to treat mastitis. JECFA did not find any specific studies linking the use of rBST with the development of antimicrobial resistance. The 78th JECFA further considered that the position expressed by the 50th JECFA remained valid, i.e. ‘that the use of rBST would not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use’.

Particular concerns are expressed by the European Commission about the fact that while the 78th JECFA states that ‘there was no evidence to suggest that the use of rBSTs would result in a higher risk to human health’ it does not explore the fact that, equally, there is no evidence suggesting the contrary.

The 22nd Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) discussed the outcome of the JECFA evaluation of rBST in April 2015. Different opinions regarding the completeness and conclusiveness of the JECFA evaluation remained even if delegations could broadly agree that JECFA had addressed the questions put to it by the Codex Alimentarius Commission (CAC). As a next step, CAC will consider the draft Codex MRLs for rBST at its 38th session which will take place on 6-11 July 2015 in Geneva.

Antimicrobial resistance is a topic of vital importance to the parent organisations of Codex. A number of closely related initiatives in multilateral bodies have been taken, in recent weeks, with a view to renew global efforts in the fight against antimicrobial resistance. The World Health Assembly endorsed, on 25 May 2015, a comprehensive global action plan to tackle AMR. The plan recognises the need for urgent action and calls upon all member nations to promptly develop their own actions to combat AMR to counter the growing threat of AMR.

Similarly, the Food and Agriculture Organisation (FAO) adopted a draft resolution on 10 June 2015 in the Commission in charge of policy and regulatory issues. This resolution was endorsed by the FAO Conference on 13 June 2015. This resolution urges FAO members to take urgent action at regional, national and local levels to mitigate risks posed by inappropriate antimicrobial usage and antimicrobial resistance in food, agriculture and the environment. The FAO's actions are aligned with those of the World Health Organisation (WHO), and together enhance the tripartite action taken by FAO and WHO, together with the World Organisation for Animal Health (OIE) as all three bodies lead the global efforts to mitigate rising AMR.

1.1.2. Terms of reference

1. Analysis of the 78th JECFA conclusions, in particular if the position taken by the 78th JECFA (explicitly stating that ‘there was no evidence to suggest that the use of rBST would result in a higher risk to human health due to the possible increased use of antimicrobial agents to treat mastitis’) is justified to support the earlier 50th JECFA conclusion, i.e. ‘that the use of rBSTs would
not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use'.

2. It would be also useful to know what, if any, type of studies/data could potentially be useful to exclude a possible link between the use of rBST and the development of antimicrobial resistance.

1.2. Interpretation of the Terms of Reference

Terms of reference (ToRs) 1 is interpreted as an analysis of the 78th JECFA conclusions, in particular discussing whether the evidence reported by JECFA report is appropriate to exclude risks to human health, linked to AMR, due to the use of rBST on dairy cows.

1.3. Recombinant Bovine Somatotropin (rBST)

Bovine somatotropin (BST) is a natural neurohormone produced by the pituitary gland of the cattle that coordinates the body growth through the regulation of protein, fat and carbohydrate metabolism. It is also an important regulator of lactation in cows. Circulating concentrations of BST are positively correlated with the level of milk production.

The development of deoxyribonucleic acid (DNA)-recombinant techniques has permitted the industrial production of recombinant bovine somatotropin (rBST) in genetically modified bacteria allowing in turn its use as a growth promoting substance or as a milk production enhancer, the latest being its principal use. The chemistry of the rBST is slightly different to the natural pituitary-derived somatotropin; they are biologically equivalent.

Due to the instability of the product in the gastrointestinal tract, rBST has to be administered by parenteral application through subcutaneous injections.

The use of rBST in lactating dairy cows is authorised in the USA and some other countries but it is prohibited in several countries, among others Canada, Australia, New Zealand, Japan, Israel and the Member States of the European Union (EU).

The commercial use of rBST was approved by the US Food and Drug Administration (FDA) in 1993 and since February 1994, rBST has been used commercially in the USA to enhance milk productivity.

Details on the treatment in the US, according to the manufacturer instructions, are provided by FDA (1993):

- Indications for use: for increased production of marketable milk in lactating dairy cows.
- Dosage form: sterile, prolonged-release injectable formulation in single-dose syringes each containing 500 mg sometribove zinc.
- Routes of administration: subcutaneous injection in the postscapular region (behind the shoulders) or ischiorectal fossa (depression on either side of the tailhead).
- Recommended Dosage: One syringe (500 mg) every 14 days; beginning of the treatment during the 9th week after calving and continuing until the end of lactation.

rBST can be used in dairy cows also for extending the length of the lactation and keeping a cow in production for 30-100 additional days.

Instructions for use of products currently on the market indicate that cows injected with sometribove zinc suspension are at increased risk for mastitis (visibly abnormal milk) and may have higher somatic cell counts.

In 1999, the Canadian authorities decided that the use of this hormone should not be permitted due animal welfare concerns associated with the use of rBST. These included an increased risk of clinical mastitis and lameness, and a reduction in the life expectancy of treated cows.
By law from 1998, 1 in the EU, ‘no other substance, with the exception of those given for therapeutic, or prophylactic purposes or for the purposes of zootechnical treatment as defined in Article 1(2)(c) of Directive 96/22/EEC (1), must be administered to an animal unless it has been demonstrated by scientific studies of animal welfare or established experience that the effect of that substance is not detrimental to the health or welfare of the animal’. In 1999, the SCAHAW (SCAHAW, 1999) concluded that BST/rBST is used to increase milk yield and that it causes substantially and significantly poorer welfare in terms of increase of production-related diseases, such as foot and leg disorders, mastitis and reproductive disorders. Moreover, it leads to reactions at the injection site.

On this basis, a ban to market BST and rBST and to administrate it in the EU has been put in place by Council Decision 1999/879/EC. 2

2. Approach to answer the terms of reference

- The approach followed to answer the ToRs and analyse the link between the use of rBST in dairy cattle and AMR in humans is briefly summarised below:

- Firstly, an assessment of JECFA-78 is performed (Section 3.1), with particular attention to the methodology used for the systematic literature review. Considerations are formulated on whether the literature search is fit for finding enough scientific evidence to support the earlier 50th JECFA conclusions, or if additional evidence on potential AMR concerns not considered by JECFA should be also assessed. The JECFA conclusions are then discussed.

- Secondly, an assessment of the existing evidence of a direct link between the use of rBST in dairy cattle and AMR in humans is performed (Section 3.2). In order to do so:
  - conclusions from relevant JECFA reports are briefly discussed, in light of elements that allow replying to the question reported there;
  - a new literature search ("EFSA literature search 1") is performed to look for evidence in the scientific literature on a direct link between the use of rBST cattle and AMR in humans (i.e.: to search studies postulating, investigating or mentioning this link or listing what the human AMR concerns linked to the use of rBST in dairy cattle might be).

- Thirdly, an assessment of the following main intermediate steps that may indirectly link the use of rBST in dairy cattle and AMR in humans (as summarised in Figure 1), is reported:
  - possible link between use of rBST and the occurrence of mastitis in dairy cattle;
  - possible link between occurrence of mastitis and the use of antimicrobials in dairy cattle;
  - possible link between treatment with antimicrobials and AMR in dairy cattle (and in dairy cattle farms);
  - possible link between AMR in dairy cattle (and in dairy cattle farms) and AMR in humans.

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Use of rBST in dairy cattle and AMR in humans

Figure 1: Direct and indirect links between the use of rBST in dairy cattle and AMR in humans. Detailed pathways leading to the development of AMR bacteria of animal origin in humans, and the exchange of resistance mechanisms and bacteria between different reservoirs are explained in Figures 3 and 4.

The evidence described in JECFA reports has been complemented with additional scientific evidence from other sources and briefly discussed to assess the plausibility of the single four steps (Section 3.3).

- Overall conclusions are finally formulated (Section 4), together with an indication on further studies that would be needed to investigate the link between the use of rBST in dairy cattle and AMR in humans, in order to answer the second ToRs of this mandate.

3. Assessment

3.1. Assessment of JECFA reports

3.1.1. rBST evaluations performed by JECFA

- rBST were evaluated by JECFA on three occasions, i.e. at the 40th, 50th and 78th meetings of JECFA (WHO, 1993a, b, 1998, 1999, 2014a, b), in this document referred to as JECFA-40, JECFA-50 and JECFA-78.
- Scope of these reports was to provide guidance on public health issues pertaining to residues of veterinary drugs in food of animal origin. The reports evaluated the safety of residues of certain veterinary drugs, including rBST.
- Main aspects were evaluated in these reports:
  - toxicological effects (the only ones evaluated also by JECFA-40);
  - the possible increased use of antibiotics to treat mastitis in cows, which leads to a higher rate of ‘violative’ drug residues in milk, possibly because of an increase in the incidence of mastitis in cows treated with rBST;
  - the possibility that increased levels of insulin-like growth factor-I (IGF-I) in the milk of cows treated with rBST might lead to increased cell division and growth of tumours in humans;
  - the potential effect of rBST on the expression of certain viruses in cattle, particularly retroviruses;
  - the possibility that incubation period of bovine spongiform encephalopathy (BSE) is shortened due to an increase in the production of pathogenic prion proteins induced by IGF-I;
  - the possibility that early exposure of human neonates and young children to milk from rBST-treated cows increases health risks (e.g. the risk of developing insulin-dependent diabetes mellitus);
  - other potential adverse health effects;
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- the need to revise or maintain the acceptable daily intake (ADI) and maximum residue limits (MRLs) for rBSTs;
- aspects of AMR associated with the use of rBSTs in relation to human health.

- In the context of this report, only the following aspects investigated by JECFA are taken into consideration:
  - ‘the increased use of antibiotics to treat mastitis in cows, which leads to a higher rate or ‘violative’ drug residues (i.e. residues exceeding regulatory limits) in milk, possibly because of an increase in the incidence of mastitis in cows treated with rBSTs’ (JECFA-50);
  - ‘are the incidences of clinically relevant mastitis different between cattle, sheep and goats treated with rBSTs compared with untreated animals? Are there differences in antimicrobial residue levels in the milk and meat products from treated compared with untreated animals?’ (JECFA-78);
  - ‘is consumption of milk or meat from rBST-treated cattle, sheep or goats associated with increased rates of morbidity and mortality in infants or in the general population compared with the equivalent age groups consuming meat or milk from untreated animals?’ (JECFA-78).

- While this report is focused on the assessment of the methodology and conclusions of JECFA-78, since JECFA-78 endorses the former conclusions by JECFA-50, relevant conclusions by JECFA-50 are also mentioned and discussed when relevant.

3.1.2. Assessment of the methodology used by JECFA-78

- In order to answer the questions received and update JECFA’s previous review of rBST at its 50th meeting, JECFA-78 (WHO, 2014a) claims that ‘the Committee undertook a systematic review of the literature to address the following questions:
  - What are the hormone levels in the milk and/or meat of cattle, goats or sheep treated with rbSTs compared with untreated animals?
  - Are the incidences of clinically relevant mastitis different between cattle, sheep and goats treated with rbSTs compared with untreated animals? Are there differences in antimicrobial residue levels in the milk and meat products from treated compared with untreated animals?
  - Are retroviral/lentiviral levels and serotype distributions different between cattle, sheep and goats treated with rbSTs compared with untreated animals?
  - Are prion levels in meat and milk and prion infectivity different between cows treated with rbSTs compared with untreated animals?
  - Is consumption of milk or meat from rbST-treated cattle, sheep or goats associated with increased rates of morbidity and mortality in infants or in the general population compared with the equivalent age groups consuming meat or milk from untreated animals?’

- The systematic review was conducted according to a protocol available as an annex to the JECFA-78 report (WHO, 2014b). The following three steps of the procedure are described in the protocol:
  1. framing the questions;
  2. systematic search of the literature;
  3. identifying relevant publications.
- These steps are briefly discussed below, with reference to the protocol used by JECFA-78. Overall, JECFA considered five issues (see the above quoted questions): two are relevant for AMR (i.e.

3 http://www.who.int/foodsafety/chem/annex_rbST_systematic_lit_search.pdf?ua=1
incidence of mastitis and antimicrobial residue levels; rates of morbidity and mortality associated with milk or meat consumption) and are briefly discussed below, while the other three are not discussed since they are not related to AMR issues (i.e. effect on hormone levels in milk and meat, effect on viral expression, and effect on prion expression in animals treated with rBST).

- When comparing the information provided in the JECFA-78’s protocol for Steps 1, 2 and 3 of the procedure followed (see below), it is not clear what search strategies were finally used in the literature search. In particular, it is not clear whether:
  - the search strings defined in Step 1 (‘framing the questions’) of the procedure are the ones finally used for performing the literature search,
  - or they were used for the selection of relevant scientific papers after a literature search was performed with the search terms defined in Step 2 (‘systematic search of the literature’) and Step 3 (‘identifying relevant publications’) of the procedure.

- Without a clear definition of the search strategies used and further details on the paper selection process, the literature search is not repeatable and a full assessment of the accuracy of the results is not possible.

**Step 1: Framing the questions**

- For all issues considered, JECFA-78 formulated a search question after defining the population, the intervention, the comparator and the outcome of interest (Population, Intervention, Comparator, Outcome (PICO) methodology). Based on this, search terms were defined.

**Question on the possible increased use of antibiotics to treat mastitis in dairy cattle**

- The search question as reported in JECFA-78 (WHO, 2014a) is the following: ‘Are the incidences of clinically relevant mastitis different between cattle, sheep and goats treated with rBSTs compared with untreated animals? Are there differences in antimicrobial residue levels in the milk and meat products from treated compared with untreated animals?’

- Within this question two issues are actually covered and mixed, the first one being related to the incidence of mastitis, and the second one to antimicrobial residues levels. Both questions are relevant in relation to AMR issues, since both might provide an indirect indication on the increased number of treatments of dairy cows with antimicrobials following to the treatment with rBST.

- The above question is translated into the following elements of a systematic review PICO question in the protocol in annex of JECFA-78 (WHO, 2014b; ‘Annex to rBSTs evaluation to describe in detail the systematic literature search process’):⁴
  - ‘Population: Cattle, sheep and goats in a given geographic region
  - Intervention: rbST administration
  - Comparator: not administered rbST
  - Outcome: Mastitis incidence in the two groups, incidence of antibiotic-treated mastitis, antibiotic residues in milk & meat
  - Search terms: (rbST & synonyms) AND (mastitis) AND (antibiotic residue)’.

- These elements are not consistent with the objective of the systematic review as claimed in the main body of the same report (WHO, 2014b – page 135), where it is stated ‘The Committee performed a systematic review of the literature concerning the effects of rBSTs on mastitis incidence and somatic cell counts, with particular reference to antimicrobial residues in milk. The literature search, as described above, for publications from 1998 to August 2013 retrieved 29 unique articles that included the term ‘somatic cell count(s)’ OR ‘antibiotic’ OR ‘mastitis’.

- Even to address the incidence of mastitis and use of rBST, the search string used led to a narrow literature retrieval. Potentially relevant studies related to the incidence of mastitis, not including

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⁴ [http://www.who.int/foodsafety/chem/annex_rbST_systematic_lit_search.pdf?ua=1](http://www.who.int/foodsafety/chem/annex_rbST_systematic_lit_search.pdf?ua=1)
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‘antibiotic residue’, have been excluded. This has reduced the sensitivity of the search. The formulation of two different search strings for the two different issues identified by JECFA would have allowed a broader retrieval of papers.

- The inclusion of the term ‘antibiotic residue’ is very specific, and it is not reported whether other relevant terms (e.g. antimicrobials) were also used in the search. Similarly, it is not indicated whether other search terms for indicating ‘mastitis’ (e.g., udder inflammation, somatic cell count) were used.

- Based on these considerations, overall the search strategy defined allows identifying only a proportion of the scientific articles related to the two issues of the question formulated by JECFA-78.

- In relation to AMR-related aspects, the question formulated by JECFA only addresses the presence of antimicrobial residue levels in milk and meat from rBST-treated animals. Other aspects, such as the possible development of AMR in dairy cattle and dairy cattle farms following to the treatment with antimicrobials are not considered.

**Question on the possible increased health risks of consuming milk or meat from rBST-treated animals versus untreated animals**

- The search question formulated by JECFA-78 is the following: ‘Is consumption of milk or meat from rBST-treated cattle, sheep or goats associated with increased rates of morbidity and mortality in infants or in the general population compared with the equivalent age groups consuming meat or milk from untreated animals?’

- A number of different search strings were defined in order to investigate several possible health concerns, among which many were focused on specific concerns related to diabetes mellitus in infants and cancer, and to milk-based infant formula. These are not considered here since they relate to issues other than AMR.

- One of the search strings defined by JECFA that may identify scientific papers related to AMR issues is the following: ‘(rBST & synonyms) AND humans NOT infants AND health’. This search string (although excluding infant-related papers) is very sensitive, but not specific to AMR-related issues.

**Steps 2 and 3: Systematic search of the literature and identifying relevant publications**

- The JECFA-78 protocol for steps 2 and 3 of the procedure indicate that the literature search for the five questions formulated by JECFA has been performed ‘using rBST and its synonyms in the following Boolean combinations: rBST AND meat, rBST AND child, rBST AND milk, rBST AND cattle’ for the years 1998-2013. The following databases of scientific articles were queried: ‘Agricola, CINAHL, Food Science Source, FSTA, EMBASE, PubMed, and GlobalHealth/CAB’.

- Synonyms used for the different terms are listed in the protocol. Inclusion/exclusion criteria for the articles retrieved are also indicated, together with the number of articles retrieved. The protocol indicates that additional searches were performed for some non-AMR related issues (i.e. toxicological studies, bioavailability and bioactivity of rBST, and IGF-I and dietary IGF-I and human health effects). Databases were populated with articles retrieved. Finally, the protocol mentions that ‘the edited databases were then searched for citations relevant to the issues above using keyword searches and also by reading each abstract’.

- The databases used in the search are appropriate.

- The timespan considered is limited as from 1998; it is not clarified how the previous scientific evidence has been taken into consideration in the overall assessment.

**3.1.3. Main conclusions of JECFA-50**

- In its evaluation, JECFA-50 (WHO, 1998, 1999) considered ‘the possible increased use of antibiotics in cows treated with rBST’, analysing results from a monitoring programme in bulk milk in the USA, and noting that ‘the percentage of milk samples discarded as a result of testing for residues of antibiotics did not change significantly after the introduction of rBSTs’ in one State and
that a 'small but statistically significant increase' was observed in some other States. It highlighted that the latter was coinciding 'with a change to a more sensitive detection method'.

- Based on this evidence JECFA concluded that 'the use of rBST would not result in a higher risk to human health due to the use of antibiotics to treat mastitis and that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use'.

3.1.4. Main conclusions of JECFA-78

- JECFA-78 (WHO, 2014a, b) was asked to re-evaluate the four analogues of natural BST (somagrebove, sometribove, somavubove, somidobove). Among other issues, JECFA was requested to consider 'new data and information related to other factors pertaining to human health, including (i) the possible increased use of antimicrobials to treat mastitis in cows' and 'aspects of antimicrobial resistance associated with the use of rBSTs in relation to human health'.

- As indicated above, JEFCA-78 undertook a review of the literature (from 1998 to August 2013). Findings were summarised in JECFA-78, in particular in relation to the association between the use of rBSTs and the incidence of clinical or subclinical mastitis, the use of antimicrobial agents, and the detection of residues of antimicrobial drugs in bulk milk tankers.

- In relation to the concern of the presence of residues of antimicrobials in milk, similarly to JECFA-50, JECFA analysed the results of a monitoring in bulk milk programme for the years 1996-2012, indicating that the percentage of milk tankers positive for antimicrobial residues has declined during this period. From their analysis, JECFA concluded that 'the available evidence suggests that in the USA, the approval of rBSTs was not associated with an increase incidence of non-compliant antimicrobial residues in bulk milk' (WHO, 2014a).

- In relation to antimicrobial resistance, JEFCA did not find 'specific studies that investigated the association between the use of rBSTs and the development of antimicrobial resistance in mastitis pathogens', and concluded that 'although bovine mastitis is considered the single most important reason for antimicrobial use in lactating dairy cows (Erskine et al., 2004) and although antimicrobial resistance in mastitis pathogens is a cause of concern (Oliver, Murinda & Jayarao, 2011; Oliver & Murinda, 2012), in the absence of properly designed studies, whether the use of rBSTs in cows of farms increases antimicrobial resistance remains speculative' and that 'there is a lack of evidence that the use of rBSTs in dairy herds contributes to antimicrobial resistance in dairy herds'.

- Finally, JECFA-78 concluded that 'available new information therefore does not change the conclusion of the fiftieth Committee meeting in regards to the risk to human health due to the use of antimicrobial agents to treat mastitis' (WHO, 2014a, b).

3.1.5. Assessment of the conclusions of JECFA-78 and JECFA-50

- JECFA-78 considered information related to the link between the use of rBST and the incidence of mastitis in cattle (see Section 3.3.1). It was reported that no significant difference was found in the incidence of mastitis between rBST-treated and untreated cows; however, in JECFA-78 there is no analysis of the data that could support this statement.

- JECFA-78 and JECFA-50 reported that the effects of rBST on the incidence of mastitis are an animal health issue and outside the terms of reference of the Committee, and no overall conclusions on the link between the use of rBST and the incidence of mastitis in dairy cattle were formulated in the 'Evaluation' section.

- Assuming that the appropriate withdrawal times for antimicrobial treatments are respected, JECFA conclusions can be supported in relation to the presence of residues of antimicrobials in bulk milk, i.e. 'that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use'.

- The assessments by JECFA are based on the analysis of the percentage of milk samples discarded as a result of testing for residues of antimicrobials. The presence or absence of residues of
antimicrobials in milk cannot be used as an indicator of the extent of antimicrobial treatments in dairy cattle, since if withdrawal periods are respected no residues would be found in milk.

- No information is provided by JECFA on the volume of antimicrobial treatments during the period analysed, and data do not allow comparing animals, or herds, using or not using rBST. Based on this, no conclusion can be drawn on whether or not the use of rBST leads to an increase in the use of antimicrobials, or on the effect of the treatment with rBST on the presence of residues in milk from dairy cattle.

- In relation to possible development of resistant bacteria in dairy cattle, only the association between the treatment with rBST and the development of resistant mastitis pathogens is discussed. The development of resistance in other pathogenic or non-pathogenic bacteria in dairy cattle and in dairy cattle farms is not discussed. JECFA-78 interprets the absence of evidence for this association as sufficient to support the previous JECFA-50 conclusions. In the absence of such evidence, the link between the use of rBST and AMR in dairy cattle should be properly investigated before being able to provide definite conclusions.

- The assessments by JECFA only consider human health concerns related to the exposure to residues of antimicrobials in milk. They do not consider other AMR-related aspects, such as the possible development of AMR in dairy cattle and dairy cattle farms following to the treatment with antimicrobials and the possible development of AMR in humans due to exposure to pathogenic and non-pathogenic resistant bacteria originating from the cattle reservoir (see Section 3.3.4).

- Based on the above, AMR-related concerns are only partially considered by JECFA, and therefore the conclusions from JECFA-50, unchanged in JECFA-78, i.e. that ‘the use of rBST would not result in a higher risk to human health due to the use of antibiotics to treat mastitis’ cannot be considered valid in relation to all of the human AMR-related concerns.

### 3.2. Assessment of the direct link between the use of rBST in dairy cattle and AMR in humans

#### 3.2.1. Evidence reported by JECFA-50 and JECFA-78

- No specific studies are reported within the JECFA reports (WHO, 1998, 1999, 2014a, b) that postulate or investigate a direct link between the use of rBST in cattle, or other ruminants, and AMR risks in humans.

#### 3.2.2. Additional scientific evidence from a literature search

- It is difficult to design and execute experiments investigating the direct link between the use of rBST in dairy cattle and AMR in humans. Rather, an indirect link should be investigated by considering and studying all the intermediate steps that may incur between the use of rBST in dairy cattle and AMR in humans (see Figure 1).

- However, in order to identify any evidence of a direct link between the use of rBST in dairy cattle and AMR in humans, a new literature search (‘EFSA literature search 1’) has been performed. The literature search has taken into account the considerations expressed above on the search questions and search terms defined by JECFA-78 (see Section 3.1). The intention of this literature search was to identify additional scientific publications that may have not been identified by JECFA-78.

- **EFSA literature search 1** was based on the following criteria:
  - Aim of the search: to identify scientific literature on the consequences of the use of BST and rBST in dairy cattle or other ruminants in the possible development of AMR in humans.
  - Population: dairy cattle or other ruminants.
  - Intervention: use of BST or rBST.
  - Comparator: not considered, in order to have a wider set of results in relation to the outcome.
Outcome: possible development of AMR in humans.

Search question: May this paper include information on the consequences of the use of (r)BST in ruminants in the development of AMR in humans?

Databases queried: Web of ScienceSM Core collection; BIOSIS Citation IndexSM, CABI: CAB Abstracts®, Chinese Science Citation Database®; Current Contents Connect®; Data Citation IndexSM; FSTA® - the food science resource, KCI-Korean Journal Database, MEDLINE®, SciELO Citation Index, Zoological Record®.

Search string: (BST OR rBST OR somatotrop* OR 'growth hormon' OR 'growth hormones’ OR 'growth hormone’ OR 'growth hormones’ OR somagrebove OR sometribove OR somavubove OR somidobove OR 'GH' OR bGH OR rBGH OR posilac) AND (bovin* OR cow* OR cattle OR bovid* OR dair* OR goat* OR sheep OR ovine* OR caprine* OR ruminant* OR heifer*) AND (human* OR public health OR consumer*) AND (resistant OR resistance* OR antibiotic* OR antimicrob*)

Fields searched: title, abstract, keywords (i.e. field ‘topic’).

Languages: no restriction

Years: 1993-2015 (the search was limited to the period after the approval of the use of sometribove in the USA, since there is a higher chance that relevant data and studies were produced after the commercialisation of the product in that country).

- The search yielded a total of 216 scientific publications; 212 after removal of duplicates.
- A first screening of all titles and abstracts was performed in parallel by two reviewers, and references were selected on the basis of the review question. Since the aim was to identify scientific publications that postulate, investigate, or mention the possible link between the use of BST or rBST in cattle and AMR in humans, no exclusion criteria based on the type of papers retrieved (e.g. primary research studies, reviews, peer-reviewed papers) were applied.
- Fifteen references were considered relevant after consensus reached by the two reviewers. Relevant information in relation to the consequences of the use of BST/rBST in ruminants in the development of AMR in humans was retrieved from the full papers. Three of these were publications by WHO on the JECFA reports, and the other 12 were scientific papers, including peer-reviewed journals and other journals discussing scientific subjects.
- Many of the papers selected and analysed included information on various aspects of the use of rBST in ruminants (especially dairy cattle), including e.g. effects on the performance, on the quality and characteristics of the derived animal products, and on the effect on health or welfare of the animals treated (Bauman et al., 1994; Dubreuil, 1997; Chilliard et al., 1998, 2001; IFST, 1998; Bonneau and Laarveld, 1999; Vicini et al., 2008; Erasmus and Webb, 2013; Collier and Bauman, 2014; Macrina et al., 2014).
- Some papers mentioned and discussed, to a different degree of detail, human safety concerns or effects in humans other than AMR-related effects, either postulating potential health concerns or arguing for the absence of any concern (Bauman et al., 1994; Dagenais, 1995; Dubreuil, 1997; Chilliard et al., 1998, 2001; Bonneau and Laarveld, 1999; Vicini et al., 2008; Erasmus and Webb, 2013; Collier and Bauman, 2014).
- In relation to AMR-related issues:
  - some studies reviewed past data/information in relation to the possible effect of the treatment with growth hormones on the diminished ability to eliminate xenobiotics because of a reduced detoxifying capacity of the liver, which may imply longer persistence of antimicrobials in animals and their products when treated (Chilliard et al., 1998, 2001; IFST, 1998; Bonneau and Laarveld, 1999;
  - some others discussed the potential risks posed by the presence of residues of antimicrobials in milk following to the use of rBST, analysing the same data assessed in JECFA reports and similarly concluding that the ‘use of rBST has not increased human exposure to milk antibiotic residues as evidenced by post approval studies with commercial herds and by national data for antibiotic residue violations and milk SCC...
(Collier and Bauman, 2014), or simply reporting JECFA’s conclusions (IFST, 1998; Erasmus and Webb, 2013).

3.3. Assessment of the indirect link between the use of rbST in dairy cattle and AMR in humans

3.3.1. Link between the use of rbST and the occurrence of mastitis in dairy cattle

Evidence reported in the JECFA-78 report

- In the ‘Increased use of antimicrobial agents to treat mastitis in cows treated with rbSTs’ section of JECFA-78 (WHO, 2014b) – page 134, the report described the results of the literature search as follow: ‘The literature search, …, for publications from 1998 to August 2013 retrieved 29 unique articles that included the term ‘somatic cell count(s)’ OR ‘antibiotic’ OR ‘mastitis’. Some studies were located that evaluated the effects of rbSTs as a treatment for mastitis or that evaluated the effects of rbSTs on animal health parameters other than mastitis. These studies were excluded as irrelevant. An additional four relevant papers identified from review articles by De Vliegher et al. (2012) and Pezeshki et al. (2010) were also included in the review (Table 8)’ – page 135.

- There is an inconsistency between the literature search strategy and selection criteria stated in the protocol in the annex of JECFA-78 report (WHO, 2014b), and the above reported literature search results, including the list of studies listed in Table 8 of the report (WHO, 2014b), e.g.: review papers were included in Table 8, whereas according to the protocol ‘review articles that did not contain primary study data’ should be excluded (‘were deemed irrelevant’).

- From the literature search performed by JECFA, the results of thirty references were assessed, listed in Table 8, and summarised in the main text as follow (page 134): ‘The meta-analysis publication by Dohoo et al. (2003) was a reanalysis of data already published prior to approval of Posilac (1989–1994) and included 53 randomized clinical trials that Monsanto had provided to Health Canada (Health Canada, 1998). These represented the experimental data considered in previous evaluations by the fortieth and fiftieth Committee meetings. This study reported a 25 % increase in incidence of mastitis in rbST-treated herds versus non-treated herds. In contrast, a systematic review by the present Committee of clinical (Brozos et al., 1998; Judge et al., 1999; Collier et al., 2001; Vallimont et al., 2001; Gulay et al., 2003, 2007; VanBaale et al., 2005) and epidemiological studies (Ruegg et al., 1998) published since then (see Table 8) found no effect of rbST on mastitis incidence, possibly due to insufficient power to detect differences in mastitis incidence and exclusive use of multiparous animals as test subjects. It was noted that many of the studies listed in Table 8 and reviewed by the Committee did not follow the label recommended use directions. Regarding the incidence of subclinical mastitis, assessed as increased somatic cell count scores in milk, the vast majority of studies reported no effect of rbST treatment on somatic cell count values (Ruegg et al., 1998; Chiofalo et al., 1999; Vallimont et al., 2001; Dohoo et al., 2003; Gulay et al., 2003, 2007; VanBaale et al., 2005; Schneider et al., 2012; USDA, 2012), although a few studies reported small, transient increases (Brozos et al., 1998; Bauman et al., 1999; Boutinaud et al., 2003)’.

- The JECFA-78 report (WHO, 2014b) highlights the limitations of the results of the performed systematic literature review (i.e.: ‘no effect of rbST on mastitis incidence was possibly due to insufficient power to detect differences in mastitis incidence and exclusive use of multiparous animals as test subjects’). It was also reported that ‘many of the studies listed and reviewed by the Committee did not follow the label recommended use directions’; in addition to that, it is important to note that the list of studies that have been selected (see Table 8) includes scientific papers also on sheep, goats and buffaloes (e.g. Chiofalo et al., 1999; Chadio et al., 2000; Feckinghaus, 2009).

- It is not clear according to which criteria the selection of papers have been carried out, and no appraisal of the quality of the studies identified by the systematic literature search was reported by JECFA-78 (WHO, 2014a, b and annex).

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1 http://www.who.int/foodsafety/chem/annex_rbST_systematic_lit_search.pdf?ua=1
There is no analysis in JECFA-78 (WHO, 2014a, b) that could support the statement that no significant difference was found in the incidence of mastitis between rBST-treated and untreated cows.

JECFA-78 reiterate the comment of JECFA-50 that the effects of rBST on the incidence of mastitis are an animal health issue and outside the terms of reference of the Committee, and no overall conclusions on the link between the use of rBST and the incidence of mastitis in dairy cattle were formulated, in the 'Evaluation' section.

Scientific evidence from other sources

U.S. Food and Drug Administration

- The Food and Drug Administration approved the use of rBST for increasing the production of milk in lactating dairy cows in the US in 1993 (FDA, 1993).
- With studies conducted in dairy cows treated groups against control (not treated) ones in the US and the analyses of the results, the FDA's Veterinary Medicine Advisory Committee (FDA, 1993) found that 'there was an association between sometribove usage and the number of cows affected with clinical mastitis: comparing the proposed use level (500 mg) to control, the relative risk of a treated animal showing signs of clinical mastitis during the treatment period was about 1.79 times that of a control animal. Moreover, the risk for contracting subclinical mastitis for treated dairy cows groups with 250 mg, 500 mg, 750 mg of sometribove were 1.56, 1.55, and 1.51, respectively, compared to controls'. Based on the analysis of the results obtained, it was therefore concluded that 'the administration of sometribove (500 mg/14 days): 1) increases the risk of clinical mastitis in both primiparous and multiparous cows; 2) increases the number of cases of clinical mastitis in both primiparous and multiparous cows; 3) increases the risk of subclinical mastitis in both parity groups; and 4) increases milk somatic cell counts in some herds'.
- In a recent study by FDA (Sechen, 2013), it was reported that 'the usage of BST on U.S. dairy operations increased from 9.4 % in 1996 to 15.2 % in 2002, where it remained in 2007, with largest usage rates each year in herds with 500 or more milk cows; moreover, 'the percentage of U.S. dairy cows with clinical mastitis increased from 1996 to 2002, and again in 2007'. The interpretation of these results was that 'although the slight increase in mastitis incidence from 1996 to 2002 might be associated with a more than doubling in the percentage of cows given BST, mastitis incidence continued a trend upwards in 2007 despite a 5 % decrease in the percentage of cows given BST. A more likely relationship might be found with the increased annual yield of milk per cow each year'. The data reported on milk yield production per cow per year was shown to continuously increase in the U.S.

Report of the Canadian Veterinary Medical Association Expert Panel on rBST (Health Canada, 1998)

- At Health Canada's request, the Canadian Veterinary Medical Association (CVMA) established an Expert Panel to review the issues of the efficacy and safety of rBST. The Canadian Panel reviewed material from the submission of the manufacturer of rBST (sometribove) to have it approved for use in Canada, and it also carried out an extensive review of the published literature on the subject. The review process focused on studies which measured clinically relevant outcomes. The effects of rBST were assessed in several areas, including milk yield and udder health. Within each area, key measures of effect were identified and all data from the literature review were extracted. These data were analysed by meta-analyses to generate overall estimates of effect. Full details of the review process with literature search protocol, the data extraction and the meta-analysis etc. are reported by Health Canada (1998) and by Dohoo et al. (2003a, b).
- From the meta-analysis on the effects of rBST in dairy cows, the Canadian Panel concluded that 'rBST does increase production with responses that varied from study to study. In primiparous Holsteins the production increase averaged 3 kg or approximately 11.3 %. In multiparous Holsteins the increase averaged 4.4 kg or approximately 15.6 %'.
- Moreover, the meta-analysis showed that 'there was approximately a 25 % increase in the risk of clinical mastitis in treated cows. It appeared as though there was also a slight increase in the prevalence of subclinical infections. However, the data relating to subclinical mastitis was limited.
Furthermore, the Panel felt that current dairy health management practices would reduce but could not eliminate the increased risk of clinical mastitis that was associated with the use of rBST. It also resulted that “rBST would produce an increase of approximately 19.4% in the total number of cases of mastitis per cow. If the producer reduced the herd size to keep total milk production constant (given that production per cow has increased) there will be approximately a 10.4% increase in the total number of cases of mastitis expected.”

- On the effect of rBST on subclinical mastitis, the Canadian Panel concluded that there was probably an increased prevalence of subclinical intramammary infections in rBST treated cows, but the magnitude of the increase was difficult to quantify.

**EU Scientific Committee on Animal Health and Animal Welfare (SCAHAW, 1999)**

- The potentially increased incidence of clinical mastitis in rBST/BST treated cows was investigated by SCAHAW in the “Report on Animal welfare aspects of the use of bovine somatotrophin” (SCAHAW, 1999). The study consisted in a comprehensive assessment of the literature on this specific topic.

- The overall conclusions of the report were that “rBST/BST use causes a substantial increase in levels of foot problems and mastitis and leads to injection site reactions in dairy cows, conditions, that are painful and debilitating, leading to significantly poorer welfare in the treated animals”. Therefore, ‘from the point of view of animal welfare, including health’, the Committee recommended that ‘BST should not be used in dairy cows’.

- Based on this evidence, the administration of BST to dairy cows was prohibited in the EU (see Section 1.3).

- The SCAHAW assessment confirmed also that ‘there is clear evidence of significant positive associations between milk yield and mastitis and other production related diseases’.

- On the effect of treatment with rBST/BST on the incidence of clinical mastitis (or of mean somatic cell count (SSC)) in dairy cows, the SCAHAW reported the findings of previous studies and reviews which seemed to achieve contrasting results. For example, Pell et al. (1992) and Millstone et al. (1994) reported increased incidence of clinical mastitis or of mean SSC, respectively, whereas no (or non-significant) effects in BST-treated cows compared to control cows resulted in other studies (e.g. Monsallier, 1991; McClary et al., 1994; Masoero et al., 1998).

- It was also emphasised that meta-analyses (e.g. Willeberg, 1993) attributed the apparent inconsistencies to the different statistical power in detecting a difference in the treatment effects: the absence of significance in the effect of rBST treatment might be often the result of a low sample size, which would limit the validity of their findings and could not justify such conclusion. On the basis of the data corrected by the meta-analyses, the SCAHAW concluded that ‘BST usage increases the risk of clinical mastitis above the risk in non-treated cows. The magnitude of this increase has been variously estimated by meta-analyses or large scale studies at 15 to 45%, 23%, 25%, 42% and 79%. These estimates were considered ‘not only statistically significant, but also biologically relevant and of considerable welfare concern’.

- If experimental field trials are designed to investigate the level of increase of mastitis in lactating dairy cows treated with rBST compared to untreated control groups, they should involve an appropriate sample size that could allow the identification of a statistically significant effect of the treatment with rBST. These studies should be conducted in commercial settings, within the same herd or between comparable dairy herds with regard to genetics and management practices.

**Scientific Committee on Veterinary Measures relating to Public Health**

- In a parallel exercise, the use of BST to dairy cows as a productivity aid to milk production was examined by the Scientific Committee on Veterinary Measures relating to Public Health (SCVMP) in 1999. In particular, the SCVMP was asked to assess the possible direct and indirect adverse effects on public health caused by the use of BST under normal conditions.

- It was concluded that ‘numerous reports have indicated that the application of recombinant growth hormones (rBST, rbST) increases productivity of dairy cows measured as total milk yield..."
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per animal per lactation period. The application of rBST therefore may result in economic benefits although no therapeutic indications have been considered in the target animal species to date.

EFSA’s assessments on animal welfare issues

- Mastitis is ‘the inflammation of the mammary gland, with an infectious or non-infectious aetiology’ which, depending on the clinical severity, can be very painful for the animal and, thus, represents a major animal welfare problem. Sub-clinical mastitis is an udder inflammation that is not visible and can have only a small effect on animal welfare (EFSA, 2009a, b).

- Factors affecting the incidence of mastitis include pathogens, genetic predisposition, management practices, quality and social factors in the herd. Housing and management hazards are more likely to cause udder problems that affect welfare than nutrition-feeding and genetic selection hazards (EFSA, 2009a).

- SCC in milk samples is commonly used to monitor clinical and sub-clinical mastitis (EFSA, 2009a; EFSA AHAW Panel, 2012). Moreover, this animal-based measure is an early indicator that can be used to predict those animals at risk of poor welfare if no change or intervention is made (EFSA AHAW Panel, 2012). At cow level, SCC of less than 250 000 cells/mL and for heifers, SCC less than 150 000 cells/mL, is used to identify udder quarters with high probability to develop intra mammary infections (EFSA, 2009b).

- The main aims of dairy cows breeding during the last decades was to improve production efficiency, with the consequence of a dramatic increase in milk yield per cow due to rapid progress in genetics and management. Selection for milk yield and milk practices imposes stress on the udder that might be vulnerable to injury and mastitis (EFSA, 2009b). Somatic cell count and clinical mastitis have a large genetic component; moreover, mastitis resistance is genetically antagonistic to high milk production traits (EFSA, 2009a, b).

Additional scientific evidence from a literature search (‘EFSA literature search 2’)

- A new literature search (‘EFSA literature search 2’) has been carried out. The aim of this second scoping literature search was to identify additional scientific evidence that could be relevant for addressing the question on the possible effect (link) of the treatment with rBST on the incidence of mastitis in dairy cows, with the main objective to find whether other systematic literature reviews (and meta-analyses), addressing the link, have been performed.

- The following elements were considered:
  - Search question: What is the impact of the use of rBST on the incidence of mastitis in lactating dairy cows?
  - PICO elements:
    - Population: lactating dairy cows
    - Intervention: treatment with rBST
    - Comparator: control group of lactating dairy cows not treated with rBST, or treated with placebo, or treated with different levels of rBST
    - Outcome: incidence of mastitis or difference in incidence of mastitis before and after rBST treatment
  - Databases queried: Web of Science™ Core collection; BIOSIS Citation IndexSM, CABI: CAB Abstracts®, Chinese Science Citation DatabaseSM, Current Contents Connect®, Data Citation IndexSM, FSTA®-the food science resource, KCI-Korean Journal Database, MEDLINE®, ScIELO Citation Index, Zoological Record®.
  - Search string: (BST OR rBST OR somatotrop* OR ‘growth hormon’ OR ‘growth hormones’ OR ‘growth hormone’ OR ‘growth hormones’ OR somagrebove OR sometribove OR somavubove OR somidobove OR nutrilac OR ‘GH’ OR BGH OR rBGH) AND (bovin* OR cow* OR cattle OR dair* OR ruminant*) AND (mastitis OR udder health OR udder pathol* OR mammary health OR somatic cell count OR ‘SCC’)
- Fields searched: title, abstract, keywords (i.e. field ‘topic’).
- Languages: English
- Years: 1998-2015 (the search was limited consistently with the starting year considered for the JECFA-78 systematic literature review).

- The search yielded a total of 245 scientific publications (240 after removal of duplicates).
- The screening of all titles and abstracts was performed by applying the following inclusion/exclusion criteria:
  - inclusion of studies that investigate the effects of rBST on mastitis (or SCC) in lactating dairy cow – both positive and negative effects;
  - exclusion of studies with: a) no full abstract reported; b) treatment with rBST/BST in combination with other substances; c) treatment with rBST for fertility investigation or for inducting lactation or for pre-partum studies; d) modelling studies; e) treatment in non-bovine species or not lactating dairy cows (e.g. sheep, goats, buffaloes, calves, beef cattle, heifers, dry cows).

- After selection, 11 references were considered to be relevant for extracting information in relation to the effects of the use of rBST in the incidence of mastitis in lactating dairy cows.

- A preliminary assessment of the results of the studies, performed in order to identify whether effects on milk yield, incidence of mastitis, incidence in mean SCC were reported, is presented in Table 1. The information whether the papers reported a meta-analysis is also indicated.

**Table 1:** Preliminary results from comparison of the references considered relevant for addressing the literature search question (no evaluation of the quality of the studies and meta-analyses were performed, thus the results that are reported should be considered indicative). Indication of the papers reporting a meta-analysis on the possible link between the use of rBST and the incidence of mastitis is included.

<table>
<thead>
<tr>
<th>Citation</th>
<th>Year of publication</th>
<th>Change in milk yield(^{(a)})</th>
<th>Change in incidence of mastitis(^{(a)})</th>
<th>Change in mean SCC(^{(a)})</th>
<th>Meta-analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bauman et al.</td>
<td>1999</td>
<td>Increased</td>
<td>NR</td>
<td>Small but significant increase in SCC linear score</td>
<td>No</td>
</tr>
<tr>
<td>Campos et al.</td>
<td>2011</td>
<td>Increased</td>
<td>Not increased</td>
<td>Not increased</td>
<td>No</td>
</tr>
<tr>
<td>Chilliard et al.</td>
<td>1998-2001</td>
<td>Increased</td>
<td>The increased incidence of mastitis may result from the increased milk yield</td>
<td>Slightly increased number (in the first weeks of lactation)</td>
<td>No</td>
</tr>
<tr>
<td>Collier et al.</td>
<td>2001</td>
<td>Increased</td>
<td>No significant effect</td>
<td>No effect</td>
<td>No</td>
</tr>
<tr>
<td>Dohoo et al.</td>
<td>2003b</td>
<td>Increased (with reference to Dohoo et al. (2003a))</td>
<td>Increased risk of clinical mastitis by approx. 25% (approximately 19.4% increase of the total number of cases of clinical mastitis per cow)</td>
<td>Apparent trend towards slightly increased SSC difficult to be quantified</td>
<td>Yes</td>
</tr>
<tr>
<td>Kim and Kim</td>
<td>2012</td>
<td>Increased</td>
<td>Not increased</td>
<td>Not increased</td>
<td>No</td>
</tr>
<tr>
<td>Masoero et al.</td>
<td>1998</td>
<td>Increased</td>
<td>NR</td>
<td>Not increased</td>
<td>No</td>
</tr>
<tr>
<td>Soliman and El-Barody</td>
<td>2014</td>
<td>Increased</td>
<td>Incidence of mastitis is due to increased milk yield</td>
<td>One study reported no effect; one study reported higher SSC</td>
<td>No</td>
</tr>
<tr>
<td>St-Pierre et al.</td>
<td>2014</td>
<td>Increased</td>
<td>No significant differences of clinical mastitis</td>
<td>No significant differences</td>
<td>Yes</td>
</tr>
<tr>
<td>VanBaale et al.</td>
<td>2005</td>
<td>Increased</td>
<td>Number of cows sent to the hospital for mastitis (97) not affected by somatropin treatments (chi(2) = 0.49)</td>
<td>Increased</td>
<td>No</td>
</tr>
</tbody>
</table>

NR: not reported; SCC: somatic cell count.

\(^{(a)}\): after treatment with rBST/BST compared with the control group or before treatment of the same group.
• From EFSA literature search 2, two systematic literature reviews using meta-analysis have been identified: Dohoo et al. (2003b) and St-Pierre et al. (2014). The results of the meta-analysis carried out by Dohoo et al. (2003a, b) have been already reported in subsection 'Report of the Canadian Veterinary Medical Association Expert Panel on rBST (Health Canada, 1998)' of this section, whereas the results of St-Pierre et al. (2014) are described in the following one.

Meta-analysis of the effects of sometribove zinc suspension on the production and health of lactating dairy cows of St-Pierre et al. (2014)

• It is a recent meta-analysis of the effects of rBST-Zn (sometribove zinc formulation—commercially available form in the US) that has been published with the aim to provide an updated review of the efficacy and safety of rBST-Zn on the production and health of lactating dairy cows.

• The studies that were included in the meta-analysis were studies published in peer-reviewed scientific journals or reviewed by a regulatory agency, which included a control group, and in which rbST-Zn was administered to dairy cows in accordance with the Food and Drug Administration (FDA)-approved label directions. The data reported on the effects of rBST-Zn in treated vs. untreated cows were statistically analysed for six domains, including ‘milk production and composition’ and ‘udder health’ (the latter was expressed as the incidence of clinical mastitis/100 cow-days at risk and the mean log SCC).

• As results, the clinical incidence rate for mastitis was calculated for 14 studies, and the logSCC was calculated for nine studies. For both variables, the results were considered by the authors heterogeneous across the studies included in the meta-analysis.

• Based on the results of the meta-analysis, St-Pierre et al. (2014) claimed that: a) ‘clinical mastitis rates did not differ significantly (P = 0.122) between cows that were and were not treated with rbST-Zn’; b) the mean ± SE logSCC response did not differ significantly (P = 0.540) between cows that were and were not treated with rbST-Zn; c) milk yields resulted to be ‘significantly greater for cows administered with rbST-Zn than those for control cows’.

• Across the 14 evaluated studies, the authors highlighted that ‘only four ones reported that rbST-Zn treated cows were significantly more likely to develop clinical mastitis than control cows’. On the basis of the results of the test for heterogeneity, it was suggested that this ‘higher risk was dependent on factors other than rbST-Zn administration’ (e.g. season, stage of lactation, parity).

• Moreover, on their results, the authors supported the concept that the positive association between milk production and higher risk/incidence of clinical mastitis can be compensated by improved management on modern commercial dairy operations.

• On these bases, the authors concluded that ‘results of the present meta-analysis indicated that administration of the rbST-Zn formulation, which is commercially available to US producers, to lactating dairy cows in accordance with the FDA-approved label directions caused an increase in milk, fat, and protein yields with no significant or unmanageable adverse effects on milk composition (percentages of fat, protein, and lactose in milk), udder health, reproduction, body condition, lameness, or culling; and that ‘the current management practices implemented by US dairy producers and veterinarians are adequate for the safe and effective commercial use of rbST-Zn’.

EFSA’s Critical Appraisal of the Systematic Reviews of Dohoo et al. (2003a, b) and St-Pierre et al. (2014)

• The two meta-analyses identified through EFSA literature search 2 (Dohoo et al. (2003a, b) and St-Pierre et al. (2014)) on the effects of rBST reported similar results in the case of milk yield (i.e. statistically significant increase) and SCC (no significant relevance) in rBST-treated cows, compared to untreated ones. On the contrary, the results on clinical mastitis were extremely different: Dohoo et al. (2003a, b) quantified an increased risk of clinical mastitis by approximately 25 % in rBST-treated cows compared to untreated ones, whereas St-Pierre et al. (2014) concluded that clinical mastitis rates did not differ significantly between the two groups of animals.
Due to the different results, EFSA critically appraised the methodology used in carrying out the two systematic reviews (SRs) of Dohoo et al. (2003a, b) and St-Pierre et al. (2014). In this exercise EFSA used a methodological approach developed internally to assess the quality of Systematic Reviews of interventions and of Extensive Literature Searches\(^6\) (see Appendices).

Both SRs suffer from methodological flaws that potentially introduced a risk of bias in the results.

From the Critical Appraisal of Dohoo’s SR it has been overall assessed that:

- The study selection process allowed exclusion of studies in a subjective way. As a consequence the results of the SR could have changed significantly and it is not possible to predict in which direction. This could have affected all the outcomes including those related to ‘clinical mastitis’, ‘somatic cell count (SCC)’ and ‘Prevalence of subclinical mastitis’ (issue No 1).

- The results of the tests ending up being statistically non-significant were inappropriately interpreted as ‘evidence of absence of an effect’ (i.e. no difference in the outcomes between treated and untreated cows). A proper interpretation would have been that results are inconclusive and no firm conclusions can be drawn. This led to a potential underestimation of the risk of adverse health effects of rBST (i.e. some of the outcomes could be truly not different, for some others test could be not enough powerful to detect a difference). The outcomes potentially affected by this issue include, but are not limited to, ‘SCC’ and ‘Prevalence of subclinical mastitis’ (issue No 2). ‘Clinical mastitis’ is not affected by this issue since results were statistically significant.

From the Critical Appraisal of St-Pierre’s SR it has been overall assessed that:

- The study selection process is not transparently reported and, in addition, allowed inclusion of studies in a selective way. As a consequence, the results of the SR could have changed significantly and it is not possible to predict in which direction. This could have affected all the outcomes, including those related to ‘clinical mastitis’ and ‘SCC’ (issue No 1). ‘Prevalence of subclinical mastitis’ was not among the outcomes reported by St-Pierre et al. (2014).

- The results of the tests ending up being statistically non-significant were inappropriately interpreted as ‘evidence of absence of an effect’ (i.e. no difference in the outcomes between treated and untreated cows). A proper interpretation would have been that results are inconclusive and no firm conclusions can be drawn. This led to a potential underestimation of the risk of adverse health effects of rBST (i.e. some of the outcomes could be truly not different, for some others test could be not enough powerful to detect a difference). The outcomes potentially affected by this issue include, but are not limited to, ‘clinical mastitis’ and ‘SCC’ (issue No 2).

Therefore, for both the SRs the risk of bias has been considered high.

Based on the meta-analyses of the effects of recombinant bovine somatotropin carried out in 1998, Dohoo et al., (2003a, b) reported that there is approximately a 25 % increase in the risk of clinical mastitis in rBST-treated cows compared to untreated cows, which resulted in an estimation of approximately 19.4 % increase in the total number of cases of clinical mastitis per cow. However, after critically appraising the SR, it cannot be excluded that the true strength of the link could in reality be lower or higher (because of the risk of bias introduced by the experts selecting studies after screening for relevance was completed; issue No 1).

The results of the recent meta-analysis of the effects of rBST-Zn carried out by St-Pierre et al. (2014) were interpreted as indicating that the risk of rBST-Zn-treated cows developing clinical mastitis did not differ from that of control cows. However, after critically appraising the SR, because of the risk of bias introduced by the selection process (issue No 1), it cannot be excluded that the true estimate of the difference could be higher or lower. In addition, as a consequence of the inappropriate interpretation of the results (issue No 2), a true effect could remain undetected, in case of insufficient power of the test, and estimated as not existing while it does in reality.

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Therefore, the test for significance of the difference in the risk for mastitis, as estimated by the authors, has to be considered inconclusive and not supporting the absence of an effect.

- Full description and details of the critical appraisal tools (CATs) are reported in the Appendices of this report.

**Additional scientific evidence on the indirect effect of rBST-treatment on the incidence of mastitis and implications on animal welfare**

- The reported effects of BST on clinical mastitis might be due to an indirect causal effect mediated through the increase in milk yield (Figure 2).

**Figure 2:** Possible links between rBST treatment and incidence of mastitis in dairy cows

- The treatment with rBST/BST aims to increase milk production in lactating dairy cows (FDA, 1993), and this effect was proved with consensus by the scientific literature (see previous sections).
- Once the nutritional standard and quality have been optimised, for producing more milk, the cow should stress the energy balance between ingestion and production phases. This is the purpose of some genetic and management practices that increase the gap between energy input and output, promoting the output. The administration of rBST/BST, where allowed, is an example of these output-oriented management practices (EFSA, 2009b).
- High producing dairy cows need to mobilise body reserve to support their milk production and, if output is increased, other biological processes, such as maintenance, movement, immune defence, may be affected (EFSA, 2009b).
- With increasing milk yield cows need to spend more time eating and thus have less time available for other activities that can address physiological and behavioural needs, such as resting (EFSA, 2009b).
- Large loads of milk in the udder inhibit cows from lying and resting; moreover, voluminous udders can cause lying discomfort, preventing the animals to express their natural behaviour and being significant restrictions to animal welfare (e.g. upright lying position with retracted hind legs and free changing of lying postures, including free stretching of the legs; EFSA, 2009b).
- Positive correlations between milk production and mastitis have been reported (Broom and Corke, 2002; Fetrow and Eicker, 2003); however, more frequent evacuation of the udder could diminish the risk of mastitis, although total milking time per day is longer and thus also the mechanical impact of the milking machine on the udder (EFSA, 2009b).
- There is evidence that increased production increases the risk of mastitis; however, the higher risk of mastitis could be limited by improving the management practices and hygiene (for details see EFSA, 2009a).
- Scientific literature findings reported the increased risk of mastitis in lactating dairy cows treated with rBST.
- Based on this evidence, the discussion whether the possible link between rBST treatment in dairy cows and increase incidence of mastitis is indirect (due to increased milk yield), or if there is a direct increased risk associated with use of the product, seems to be irrelevant. In fact, even if the effect is indirect, it still represents an effect of administration of the drug. Therefore, it is
appropriate to conclude that ‘whether this effect is direct or indirect does not alter the welfare concerns’ (SCAHAW, 1999).

3.3.2. Link between the occurrence of mastitis and the use of antimicrobials in dairy cattle

Evidence reported in the JECFA reports

- JECFA-78 referred to one past study and indicated that ‘bovine mastitis is considered the single most important reason for antimicrobial use in lactating dairy cows (Erskine et al., 2004).

Scientific evidence from other sources

- Some aspects related to this issue were already considered and discussed in the past by the European Commission Scientific Committees:
  - When discussing the effect of rBST on clinical mastitis, the SCVMP (1999) indicated that ‘Treatment of clinical mastitis cases with antimicrobials is not limited to those cases which may be classified as severe, although such cases are probably more likely to receive systemic treatment. Also mild clinical cases are often treated with local application of antimicrobials, such as the application of formulations for intramammary use. Even cases of subclinical mastitis are sometimes treated with antimicrobials, depending on other factors in the herd, as are cows being dried off before calving (Radostits et al., 1994). The result is that mastitis is the one condition in dairy cows which is associated with use of the largest amount of antimicrobials. It is therefore not surprising, that by far the most frequent reason for residue violations in milk are related to mastitis treatment (Leslie and Keefe, 1998). This applies in particular in cases where the principles of Good Clinical Practice are not respected.

  - In addition, SCVMP (1999) anticipated that ‘with an increase of the incidence of bovine mastitis more veterinary medicinal products will be used’.

  - SCAHAW (1999), when discussing animal welfare aspects of the use of BST, also indicated that ‘BST increases the frequency of certain disease conditions such as mastitis and foot problems in cows. These conditions are normally treated using veterinary medicines. Hence BST is leading, on average to the increased use of veterinary medicines’.

- In the scientific literature, mastitis is recognised to be the main condition for which antimicrobials are administered to dairy cattle. For example:
  - White (2006) indicated that ‘mastitis remains the most common cause of antibacterial use on dairy farms, as therapy is a major component and a primary tool for mastitis control in lactating and dry cows’;

  - Oliver and Murinda (2012) indicated that, despite other possible measures for the control of mastitis, cows developing mastitis often require an intervention with antimicrobials;

  - Supré et al. (2014) also indicated that antimicrobial drugs are frequently used for treatment of clinical and subclinical mastitis;

  - De Briyne et al. (2014) conducted a survey among 3 004 veterinarians in the 25 European countries to gather information about antimicrobials prescribing habits. Feedback obtained from this survey indicated that mastitis is the most common reason for administering antimicrobials, accounting for 40 % of the treatments with antimicrobials in cattle.

- Generally, in the case of mastitis, antimicrobial treatments are administered as intramammary inoculations. Several intramammary preparations are available for use, often combining two or more active compounds, including third and fourth generation cephalosporins, and with a wide indication (Suojala et al., 2013; De Briyne et al., 2014). Active antimicrobial substances included in intramammary preparations in the EU include associated amoxicillin and clavulanic acid and cefquinome.
According to EMA-ESVAC (2014), the percentage of antimicrobials used in veterinary medicine\(^7\) for food-producing animals (including horses) that are sold as intramammary preparations in 2012 in 26 EU/EAA countries was 0.7 %, which increased up to 14.6 % when considering only third and fourth generation cephalosporins.\(^8\) This shows for example the important extent of the treatments with cephalosporins in lactating dairy cows, which is the only species for which cequinoem is registered for use as intramammary suspension.

Although more rarely, antimicrobials can also be administered as systemic preparations, in particular for severe or chronic cases of mastitis, including several active compounds such as fluoroquinolones and third/fourth generation cephalosporins\(^10\) (Roberson, 2012; Suojala et al., 2013; Bergonier, 2014).

In summary, it seems clear that the occurrence of mastitis in dairy cattle leads to the treatment with antimicrobials, often administered topically, i.e. intramammary administration, and more rarely systemically.

It is not clear whether mastitis potentially caused by the use of rBST would qualify for treatment with antimicrobials, and how much the use of rBST contributes to the overall mastitis problem. Therefore, it is difficult to quantify how much of the antimicrobial treatments might be due to the use of rBST in dairy cattle.

Additional studies should investigate whether mastitis possibly induced by the use of rBST would qualify for antimicrobial treatments.

### 3.3.3. Link between the treatment with antimicrobials and AMR in dairy cattle (and in dairy cattle farms)

**Evidence reported in JECFA reports**

- JECFA-78 referred to past studies and indicated that ‘antimicrobial resistance in mastitis pathogens is a cause of concern (Oliver, Murinda & Jayarao, 2011; Oliver & Murinda, 2012).’

- The link between the treatment with antimicrobials and AMR in dairy cattle and dairy cattle farms is not considered further by JECFA-78, which rather included some conclusions in relation to the link between the use of rBST in cattle and the development of AMR in mastitis pathogens, discussed later in this document (see Section 3.3.5).

**Scientific evidence from other sources**

- Some aspects related to this issue were already considered and discussed in the past by the European Commission Scientific Committees.
  - The SCVMP (1999) indicated that one of the ‘public health reasons for limiting as far as possible the use of antimicrobials in dairy cows’ includes the risk of ‘an increased selection of bacteria resistant to antimicrobials’. It indicated that ‘the increased use of antimicrobial substances in the treatment of rBST related mastitis might lead to the selection of resistant bacteria’ and that ‘recent publications referring to the specific issue of bacterial resistance following mastitis related use of antimicrobials vary in their evaluation of the phenomenon (Hillerton 1998, Sandgren 1998, Wegener 1998, Aarestrup and Jensen 1999).’
  - In that occasion the SCVMP (1999) concluded that ‘secondary risks associated with the use of rBST in dairy cows’ include ‘an increased use of antimicrobial substances in the treatment of rBST related mastitis which might lead to an increased risk of residue formation in milk and to the selection of resistant bacteria’.

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\(^7\) All pharmaceutical forms and medicated feed except dermatological preparations and preparations for sensory organs are taken into account, including antimicrobial agents for intestinal use, intrauterine use, systemic use, intramammary use, and used as antiparasitic agents (EMA-ESVAC, 2014).

\(^8\) Third and fourth generation cephalosporins and fluoroquinolones are among the critically important antimicrobials for human medicine, as defined by WHO (2012).
Similar considerations were expressed by the Scientific Committee on Animal Health and Animal Welfare (SCAHAW, 1999), who indicated that ‘BST is leading, on average to the increased use of veterinary medicines. This increased use allows more opportunity for the development of resistance to antimicrobials in pathogens on farms.’

SCAHAW (1999) also concluded that ‘increased antimicrobial usage may lead to resistance to antimicrobials with consequences for the health of humans, cattle and other animals’.

- The most direct cause for the development of AMR (in animals as well as in humans) is the exposure to antimicrobial agents though medical treatments.

- Without specifically referring to the administration of antimicrobials for the treatment of mastitis in cattle, several studies have analysed the link between the use of antimicrobial agents and the development of AMR in both animals and humans. A report recently published by ECDC, EFSA and EMA (2015) analysed the possible associations between the consumption of antimicrobial agents and the occurrence of AMR in food-producing animals and in humans:
  - these analyses were performed for selected combinations of antimicrobial agents and bacteria for which data are gathered by monitoring programmes carried out annually in EU Member States;
  - ECDC, EFSA and EMA (2015) did not analyse data related to cattle specifically, but rather from all food-producing animals jointly, and data used did not refer to treatments for specific purposes (e.g. mastitis) or specific administration routes (e.g. systemic versus topical). Therefore, the analysis might not be fully applicable in the framework of the specific case discussed here. However, some general conclusions from the this report provide updated evidence of the relationship between the treatment with antimicrobials and the development of AMR, both in animals and in humans. In particular, this report indicated that ‘in both humans and animals, positive associations between consumption of antimicrobials and the corresponding resistance in bacteria were observed for most of the combinations investigated’.

- As indicated in Section 3.3.2, generally, in the case of mastitis, antimicrobial treatments are administered as intramammary inoculations.

- Resistance in mastitis pathogens has been reported in several studies, also recently (Suojala et al., 2013; Ferrari and Maraboli, 2014; Idriss et al., 2014; Jagielski et al., 2014; McDougall et al., 2014; Robles et al., 2014; Ruegsegger et al., 2014; Seixas et al., 2014).

- The effect of the use of antimicrobials in increasing resistance in mastitis pathogens has been discussed in several papers, and studies have shown that bacteria isolated from cases of mastitis have developed resistance to antimicrobials that have been extensively used over the years in dairy cattle (Saini et al., 2012; Suojala et al., 2013; Beuron et al., 2014; da Costa Krewer et al., 2015).

- As indicated in Section 3.3.2, although more rarely, antimicrobials can be also administered as systemic preparations, in particular for severe or chronic cases of mastitis.

- When considering the possible development of AMR due to the treatment of mastitis with antimicrobials, the main concerns to be considered in the cattle treated are linked to:
  - the development of AMR bacteria at the level of mammary glands, especially as a result of intramammary administration of antimicrobials (both mastitis pathogens and non-pathogenic bacteria might develop resistance);
  - the development of AMR in bacteria in the intestinal tract of animals as a result of systemic administration (animal pathogens, zoonotic pathogens or commensal bacteria might develop resistance).

- More in general, the treatment with antimicrobials can have a direct or indirect effect in increasing the selection pressure of the bacterial population of the farm environment and of other animals in the farm (see also Section 3.3.4), due to, for example:
  - the disposal of the milk during the withdrawal times;
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- the possible use of the milk to feed calves or other animals;
- the spilling of milk from mammary glands to the environment;
- the disposal of animal manure.

- In particular, in relation to the possible use of waste milk to feed calves, Brunton et al. (2012) conducted a survey on feeding practices in dairy farms, and reported that 83% of the respondents reported feeding calves with waste milk from cows with mastitis, often including the first milk after antimicrobial treatment. Waste milk can contain both residues of antimicrobials and resistant bacteria (Randall et al., 2014). Aust et al. (2013) investigated the effect of the use of waste milk fed to calves in the development of AMR bacteria isolated from the faeces of calves, and found that resistant Escherichia coli isolates were significantly higher in calves fed with both pasteurised and non-pasteurised waste milk compared to bulk milk. Duse et al. (2015) reported a significantly higher resistance in E. coli isolated from calves when fed with waste milk of cows treated with antimicrobials during lactation compared to when such milk was eliminated.

- The extent to which the administration of antimicrobials for the treatment of mastitis contributes to the overall development of AMR in dairy cattle and dairy cattle farms is not known, and should be investigated.

- Similarly, it is not possible to quantify how much the treatments due to mastitis caused by the use of rBST in cattle can contribute to the problem.

- In addition, the different role of topical versus systemic administration of antimicrobials to treat mastitis in the development of AMR in dairy cattle and dairy cattle farms should be studied and clarified.

3.3.4. Link between AMR in dairy cattle (and in dairy cattle farms) and AMR in humans

Evidence reported in JECFA reports

- As discussed earlier in this document, potential human risks related to AMR considered by JECFA only include the presence of residues of antimicrobials in products obtained from animals treated with rBST, in particular milk from dairy cattle (see Section 3.1). No other pathways (e.g. exposure to resistant bacteria through direct contact with animals, food, and environment) are considered, since only resistance ‘arising from human exposure to residues of antimicrobial agents in edible foods is relevant to the work of JECFA’ (WHO, 2014a).

Scientific evidence from other sources

- Some aspects related to this issue were already considered and discussed in the past by the European Commission Scientific Committees. As reported above, SCAHAW (1999) concluded that ‘increased antimicrobial usage may lead to resistance to antimicrobials with consequences for the health of humans, cattle and other animals.’

- There can be many pathways leading to the development of AMR in humans. These in particular include:
  - exposure to antimicrobials;
  - exposure to resistant bacteria (both pathogenic and non-pathogenic bacteria), which can be a source of AMR genes.

- Here it is only considered the exposure that originates, directly or indirectly, from animal sources, with specific reference to exposure that might follow treatments for bovine mastitis. Therefore, for example, exposure of humans to antimicrobials as a consequence of a clinical treatment in humans is not discussed.

- In relation to human AMR following the exposure to antimicrobials for reasons other than medical treatments, humans might be exposed to active compounds through residues of antimicrobials in products of animal origin. This exposure can lead to adverse effects on the human microflora through two main mechanisms (Tollefson et al., 2006):
disruption of the colonisation barrier in the human intestine, which can be more easily subject to invasion and overgrowth of pathogenic bacteria;

- development of resistance with an increase of the intestinal population of AMR bacteria.

- Exposure through residues of antimicrobials is the only pathway considered by JECFA. No other pathways are considered since they were out of the scope of JECFA-78 report, as indicated above. Therefore, AMR-related concerns are only partially considered by JECFA.

- In relation to AMR following the exposure to resistant bacteria, AMR in humans may develop through two main pathways (Tollefson et al., 2006; EFSA, 2008; ECDC EFSA and EMA, 2015), including infection with zoonotic resistant bacteria and with non-pathogenic bacteria, which may transfer AMR genes to pathogenic bacteria within the human intestine (see Figure 3).

**Figure 3:** Pathways leading to the development of AMR bacteria of animal origin in humans (adapted from Tollefson et al., 2006)

- Humans can be exposed to animal AMR bacteria in different ways, and in particular through:
  - direct contact with animals;
  - food of animal origin;
  - food of non-animal origin (contaminated through the environment);
  - contaminated environment.

- Animal AMR bacteria can be also spread in animal populations and in the environment, and not necessarily be acquired by humans from the animal species that originally developed the resistance. Recently, ECDC, EFSA and EMA (2015) discussed in detail these possible pathways (see Figure 4).
A quantification of the relative contribution of the different sources to the overall human exposure is extremely difficult to provide due to the possible interrelations among these transmission pathways.

TATFAR, the Transatlantic Taskforce on Antimicrobial Resistance, has been established in 2009 with the goal of improving cooperation between the US and the EU. TATFAR\(^9\) indicated that ‘the use of antibiotic drugs in animals selects for antimicrobial resistance; however, the mechanism by which this resistance may be transferred to man and the extent of the threat that this represents to human health is less clear’. It recommended the ‘establishment of a joint working group of international subject matter experts. One of its aims would be to identify key knowledge gaps in understanding the transmission to man of antimicrobial resistance arising as a result of the use of antimicrobial drugs in animals.’

In relation to the role of foods specifically, EFSA issued a scientific opinion on Food-borne antimicrobial resistance as a biological hazard (EFSA, 2008).

That opinion proposed a framework to assess the risk of acquisition of AMR bacteria or bacteria-borne AMR genes through the food chain. In particular, EFSA (2008) developed a simplified approach to assess the extent to which food serves as a source of AMR, allowing to compare and rank the food types for specific AMR bacteria (i.e. bacterial species resistant to a particular antimicrobial). Before being able to perform a quantitative risk assessment for all AMR bacteria, a number of uncertainties need to be further investigated. Key uncertainties, data limitations and problematic issues are discussed in detail elsewhere (Snary et al., 2004; EFSA, 2008), and include for example data on:

- survival and multiplication of AMR bacteria in food;
- whether AMR bacteria are more pathogenic than susceptible bacteria;
- role of the susceptibility of consumers;
- control interventions possibly applied;

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- dose-response, severity of illness, treatment failure for the different antimicrobial-bacteria combinations;
- role of commensals and bacteria intentionally added to food processing.

The application of the framework developed by EFSA (2008) to the antimicrobial-bacteria combinations that may originate from cattle would provide more information on the relative role of all foods as a source of AMR. However, this would not provide direct information in relation to the extent to which the use of rBST in dairy cattle contributes to the problem.

More in general, when considering the specific issue covered here, i.e. the possible transmission to humans of cattle AMR bacteria possibly developed as an indirect consequence of the use of rBST, it is not possible to quantify the relative contribution of the use of rBST in cattle to the overall exposure of humans to AMR bacteria through all possible pathways.

Evidence is available on the possible role of milk and meat from cattle as a vehicle for the spread of AMR bacteria to humans (Tacket et al., 1985; Villar et al., 1999; Olsen et al., 2004; Dechet et al., 2006; Varma et al., 2006; Murphy et al., 2007; Oliver et al., 2009; Van Kessel et al., 2013).

There is a need to further understand and investigate the transmission pathways of AMR from animals to humans, and to quantify the risk that the different pathways may imply for humans.

Source attribution studies are needed to understand:
- the extent to which the cattle reservoir contributes as a source of human AMR bacteria;
- the relative contribution of the different pathways to the transmission of AMR bacteria from the cattle reservoir to humans (e.g. cow's milk, beef, food from other animal species, food of non-animal origin, environment, direct contact with animals, etc.).

3.3.5. Plausibility of the indirect link between the use of rBST in cattle and AMR in humans

Additional evidence reported in JECFA reports

- Additional relevant information is reported by JECFA-78 in relation to some aspects of the use of rBST.
- The first one concerns the association between the use of rBST and the use of antimicrobial agents. JECFA-78 indicated that this association could not be analysed because of the unavailability of data on the use of antimicrobials agents to treat mastitis in farms using and not using rBST. Such data should be collected and analysed.
- The second relates to the association between the use of rBST and AMR in dairy cattle herds, for which JECFA-78 ‘did not find specific studies that investigated the association between the use of rBSTs and the development of antimicrobial resistance in mastitis pathogens’. JECFA-78 also referred to past studies, reporting that ‘bovine mastitis is considered the single most important reason for antimicrobial use in lactating dairy cows (Erskine et al., 2004)’ and that ‘antimicrobial resistance in mastitis pathogens is a cause of concern (Oliver, Murinda & Jayarao, 2011; Oliver & Murinda, 2012).’ From the above information it was concluded that ‘in the absence of properly designed studies, whether the use of rBSTs in cows of farms increases antimicrobial resistance remains speculative’ and that ‘there is a lack of evidence that the use of rBSTs in dairy herds contributes to antimicrobial resistance in dairy herds’.
- Although there might be a lack of direct evidence about the link between the use of rBST and AMR in dairy cattle herds, it is considered that:
  - in the absence of such evidence three intermediate steps should be considered, i.e. the link between the use of rBST and the occurrence of mastitis, between the occurrence of mastitis and the use of antimicrobials, and between the use of antimicrobials and the development of AMR in dairy cattle herds (see Sections 3.3.1-3.3.3);
o epidemiological studies (e.g. prospective or retrospective cohort studies, or case-control studies) should be performed to assess the association between the treatment with rBST and the development of AMR in cattle dairy herds.

- Before being able to provide definite conclusions, both issues discussed above, i.e. the link between the use of rBST and the treatment with antimicrobials and the link between the use of rBST and AMR in dairy cattle, should be properly investigated.

**Strength of evidence and uncertainties**

- Based on the evidence discussed in JECFA reports, and additional scientific evidence discussed in Sections 3.3.1-3.3.4, Table 2 aims at providing a qualitative assessment of the strength of evidence (high, medium, low strength) with regard to the four intermediate steps linking the use of rBST in cattle and AMR in humans. It also qualitatively evaluates the uncertainty associated with the four steps (high, medium, low uncertainty). Finally, it highlights that quantification of the links is needed.

- The strength of the evidence with regard to each one of the four intermediate steps linking the use of rBST in dairy cattle and AMR in humans discussed above is considered to be medium to high when assessing the steps singularly.

- Therefore, assuming that:
  - treatment with rBST can lead to an increased incidence of mastitis in dairy cattle,
  - cases of mastitis are usually treated with antimicrobials,
  - use of antimicrobials can lead to development of AMR in dairy cattle (and in dairy cattle farms),
  - AMR in humans may derive from both the exposure to AMR bacteria/genes of cattle origin and residues of antimicrobials,

it is concluded that an increase of AMR in humans following to the use of rBST in dairy cattle is plausible.

- Current knowledge does not allow quantifying the four links discussed, and additional studies are needed for their quantification.

- An estimation of the contribution of the use of rBST to human AMR could be obtained by combining the results obtained from:
  - studies investigating the extent to which the use of rBST may contribute to the development of AMR in dairy cattle, and
  - source attribution studies, investigating the extent to which the cattle reservoir contributes as a source of human AMR bacteria and the relative contribution of the different pathways to the transmission of AMR bacteria from the cattle reservoir to humans.
### Table 2: Assessment of the strength of evidence and uncertainties associated with the indirect link between the use of rBST in cattle and AMR in humans

<table>
<thead>
<tr>
<th>Step 1: rBST</th>
<th>Step 2: mastitis cattle</th>
<th>Step 3: antimicrobial use cattle</th>
<th>Step 4: AMR cattle</th>
<th>AMR humans</th>
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<tbody>
<tr>
<td><strong>Evidence of link demonstrated by one or more studies</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Strength of evidence of the link</strong></td>
<td>high</td>
<td>high</td>
<td>high</td>
<td>medium</td>
</tr>
<tr>
<td><strong>Uncertainty associated with the link</strong></td>
<td>medium</td>
<td>low</td>
<td>low</td>
<td>medium</td>
</tr>
<tr>
<td><strong>Further studies are needed to quantify the link</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
4. Conclusions

4.1. Answer to Term of Reference 1

4.1.1. With regard to methodology

- When comparing the information provided in the JECFA-78’s protocol for Steps 1, 2 and 3, it is not clear what search strategies were finally used in the literature search.

- Without a clear definition of the search terms used and further details on the paper selection process, the literature search is not repeatable and a full assessment of the accuracy of the search strategy is not possible.

- The search strategy followed allows identifying only a proportion of the scientific articles related to the questions formulated in JECFA-78, since:
  - in relation to the incidence of mastitis in animals treated or not treated with rBST, the search string also included ‘antibiotic residue’, which led to a narrow literature retrieval. Potentially relevant studies related to the incidence of mastitis, which did not include ‘antibiotic residue’, have been excluded. This has reduced the sensitivity of the search.
  - It is not reported whether additional search terms other than ‘antibiotic’ (e.g. antimicrobials) were also used.
  - Similarly, it is not indicated whether other search terms related to ‘mastitis’ (e.g., udder inflammation, somatic cell counts) were used.

- The search strategy may not allow identifying papers related to all AMR-related concerns, since:
  - in relation to possible increased use of antimicrobials to treat mastitis in dairy animals, it only addresses the presence of antimicrobial residue levels in milk and meat from rBST-treated animals, while other aspects, such as the possible development of AMR in dairy cattle and dairy cattle farms following to the treatment with antimicrobials are not considered;
  - in relation to possible increased health risks of consuming milk or meat from rBST-treated animals versus untreated animals, it mainly addresses health concerns other than AMR and the search terms defined are not specific to AMR-related concerns.

4.1.2. With regard to JECFA-78 and JECFA-50 conclusions

- JECFA-78 considered information related to the link between the use of rBST and the incidence of mastitis in cattle. It was reported that no significant difference was found in the incidence of mastitis between rBST-treated and untreated cows; however, in JECFA-78 there is no analysis of the data that could support this statement.

- JECFA-78 and JECFA-50 reported that the effects of rBST on the incidence of mastitis are an animal health issue and outside the terms of reference of the Committee, and no overall conclusions on the link between the use of rBST and the incidence of mastitis in dairy cattle were formulated in the ‘Evaluation’ section.

- Assuming that the appropriate withdrawal times for antimicrobial treatments are respected, JECFA conclusions can be supported in relation to the presence of residues of antimicrobials in bulk milk, i.e. ‘that the increased potential for the presence of drug residues in milk could be managed by practices currently in use by the dairy industry and by following the drug manufacturers’ directions for use’.

- The assessments by JECFA are based on the analysis of the percentage of milk samples discarded as a result of testing for residues of antimicrobials. The presence or absence of residues of antimicrobials in milk cannot be used as an indicator of the extent of antimicrobial treatments in dairy cattle, since if withdrawal periods are respected no residues would be found in milk.
• No information is provided by JECFA on the volume of antimicrobial treatments during the period analysed, and data do not allow comparing animals, or herds, using or not using rBST. Based on this, no conclusion can be drawn on whether or not the use of rBST leads to an increase in the use of antimicrobials, or on the effect of the treatment with rBST on the presence of residues in milk from dairy cattle.

• In relation to possible development of resistant bacteria in dairy cattle, only the association between the treatment with rBST and the development of resistant mastitis pathogens is discussed. The development of resistance in other pathogenic or non-pathogenic bacteria in dairy cattle and in dairy cattle farms is not discussed. JECFA-78 interprets the absence of evidence for this association as sufficient to support the previous JECFA-50 conclusions. In the absence of such evidence, the link between the use of rBST and AMR in dairy cattle should be properly investigated before being able to provide definite conclusions.

• The assessments by JECFA only consider human health concerns related to the exposure to residues of antimicrobials in milk. They do not consider other AMR-related aspects, such as the possible development of AMR in dairy cattle and dairy cattle farms following to the treatment with antimicrobials and the possible development of AMR in humans due to exposure to pathogenic and non-pathogenic resistant bacteria originating from the cattle reservoir.

• Based on the above, AMR-related concerns are only partially considered by JECFA, and therefore the conclusions from JECFA-50, unchanged in JECFA-78, i.e. that ‘the use of rBST would not result in a higher risk to human health due to the use of antibiotics to treat mastitis’ cannot be considered valid in relation to all of the human AMR-related concerns.

4.1.3. With regard to the link between the use of rBST in dairy cattle and AMR in humans

• No specific studies are reported within JECFA reports that postulate or investigate a direct link between the use of rBST in dairy cattle, or other ruminants, and AMR in humans.

• Similarly, no such studies were identified through an additional literature search performed in the framework of this mandate (‘EFSA literature search 1’).

• Among the articles identified through this additional literature search, some discuss AMR-related issues. In particular:
  o some reviewed past data/information in relation to the possible effect of the treatment with growth hormones on the diminished ability to eliminate xenobiotics because of a reduced detoxifying capacity of the liver, which may imply longer persistence of antimicrobials in animals and animal products when treated;
  o one study discussed the potential risks posed by the presence of residues of antimicrobials in milk following to the use of rBST, analysing the same data assessed in JECFA reports and similarly concluding that the ‘use of rBST has not increased human exposure to milk antibiotic residues’.

• It would be difficult to design and execute single experiments investigating the direct link between the use of rBST in dairy cattle and AMR in humans. Rather, an indirect link should be investigated by considering and studying all the intermediate steps that may incur between the use of rBST in dairy cattle and AMR in humans.

• The intermediate steps that may incur between the use of rBST in dairy cattle and AMR in humans include a possible link between:
  o use of rBST and occurrence of mastitis in dairy cattle;
  o occurrence of mastitis and use of antimicrobials in dairy cattle;
  o treatment with antimicrobials and AMR in dairy cattle (and in dairy cattle farms);
  o AMR in dairy cattle (and in dairy cattle farms) and AMR in humans.
• The strength of the evidence with regard to each one of the four intermediate steps linking the use of rBST in dairy cattle and AMR in humans discussed above is considered to be medium to high when assessing the steps singularly.

• Therefore, assuming that:
  o treatment with rBST can lead to an increased incidence of mastitis in dairy cattle,
  o cases of mastitis are usually treated with antimicrobials,
  o use of antimicrobials can lead to development of AMR in dairy cattle (and in dairy cattle farms),
  o AMR in humans may derive from both the exposure to AMR bacteria/genes of cattle origin and residues of antimicrobials,

it is concluded that an increase of AMR in humans following to the use of rBST in dairy cattle is plausible.

Current knowledge does not allow quantifying the four links discussed, and additional studies are needed for their quantification.

4.2. Answer to Term of Reference 2 (Type of studies/data that could potentially be useful to exclude a possible link between the use of rBST and the development of AMR)

• With regard to the link between the use of rBST and the occurrence of mastitis in dairy cattle:
  o If experimental field trials are designed to investigate the level of increase of mastitis in lactating dairy cows treated with rBST compared to untreated control groups, they should involve an appropriate sample size that could allow the identification of a statistically significant effect of the treatment with rBST. These studies should be conducted in commercial settings, within the same herd or between comparable dairy herds with regard to genetics and management practices.
  o A systematic literature review to quantify the effect of the treatment with rBST on the incidence of clinical and sub-clinical mastitis in dairy cows could be carried out, according to the methodology described in the EFSA's Guidance (EFSA, 2010).

• With regard to the link between the occurrence of mastitis and the use of antimicrobials in dairy cattle:
  o additional studies should investigate whether mastitis possibly induced by the use of rBST would qualify for antimicrobial treatments.

• With regard to the link between the treatment with antimicrobials and AMR in dairy cattle (and in dairy cattle farms), the following issues should be further investigated:
  o the extent to which the administration of antimicrobials for the treatment of mastitis contributes to the overall development of AMR in dairy cattle and dairy cattle farms;
  o how much the treatments due to mastitis caused by the use of rBST in cattle can contribute to the problem;
  o the different role of topical versus systemic administration of antimicrobials to treat mastitis in the development of AMR in dairy cattle and dairy cattle farms.

• With regard to the link between AMR in dairy cattle (and in dairy cattle farms) and AMR in humans, the following issues should be further investigated:
  o the comparison of the presence of residues in milk of dairy cattle originating from animals, or farms, using or not using rBST;
  o the extent to which the cattle reservoir contributes as a source of human AMR bacteria;
Use of rBST in dairy cattle and AMR in humans

- the relative contribution of the different pathways to the transmission of AMR bacteria from the cattle reservoir to humans (e.g. cow’s milk, beef, food from other animal species, food of non-animal origin, environment, direct contact with animals, etc.).

- With regard to the association between the use of rBST and the use of antimicrobials in dairy cattle discussed by JECFA-78, data comparing the use of antimicrobials to treat mastitis in farms using and not using rBST should be collected and analysed.

- With regard to the conclusions of JECFA-78 on the association between the use of rBST and the development of AMR in dairy cattle herds:
  - in the absence of evidence, three intermediate steps should be considered, i.e. the link between the use of rBST and the occurrence of mastitis, between the occurrence of mastitis and the use of antimicrobials, and between the use of antimicrobials and the development of AMR in dairy cattle herds;
  - epidemiological studies (e.g. prospective or retrospective cohort studies, or case-control studies) should be performed to assess the association between the treatment with rBST and the development of AMR in cattle dairy herds.

- An estimation of the contribution of the use of rBST to human AMR could be obtained by combining the results obtained from:
  - studies investigating the extent to which the use of rBST may contribute to the development of AMR in dairy cattle, and
  - source attribution studies, investigating the extent to which the cattle reservoir contributes as a source of human AMR bacteria and the relative contribution of the different pathways to the transmission of AMR bacteria from the cattle reservoir to humans.

References


Campos BG, Coelho SG, Quintao AML, Rabelo E, Machado TS and Silper BF, 2011. Use of bovine somatotropine (BST) 500 mg in crossbred Bos taurus * Bos indicus cows every 12 or 14 days. A Hora Veterinaria, 30, 8–13.


Oliver SP, Boor KJ, Murphy SC and Murinda SE, 2009. Food Safety Hazards Associated with Consumption of Raw Milk. Foodborne Pathogens and Disease, 6, 793–806.


Supré K, Lommelen K and De Meulemeester L, 2014. Antimicrobial susceptibility and distribution of inhibition zone diameters of bovine mastitis pathogens in Flanders, Belgium. Veterinary Microbiology, 171, 374–381.


Abbreviations

ADI: Acceptable Daily Intake
AMR: Antimicrobial Resistance
BCS: Body Condition Score
BSE: Bovine Spongiform Encephalopathy
BST: Bovine somatotropin
CAC: Codex Alimentarius Commission
CAT: Critical Appraisal Tool
CCRVDVF: Codex Committee on Residues of Veterinary Drugs in Food
CI: confidence interval
CVMA: Canadian Veterinary Medical Association
DNA: Deoxyribonucleic Acid
ECDC: European Centre for Disease Prevention and Control
EFSA: European Food Safety Authority
ELS: Extensive literature search
EMA: European Medicines Agency
ESVAC: European Surveillance of Veterinary Antimicrobial Consumption
EU: European Union
FAO: Food and Agriculture Organization
FCM: Fat Corrected Milk
FDA: US Food and Drug Administration
IGF: Insulin-like Growth Factor
JECFA: Joint Expert Committee on Food Additives
MRL: Maximum Residue Limit
OIE: World Organisation for Animal Health
OR: Odds ratio
PICO: Population, Intervention, Comparator, Outcome
rBST/rbST: Recombinant bovine somatotropin
rBST-Zn: sometribove zinc formulation
RCT: Randomised Control Trials
rr: rate ratio
SCAHAW: EU Scientific Committee on Animal Health and Animal Welfare
SCC: Somatic Cell Count
SCVMP: Scientific Committee on Veterinary Measures relating to Public Health
SE/se: Standard error
SR: Systematic review
TATFAR: Transatlantic Taskforce on Antimicrobial Resistance
ToR: Term of reference

WHO: World Health Organization
Appendix A – Critical Appraisal of Dohoo’s and St-Pierre’s systematic reviews

Critical appraisal of Dohoo’s systematic review

The documents that have been appraised are: the report of Health Canada (1998) and the papers by Dohoo et al. (2003a, b).

The detailed appraisal is provided in Appendix B. A summary of the main weaknesses is reported in the paragraph that follows.

Main weaknesses and potential impact on the conclusions

- It is not clear whether a Protocol for the systematic review (SR) was set up *a priori*. This could have introduced a bias in case crucial decisions have been taken after looking at the results of the studies.
- Results of the screening process do not seem to be consistent with the eligibility criteria:
  - Experts could select ‘key studies’ once the screening process for relevance was ended. The criteria used for this further screening are not specified;
  - When reporting the results of the screening, the use of sentences like ‘most articles in non-research journals (were excluded)’, ‘most foreign language papers (were excluded)’ raises questions on whether the eligibility criteria have been applied consistently to all the studies retrieved (criteria for exclusion included: studies not published in peer-reviewed publications, not written in English).
  - Furthermore, there is no evidence that the screening process has been performed independently by at least two reviewers in parallel.
  Therefore the risk of ‘selection bias’ is high.
- There is no evidence that the data extraction has been performed in parallel or checked for correctness by a second reviewer. Some mistakes could have been introduced while extracting data.
- Methodological quality of the studies has not been assessed systematically and consistently. Result of the assessment has not been included in the meta-analysis. The consequence of this is a potential risk of bias in the results if some of the studies are seriously biased and they strongly influence the pooled estimates.
- Conclusions and interpretation are affected by the following issue:
  - The comparison between treated and untreated animals, as far as health outcomes are concerned, is performed using a test of difference instead of a test of equivalence. This could still be acceptable provided that results of the test are correctly interpreted. Indeed test designed in order to prove that a difference exists cannot be used to assess the complementary hypothesis, i.e. ‘absence of evidence cannot be interpreted as evidence of absence’. Conclusions drawn on health outcomes (including mastitis incidence and SCC) suffer of the above-mentioned fundamental flow. For any test that was not significant, therefore inconclusive, conclusions are reported as there was no evidence of an effect of the treatment (e.g. authors state ‘the meta-analyses of SCC data did not show much evidence of an effect of rBST’).
  This flaw affects only outcomes that are not statistically significant introducing potentially an underestimation of the risk associated with rBST.
  To illustrate this, Table A1 provides the list of mastitis related outcomes, with number of studies, estimate of the effect, p-value of the test, whether they can be affected by the issue.
Table A1: Mastitis-related outcomes (data from Dohoo et al., 2003b)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Number of studies</th>
<th>Estimate of the effect (treated versus untreated)</th>
<th>p-value (random effect model)</th>
<th>Outcome potentially affected by the issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical mastitis (Incidence rate ratio)</td>
<td>18</td>
<td>1.24</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Clinical mastitis (rate ratio)</td>
<td>29</td>
<td>1.27</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Somatic Cell Count — linear score</td>
<td>8</td>
<td>0.08</td>
<td>0.15</td>
<td>X</td>
</tr>
<tr>
<td>Somatic Cell Count — log10</td>
<td>11</td>
<td>0.02</td>
<td>0.25</td>
<td>X</td>
</tr>
<tr>
<td>Prevalence of subclinical mastitis (rate ratio)</td>
<td>7</td>
<td>1.07</td>
<td>0.60</td>
<td>X</td>
</tr>
</tbody>
</table>

For instance, the result of the test for the Somatic Cell Count linear score (SCC – linear score) should be interpreted as inconclusive (p-value above 0.05), not as ‘evidence of absence of an effect’. This could be due to the limited number of studies assessing this outcome or to a true absence of the effect. However it is not possible to assess it.

- More details are reported in Appendix B.

Overall assessment

- In summary the SR from Dohoo et al. (2003a, b) suffers from methodological flaws that potentially introduced a risk of bias in the results.
- The first main issue relates to the study selection process that allowed exclusion of studies in a subjective way. As a consequence the results of the SR could have changed significantly and it is not possible to predict in which direction.
- Secondly, the results of the tests ending up being statistically non-significant were inappropriately interpreted as ‘evidence of absence of an effect’ (i.e. no difference in the outcomes between treated and untreated cows). A proper interpretation would have been that results are inconclusive and no firm conclusions can be drawn. This led to a potential underestimation of the risk of adverse health effects of rBST (i.e. some of the outcomes could be truly not different, for some others test could be not enough powerful to detect a difference).

Critical appraisal of St-Pierre’s systematic review

The document that has been appraised is St-Pierre et al. (2014). The detailed appraisal is provided in Appendix C. A summary of the main weaknesses is reported in the paragraph that follows.

Main weaknesses and potential impact on the conclusions

- It is not clear whether a Protocol for the SR was set up a priori. This could have introduced a bias in case crucial decisions have been taken after looking at the results of the studies.
- Results of the screening process do not seem to be consistent with the eligibility criteria:
  - studies meeting eligibility criteria were added by experts after the process of screening for relevance ended;
  - the list of excluded studies and motivation for exclusion is given only for a subset of studies (those included in Dohoo SR). Therefore it is not possible to assess the compliance of the screening process to the eligibility criteria;
  - the number of ‘studies identified through a database search’ as reported in Figure 1 is not plausible given the search string (69 is too small);
Furthermore there is no evidence that the screening process has been performed independently by at least two reviewers in parallel.

Therefore the risk of ‘selection bias’ is high.

- Methodological quality of the studies has not been assessed and, therefore, not included in the meta-analysis. The consequence of this is a potential risk of bias in the results if some of the studies are seriously biased and they strongly influence the pooled estimates.

- Publication bias was not addressed. As far as the risk of mastitis and other health and welfare outcomes are concerned, the publication bias would probably result in an overestimation of the risk (studies with positive outcomes have higher probability to be published).

- Conclusions do not reflect fully the results of the analysis carried out:
  - Conclusions are drawn not only on the basis of the results of the meta-analysis but using data extracted from other sources too. It is not specified how these additional sources were identified;
  - the interpretation of the results of the test for heterogeneity as far as the mastitis is concerned is inappropriate. The only interpretation possible is that studies exhibit lack of homogeneity in the results that could be due to different reasons (e.g. study setting, seasonality, population etc.). In no way conclusions can be drawn on dependency/independency of the mastitis risk on rbST–Zn administration.
  - The comparison between treated and untreated animals, as far as health outcomes are concerned, is performed using a test of difference instead of a test of equivalence. This could still be acceptable provided that results of the test are correctly interpreted. Indeed test designed in order to prove that a difference exists cannot be used to assess the complementary hypothesis, i.e. ‘absence of evidence cannot be interpreted as evidence of absence’. Conclusions drawn on health outcomes (including mastitis incidence and SCC) suffer of the above-mentioned fundamental flaw. For any test that was not significant, therefore inconclusive, conclusions are reported as there was no evidence of an effect of the treatment (e.g. authors state ‘the meta-analyses of SCC data did not show much evidence of an effect of rbST’).

This flaw affects only outcomes that are not statistically significant introducing potentially an underestimation of the risk associated with rbST.

To illustrate this, Table A2 provides the list of mastitis related outcomes, with number of studies, estimate of the effect, p-value of the test, whether they can be affected by the issue.

**Table A2: Mastitis-related outcomes (data from St-Pierre et al., 2014)**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Number of studies</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>p-value (random effect model)</th>
<th>Outcome potentially affected by the issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical mastitis</td>
<td>14</td>
<td>1.249</td>
<td>0.942 - 1.655</td>
<td>0.122</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Number of studies</th>
<th>Mean (se) response difference</th>
<th>95% Confidence interval</th>
<th>p-value (random effect model)</th>
<th>Outcome potentially affected by the issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic Cell Count — log10</td>
<td>9</td>
<td>−0.034 (0.055)</td>
<td>−0.141 to 0.074</td>
<td>0.540</td>
<td>X</td>
</tr>
</tbody>
</table>

For instance, the result of the test for the Somatic Cell Count in logarithmic scale (SCC − log10) should be interpreted as inconclusive (p-value above 0.05), not as ‘evidence of
absence of an effect’. This could be due to the limited number of studies assessing this outcome or to a true absence of the effect. However it is not possible to assess it.

- More details are reported in Appendix C.

**Overall assessment**

- In summary the SR from St-Pierre et al. (2014) suffers from methodological flaws that potentially introduced a risk of bias in the results.

- The first main issue relates to the study selection process that is not transparently reported and, in addition, allowed inclusion of studies in a selective way. As a consequence the results of the SR could have changed significantly and it is not possible to predict in which direction.

- Secondly, the results of the tests ending up being statistically non-significant were inappropriately interpreted as ‘evidence of absence of an effect’ (i.e. no difference in the outcomes between treated and untreated cows). A proper interpretation would have been that results are inconclusive and no firm conclusions can be drawn. This led to a potential underestimation of the risk of adverse health effects of rBST (i.e. some of the outcomes could be truly not different, for some others test could be not enough powerful to detect a difference).
Table A3: Comparison of the potential sources of differences in the conclusions between the Dohoo’s and St-Pierre’s systematic reviews (SRs)

<table>
<thead>
<tr>
<th>Objective</th>
<th>Dohoo’s SR</th>
<th>St-Pierre’s SR</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>The objective of this study was to summarize information available in the literature on the effects of recombinant bovine somatotropin (rBST) on both measures of productivity and health in dairy cattle. More specifically on milk production, milk composition, dry matter intake, and body condition score (BCS). A companion article will present the results of the effects of rBST on health, reproductive performance, and culling parameters.</td>
<td>To provide an updated evaluation of the efficacy and safety of sometribove zinc suspension (rbST-Zn), a form of recombinant bovine somatotropin, in lactating dairy cows. As for the ‘update’ they refer to the SR performed by Health Canada. Formed at the request of Health Canada, the mandate for the CVMA expert panel was to determine whether the use of rbST in accordance with its label directions would increase milk production without resulting in serious health problems that could not be adequately controlled by cattle management practices implemented at that time.</td>
<td>Objective of the SR is broader in Dohoo. All formulations and all administration routes and doses are included.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population</th>
<th>Dairy cows</th>
<th>Lactating dairy cows</th>
<th>Larger population considered in Dohoo</th>
</tr>
</thead>
</table>


<p>| Search string | The searches always included ‘(rBST or somatotropin or somatotrophin or growth hormone) and (bovine or cow or cows or cattle or dairy)’. For each topic area the following words were used along with the above using ‘and’ as a connector. (Note an * indicates that all words starting with the identified letters would be found. For example, ‘cull*’ would locate cull, culls, culled, culling, etc.) o Efficacy - ‘(efficacy or response or milk or production or yield)’ o Udder Health - ‘(udder or mastitis or mammary)’ o Reproduction - ‘(reproduction or pregnancy or calving or abortion or conception or gestation or birth or calf health)’ o Feet and Legs - ‘(feet or foot or leg or legs or hoof or hooves or joint or joints or lame* or | bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, bovine growth hormone (no syntax provided) | Search strategy is very sensitive in St-Pierre more focused in Dohoo. |</p>
<table>
<thead>
<tr>
<th><strong>Dohoo’s SR</strong></th>
<th><strong>St-Pierre’s SR</strong></th>
<th><strong>Comments</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>knee or knees or laminitis)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o General Health was broken into two categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Digestive Disorders - ‘(digest* or disorder or disorders or diarrhea or bloat or indigest* or off feed or ketosis or acetonemia)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Other - ‘(immun* or metabol* or disorder or disorders or reaction or reactions or inject* or medica* or treatment or treatments or ill* or general health or lesion or lesions)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Culling - ‘(cull*)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Drug Interactions - ‘(drug* or interaction or interactions or prostaglandin or prostaglandins or side effect or effects or reaction or reactions)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Nutrition - ‘(nutrition* or feed* or rotation or rotations or nutrient*)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Body Condition - ‘(BCS or body condition score or weight or condition)’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o Animal Welfare - ‘(welfare or concerns or wellbeing or behaviour)’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Intervention**

- rbST formulations: All
- Doses/administration routes: All

- rbST formulations: only those approved for use in US (sometribove zinc suspension rbST-Zn)
- Doses/administration routes: only those labelled by producer

Investigation of the substance in all its formulations and route/doses of administration in Dohoo. Investigation of only sometribove zinc suspension rbST-Zn administered according to label in St-Pierre.
## Appendix B — Details of the critical appraisal of Dohoo’s Systematic Review

### Systematic Review (SR) Critical Appraisal Tools (CAT)

<table>
<thead>
<tr>
<th>#</th>
<th>Appraisal question</th>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Review question and eligibility criteria for study selection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><strong>Was the review question clearly formulated?</strong>&lt;br&gt;Formulating the review question means properly translating the review question into ‘PICO’ elements (Population(s); Intervention(s); Comparator(s); Outcome(s) (including adverse effects where relevant)):</td>
<td>Health Canada Report (Health Canada, 1998)&lt;br&gt;Review the scientific data used by the Bureau of Veterinary Drugs to determine that Nutrilac (rbST) when used in accordance with its label directions will increase milk production without resulting in serious health problems which cannot be adequately controlled by current cattle management practices.</td>
<td>□ Definitively appropriate&lt;br&gt;□ Probably appropriate&lt;br&gt;□ Probably not appropriate&lt;br&gt;□ Definitively not appropriate&lt;br&gt;□ Not applicable</td>
<td>Three of the four PICO elements are described:&lt;br&gt;P: dairy cattle&lt;br&gt;I: rbST&lt;br&gt;C: Comparator not defined&lt;br&gt;O: effects on productivity and health. List of specific outcomes provided: milk production, milk composition, dry matter intake, and body condition score (BCS). Health, reproductive performance, and culling parameters. Inconsistency between objectives stated in the report and the paper.</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Were the eligibility criteria related to study characteristics appropriate and clearly defined a priori?</strong>&lt;br&gt;Appropriately defining a priori the study(s) that shall be selected for inclusion in the review, implies that they:</td>
<td>Dohoo1: References were removed if:&lt;br&gt;• the title indicated that the study pertained to a species other than dairy cattle,&lt;br&gt;• pertained to the use of the drug in ages other than lactating cows,&lt;br&gt;• were specifically related to the use of the product in tropical environments,&lt;br&gt;• were related to the mechanism of action of the drug or to the potential human health effects,&lt;br&gt;• were not published in peer-reviewed publications,&lt;br&gt;• they were not written in English.</td>
<td>□ Definitively low risk of bias&lt;br&gt;□ Probably low risk of bias&lt;br&gt;□ Probably high risk of bias&lt;br&gt;□ Definitively high risk of bias&lt;br&gt;□ Not applicable</td>
<td>Only Randomised Control Trials (RCT) were considered and justification for exclusion of observational studies is provided in the report. This criterion was not included in the eligibility criteria though. Overall eligibility criteria are fine. It is not clear whether they have been defined a priori.</td>
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</table>
# Appraisal question | Information as reported | Appraisal | Rationale for the appraisal
--- | --- | --- | ---
Studies, or e.g. allocation concealment was also considered as inclusion criteria (for other study designs alternative items will be relevant). Study-specific elements should be considered such as e.g. length of follow-up. | Process, to create the literature base upon which the review was carried out. | □ Definitively appropriate | 

If the eligibility criteria for study selection are not defined a priori, there is a risk of introducing a bias due to selective inclusion/exclusion of studies influenced by the results of such studies.

**Dohoo1** - **Section Outcome parameters evaluated/data extraction**
Data were only extracted from reports of randomized clinical trials although the studies need not have been conducted in a blind manner.

**Health Canada Report - Section 3.5.2**
All of the rbST studies included in meta-analyses employed the same general study design (randomized clinical trial).

3. **Did the reviewers avoid the use of report characteristics as eligibility criteria? If applied, were these criteria defined a priori?**
   - In principle all studies should be considered both from peer-reviewed studies and grey literature. If limits are applied such as publication type, language, years, geographical/political area, etc... these should be documented, justified and defined *a priori*.
   - See above.

* □ Definitively appropriate
  □ Probably appropriate
  □ Probably not appropriate
  □ Definitely not appropriate
  □ Not applicable

Some eligibility criteria are based on language and type of publication. Among grey literature only set of study reports submitted by Monsanto to Health Canada as part of the drug approval process have been included. No justifications have been given for the limitation to report time (1984-1991) but overall it seems acceptable since it covers a significant period of time before acceptance of the drug (1993).
### Use of rBST in dairy cattle and AMR in humans

**Appraisal question**

B. Search process

4. **Was the extensive literature search (ELS) performed in an appropriate way?**
   Please refer to ELS CAT and give an overall appraisal of the appropriateness of the ELS done.

- **Information as reported**
  
  
  The searches always included ‘(rBST or somatotropin or somatotrophin or growth hormone) and (bovine or cow or cows or cattle or dairy)’. For each topic area the following words were used along with the above using ‘and’ as a connector. (Note an * indicates that all words starting with the identified letters would be found. For example, ‘cull*’ would locate cull, culls, culled, culling, etc.)

  - Efficacy - ‘(efficacy or response or milk or production or yield)’
  - Udder Health - ‘(udder or mastitis or mammary)’
  - Reproduction - ‘(reprodu* or pregnancy or calving or abortion or conception or gestation or birth or calf health)’
  - Feet and Legs - ‘(feet or foot or leg or legs or hoof or hooves or joint or joints or lame* or knee or knees or laminitis)’
  - General Health was broken into two categories
  - Digestive Disorders - ‘(digest* or disorder or disorders or diarrhea or bloat or indigest* or off feed or ketosis or acetonemia)’
  - Other - ‘(immun* or metabol* or disorder or disorders or reaction or reactions or inject* or medica* or treatment or treatments or ill* or general health or lesion or lesions)’
  - Culling - ‘(cull*)’
  - Drug Interactions - ‘(drug* or interaction or interactions or prostaglandin or prostaglandins or side effect or effects or reaction or reactions)’
  - Nutrition - ‘(nutrition* or feed* or rotation or rotations or nutrient*)’
  - Body Condition - ‘(BCS or body condition score or weight or condition)’
  - Animal Welfare - ‘(welfare or concerns or wellbeing or behaviour)’

- **Appraisal**
  
  - □ Definitively appropriate
  - □ Probably appropriate
  - □ Probabably not appropriate
  - □ Definitively not appropriate
  - □ Not applicable

  The search strategy is provided with details and overall is appropriate to meet the objectives of the SR.
  
  Some spelling variants have been used. Some additional synonym could have been added for the substance.
  
  The combination of databases seems to cover the topic appropriately.

  For a detailed appraisal look at the CAT for ELS.

### C. Study selection process

5. **Were preventative steps taken to minimise bias and errors in the study selection process?**

- **Information as reported**
  
  Health Canada Report: Section 3.6

- **Appraisal**
  
  - □ Definitively appropriate
  - □ Probably

  Not clear whether two-step process was performed (screening abstract and title)
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<td>Study selection is normally a 2-step process: (1) rapid assessment of titles and abstracts to exclude obviously irrelevant records and (2) examination of full-text documents. The assessment should be done evaluating:</td>
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<td>• whether the selection was carried out by at least 2 mutually independent reviewers, in parallel. It is also acceptable to have 2 reviewers in the 2nd step only (i.e. examination of full-text documents), provided that in the first step an over-inclusive approach was applied;</td>
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<td>• the presence of a clearly defined consensus procedure for disagreements (e.g. discussion or involvement of another reviewer).</td>
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<td>Ideally the eligibility criteria should be pilot-tested by the reviewers on a subset of records and re-formulated if prone to misinterpretation.</td>
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<td>□ Probably not appropriate</td>
<td>No mention of procedures for resolving disagreement in the reviewing process.</td>
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<td>□ Definitively not appropriate</td>
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<td>□ Not applicable</td>
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<td>and how many reviewers screened papers for relevance in parallel.</td>
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Use of rBST in dairy cattle and AMR in humans

Appraisal question 6. Were the results of the study selection process consistent with the eligibility criteria previously defined?

Assessment should be done evaluating the following items:
- total number of records identified;
- number of records and studies excluded at each stage;
- list of excluded studies, and primary reasons for exclusion after examination of full-text documents (e.g. not relevant, duplicate study, etc.).

Assessment is easier when the necessary information is provided in a flow diagram.

Information as reported: Health Canada Report – Section 3.6

A total of 1,777 references were identified, using the above literature search strategies. All studies reported in the Monsanto submission were added to the reference list. References were then deleted if any of the following criteria applied to the reference: 1. non-bovine species (e.g. relating to dairy goats); 2. beef cattle papers; 3. use in calves; 4. use in growing heifers; 5. pre-parturition use; 6. use in tropical environments; 7. mechanism of action (as opposed to effects); 8. effects on human health; 9. most commentaries, news articles, books; 10. most conference proceedings before 1995; 11. most articles in non-research journals; 12. most Agricultural Experimental Station Bulletin publications; 13. most foreign language papers. Following deletion of the papers meeting the criteria above 242 ‘relevant’ articles remained (Appendix 4). Each Panel member reviewed the list and identified studies which they felt were ‘key’ to the review. A total of 83 reports were ultimately identified as ‘key’ and these are listed separately in Appendix 5. They included 59 reports in the published literature and 24 studies reported only in the Monsanto submission provided by HC.

Dohoo1 – Section ‘Material and methods: Literature review’

The resulting list of 242 potentially relevant articles was reviewed by all of the panel members and a subset consisting of previous review papers plus all articles that any panel member felt was likely to contain results from randomized clinical trials was identified. These manuscripts were then combined with the set of study reports submitted by Monsanto to Health Canada as part of the drug approval process, to create the literature base upon which the review was carried out. Following deletion of the papers meeting the criteria above 242 ‘relevant’ articles remained (Appendix 4).

Dohoo1 – Section ‘Results and discussion: Literature review’

From the original 1,777 articles identified by the literature review, 242 were considered potentially relevant and were examined by all panel members. Of these, 60 were identified as useful review articles or reports likely to contain data from a randomized controlled clinical trial. These manuscripts were combined with 26 study reports submitted by Monsanto as part of their submission to Health Canada to form the literature base for this review. Of these 86 reports, 53 provided data for use in 1 or more meta-analyses.

Appraisal

□ Definitively appropriate
□ Probably appropriate
□ Probably not appropriate
□ Definitively not appropriate
□ Not applicable

Rationale for the appraisal

The number and list of studies identified in each step of the screening process is provided. Detailed reasons for exclusion are not provided for all the steps of the screening process. Key studies selected by Panel experts after the process of screening for relevance ended. Not clear according to which criteria. The use of sentences like ‘most articles in non-research journals’, ‘most foreign language papers’ raises questions on whether the eligibility criteria have been applied consistently to all the studies retrieved.
# Appraisal question | Information as reported | Appraisal | Rationale for the appraisal
---|---|---|---
7. **Was data extraction carried out appropriately and adequately? Was the approach defined a priori?**

Assessment should be done evaluating:

- if data extracted are relevant for the PICO elements;
- if relevant data on study subjects, intervention and control groups were identified and extracted;
- if relevant study outcomes and related measures of precision were extracted (i.e. either summary data on each intervention group or effect estimates with confidence intervals) and, if not available, proxy information was used;
- if sources of ‘clinical’ heterogeneity (i.e. related to the PICO elements) across the included studies were explored and the relevant data extracted;
- if sources of methodological heterogeneity (i.e. related to the study design characteristics) across the included studies were explored and the relevant data extracted (e.g. parallel vs. cross-over, sample size, follow-up period);
- if any assumptions related to data extraction were justified;
- if data extraction was harmonised across the different studies from which the data were extracted (e.g. measurement unit, data transformation and calculations applied when information were missing but could be derived are detailed in HC report and performed by a single person).

**Health Canada Report – Session 3.7 ‘Data extraction’**

The 83 key studies identified above were divided among Panel members for review and data extraction. The review and data extraction process involved two steps. First, basic information about the study (e.g. location, number of cows/herds, dose of rBST, etc.) was recorded on a cover sheet for each study. At the bottom of each cover sheet observations, comments and general conclusions about the study were recorded as the reviewer felt was appropriate. The complete set of these cover sheets is included in Appendix 7. For the data extraction portion of the review process, the following key parameters were identified by the Panel.

Data were extracted in relation to eight domains: efficacy, udder health, reproduction, feet and legs, general health, body condition score, culling, nutrition.

The outcomes of interest for the udder health domain were: clinical mastitis (cm) incidence rate ratio, cm- incidence rate difference, cm-risk ratio, prevalence-quarter intra-mammary infection, prev-SCC-log, prev-SCC-lin, discard milk days.

If a study reported quantitative data for any of these key parameters the following information was recorded (if available):

- the parameter of interest;
- the standard error or confidence interval of the parameter;
- the P value from the test of significance of the treatment effect;
- whether or not the parameter estimate had been adjusted for level of milk production.

- □ Definitively appropriate
- □ Probably appropriate
- □ Probably not appropriate
- □ Definitively not appropriate
- □ Not applicable

List of relevant data to extract and instructions on how to extract were provided to experts. They were defined before starting the data extraction. All the PICO elements extracted. Transformations and calculations applied when information were missing but could be derived are detailed in HC report and performed by a single person. Information on sources of funding in the individual studies were extracted. Sources of ‘clinical’ and methodological heterogeneity across the included studies were explored and the relevant data extracted.
Use of rBST in dairy cattle and AMR in humans

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<td>8.</td>
<td><strong>Were preventative steps taken to minimise bias and errors in the data extraction process?</strong></td>
<td>Health Canada Report – Section 3.7 'Data extraction’</td>
<td>□</td>
<td>Process to check correctness of data extraction process and consensus procedure not mentioned. Clear instructions provided to the experts. Harmonization guaranteed. However, there is no duplication in the process.</td>
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<td></td>
<td>Assessment should be done by evaluating:</td>
<td>The 83 key studies identified above were divided among Panel members for review and data extraction</td>
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<td>• if data extraction was carried out in parallel by at least two mutually independent reviewers;</td>
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<td>• the presence of a clearly defined consensus procedure for disagreements (e.g. discussion or involvement of another reviewer);</td>
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<td>• the presence of a procedure for obtaining and/or confirming data from researchers;</td>
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<td>• if duplicate studies were identified and data extracted only once, with appropriate explanations.</td>
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<td>Ideally the data extraction forms should be pilot-tested by the reviewers on a subset of records and improved if prone to misinterpretation.</td>
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</table>
# Appraisal question | Information as reported | Appraisal | Rationale for the appraisal
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9. Was the methodological quality of the studies included in the review appropriately and adequately appraised? Was the approach defined a priori? | Health Canada Report – Section ‘3.5.4 Study Quality’

While it is possible to include subjective assessments of study quality in a meta-analysis, this was not done in this review

Health Canada Report – Section ‘3.7 Data extraction’

At the bottom of each cover sheet observations, comments and general conclusions about the study were recorded as the reviewer felt was appropriate | □ Definitively appropriate
□ Probably appropriate
□ Probably not appropriate
□ Definitively not appropriate
□ Not applicable | Appraisal of methodological quality done in an informal and not harmonised way. Each experts used different criteria to evaluate quality of the studies. The results of this narrative appraisal were not used in the meta-analysis (e.g. performing sensitivity analysis or sub-group analysis).

The elements to consider for appraising the methodological quality of the studies included in the review vary depending upon the study designs. Different critical appraisal tools are available and all should be considered in view of the subject of the review and, if needed, adapted.

---

10 A tool for appraising RCTs in the EFSA context is the SAS Critical Appraisal Tool for RCTs (to be published in EFSA’s website).
Use of rBST in dairy cattle and AMR in humans

10. Were preventative steps taken to minimise bias and errors when appraising the methodological quality of the studies included in the review?
Assessment should be done evaluating:
• if quality assessment was carried out in parallel by at least two mutually independent reviewers;
• the presence of a clearly defined consensus procedure for disagreements (e.g. discussion or involvement of another reviewer).

Ideally the appraisal tool should be pilot-tested, also to maximise consistency of application between different reviewers.

F. Data analysis and synthesis of results

11. Was the analysis and synthesis of the individual effect estimates properly undertaken? Was the approach defined a priori?
- If a meta-analysis was carried out, it should be assessed if it was appropriate to calculate the pooled estimate;
- Meta-analysis should be appraised evaluating:
  a) if the effect measure chosen was appropriate for the data type of the outcomes measured in the individual studies (e.g. for continuous variables, differences of means; for dichotomous variables, risk ratios);
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<td>b)</td>
<td>if the appropriate pair-wise comparisons of intervention groups were made; ¹¹</td>
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<td>c)</td>
<td>if the units of analysis were correctly identified (i.e. the analysis must take into account the level at which randomisation occurred as in most circumstances the number of observations in the analysis should match the number of ‘units’ that were randomised). Thus carefulness is required for study designs like cluster-randomised trials, cross-over trial or multiple sites trials);</td>
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<td>d)</td>
<td>if statistical heterogeneity was appropriately identified and quantified (e.g. Q test, I²);</td>
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<td>e)</td>
<td>if a consistent model was chosen and justified (i.e. Fixed Effect Model/Random Effect Model).</td>
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<td>f)</td>
<td>if missing data, when existing (i.e. entire studies, outcomes, summary data for an outcome, individual participants from summary data) were properly handled and the related assumptions clarified;</td>
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- If the results could not be pooled using meta-analysis, it should be appraised if:

¹¹ From the Cochrane Handbook: 'The comparisons addressed in the review should relate clearly and directly to the questions or hypotheses that are posed when the review is formulated' and can be translated into the following questions: - What are the experimental and control (comparator) interventions of interest? - Does the intervention have variations (e.g. dosage/intensity, mode of delivery, personnel who deliver it, frequency of delivery, duration of delivery, timing of delivery)?
Use of rBST in dairy cattle and AMR in humans

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<td>o a systematic approach was applied, i.e. the same elements were described for each study; o the studies were organised in groups or clusters, to enable identification of patterns in results (for this purpose tables and graphics - i.e. forest plots, can be provided).</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitively not appropriate □ Not applicable</td>
<td></td>
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<td></td>
<td>• If the approach to data analysis and synthesis was defined a priori.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitively not appropriate □ Not applicable</td>
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<td>12.</td>
<td>If there was the need or opportunity to perform additional analyses (e.g. sensitivity or subgroup analyses, meta-regression), were they performed? Was the approach defined a priori? Assessment should be done evaluating: • if, when enough data were available for subgroup analysis or meta-regression, such analyses were performed. The reasons for performing or not performing the analyses should be explained and the results clearly discussed; • if sensitivity analysis was conducted to test the robustness of the results with respect to any assumptions and decisions that were made in the review (e.g. related to the search, the eligibility criteria, the approach taken to handle missing data, the methodological quality of the</td>
<td>For each outcome parameter for which there were several valid estimates available from the literature, a minimum of 4 meta-analyses were carried out. First, a fixed effect meta-analysis [...] was carried out using data only from studies in which sometribove had been the product used..... Subsequently, a random effects analysis based on the method of DerSimonian and Laird was carried out.... A 2nd pair of analyses (fixed and random effects) was subsequently carried out using all of the studies (regardless of rBST formulation) in which the parameter had been reported. A 3rd set of analyses was carried out using studies based on formulations other than sometribove. In addition, separate meta-analyses were carried out for the different age groups of cows (primiparous versus multiparous), where warranted....... Meta-regression analyses (7) were used to evaluate the effects of product formulation (sometribove or other formulations), parity of cows (primiparous, multiparous, or all combined), study size (number of cows), study precision (standard error of estimate), publication source (peer-reviewed or company report), duration of treatment (days), and expected daily dosage (mg/d) on each outcome of interest. These analyses used a weighted regression to determine if there was any evidence of a linear relationship between the observed result and the factor being investigated (study size). Each factor was investigated separately as there were too few studies for any of the outcomes of interest to attempt multivariable analyses.</td>
<td></td>
<td>Not clear whether sensitivity and sub-group analysis planned a priori. The sub-group analysis and meta-regression were performed in order to assess impact on the results of different factors.</td>
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### # Appraisal question | Information as reported | Appraisal | Rationale for the appraisal
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study, sources of funding of the individual studies, etc.);

- if the approach (i.e. when to perform a sub-group and/or sensitivity analysis and how) was defined *a priori.*

#### G. Reporting bias

**13. Was risk of publication bias addressed? Was the approach defined *a priori?***

Risk of publication bias can be addressed in the review by using a combination of graphical aids (e.g. funnel plot, other available tests) and/or statistical tests (e.g. Egger regression test). The approach should be defined *a priori.*

For assessing publication bias, the authors of the review could also consider the source of funding of the individual studies included in the review.

Under this appraisal item other types of reporting biases could be assessed, e.g.:

- Publication bias: the publication or non-publication of research findings, depending on the nature and direction of the results;
- Time lag bias: the rapid or delayed publication of research findings, depending on the nature and direction of the results;
- Multiple (duplicate) publication bias: the multiple or singular publication of research findings, depending on the nature and direction of the results.

*Dohoo2 – Session ‘Material and methods – meta-analysis’*

The possibility of publication bias influencing the study results was evaluated using both Begg’s (4) and Egger’s (5) tests. The influence of individual studies on the overall results was evaluated using an influence plot. All analyses were carried out using a statistical program (Stata, Version 7; Stata Corporation, College Station, Texas, USA) (6).

*Dohoo2 – Session ‘Results and discussion – general results’*

Publication bias was not likely a serious concern in these meta-analyses for 3 reasons. Firstly, both published and unpublished results (company reports) were included in the analyses. Secondly, most of the data on health-related parameters was derived from studies designed primarily to evaluate the effects of rBST on production parameters. Consequently, lack of a significant effect for a health outcome would not likely have influenced the decision about publication. Finally, lack of significance of a health effect resulting from treatment with rBST would probably have been considered a ‘good’ result by most authors, so it would not have reduced the probability of publication. Nevertheless, publication bias was evaluated using standard techniques but, for the sake of brevity, results of this evaluation are only presented in subsequent sections if evidence of this bias was observed.
### Appraisal question

- Location bias: the publication of research findings in journals with different ease of access or levels of indexing in standard databases, depending on the nature and direction of results;
- Citation bias: the citation or non-citation of research findings, depending on the nature and direction of the results;
- Language bias: the publication of research findings in a particular language, depending on the nature and direction of the results;
- Outcome reporting bias: the selective reporting of some outcomes but not others, depending on the nature and direction of the results.

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<td>direction of the results;</td>
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<td>Location bias: the publication of research findings in journals with different ease of access or levels of indexing in standard databases, depending on the nature and direction of results;</td>
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<td>Citation bias: the citation or non-citation of research findings, depending on the nature and direction of the results;</td>
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<td>Language bias: the publication of research findings in a particular language, depending on the nature and direction of the results;</td>
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<td>Outcome reporting bias: the selective reporting of some outcomes but not others, depending on the nature and direction of the results.</td>
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| 14 | Did the conclusions reflect the results of the review and any limitation in the process? | Assessment should be done evaluating if the conclusions took into account the following aspects:  
- the pooled and/or individual studies estimates (e.g. via appropriate forest plot);  
- if performed, the results of the additional analyses;  
- the results of the assessment of methodological quality of the included studies.  
- any limitations in the process, e.g. limitations of the search, limited number of reviewers involved in the selection and data extraction process, etc.  

If the quality of the body of evidence was evaluated in the review, this should be reflected by the conclusions.  

Dohoo2 – Session ’Results and discussion – Udder health: subclinical mastitis  
In general, the meta-analyses of SCC data did not show much evidence of an effect of rBST.   
Dohoo2 – Session ’Results and discussion – Other health problems (p. 261)  
Two other specific health issues, which were considered in more detail, were the occurrence of injection site reactions and the effect of rBST on metabolic diseases.  |

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<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definitively appropriate</td>
<td>Conclusions reflect the results except for the assessment of health outcomes (including mastitis incidence) where there is a fundamental issue. Absence of evidence is not evidence of absence. Therefore test designed in order to prove that a difference exists cannot be used to assess the complementary hypothesis (i.e. no differences exist, meaning e.g. that incidence of mastitis is not different in treated and untreated cows). The consequence is that if test is not able to demonstrate that a difference exists it has to be considered inconclusive whereas in the SR conclusion it is reported as there is no concern.</td>
</tr>
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<td></td>
<td>Probably appropriate</td>
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<td></td>
<td>Probably not appropriate</td>
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<tr>
<td></td>
<td>Definitely not appropriate</td>
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<tr>
<td></td>
<td>Not applicable</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>15</th>
<th>If a protocol was provided, are appropriate justifications given for any described deviations from the protocol?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definitively appropriate</td>
<td>No clear whether protocol provided</td>
</tr>
<tr>
<td></td>
<td>Probably appropriate</td>
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<td>Probably not appropriate</td>
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<td></td>
<td>Definitely not appropriate</td>
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<tr>
<td></td>
<td>Not applicable</td>
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</tbody>
</table>

---
Use of rBST in dairy cattle and AMR in humans

I. Additional comments

16. Add here any aspects that should be outlined and are not covered above.

- The Canadian Veterinary Medical Association (CVMA) expert panel was composed by domain experts, only: collectively they had expertise in epidemiology, dairy health management, dairy nutrition, livestock management and animal welfare, and clinical pharmacology.
- There was a supervision of the declarations on conflicts of interest from Health Canada who was responsible for reviewing the declarations and determining that no Panel members had conflicts of interest.

Extensive Literature Search Critical Appraisal Tools

A. Assessing the search strategy

1. Was the review question appropriately translated into search concepts?

   Assessment should be done considering whether the review question was clearly defined and translated into correct search concepts.
   In many cases where the search aims to retrieve primary research studies, the review question should be translated into clear and appropriate key elements (i.e. PICO/PECO, PIT, and PO) combined (where necessary or possible) with study design.

   The search was using P and I and then combining them with appropriate search concepts.

2. Was the search string an optimal combination of the search concepts for sensitivity and precision?12

   Assessment should consider whether:
   - ‘too many’ search concepts were used (for example, in a PICO question, if all the four key elements have been used and the number of results yielded is very limited this indicates potentially low sensitivity);
   - any of the search concepts were too narrow or too broad (ideally separate searches should be conducted for each considered search concept and then combined. All preliminary searches shall be reported).
   The process for defining the definitive search should be documented and discussed. For instance, if there were three rounds of comments on a

12 Precision refers to the proportion of relevant records among all the records retrieved by a search strategy (relevant records retrieved/all records retrieved). Sensitivity refers to the proportion of relevant records retrieved by a search strategy (relevant records retrieved/total relevant records) (Glanville et al., 2014. Technical Manual for Performing Electronic Literature Searches in Food and Feed Safety).
Use of rBST in dairy cattle and AMR in humans

<table>
<thead>
<tr>
<th>#</th>
<th>Appraisal question</th>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>draft strategy the number of rounds should be reported and the search agreed in the final round should be documented;</td>
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<td></td>
<td>• the search appears to retrieve too many or too few records.</td>
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<tr>
<td>3.</td>
<td><strong>Were the appropriate free-text terms (i.e. terms in the title and abstract) identified for each search concept?</strong> Assessment should consider whether, for each search element, the search included:</td>
<td>Section 3.6 of report</td>
<td>□ Definitively appropriate</td>
<td>Probably the search could have been broadened introducing some additional synonyms for the substance (e.g. &quot;sometribove&quot;). However, overall the strategy appears appropriate.</td>
</tr>
<tr>
<td></td>
<td>• all possible synonyms (e.g. welfare, wellbeing, etc.);</td>
<td></td>
<td>□ Probably appropriate</td>
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<tr>
<td></td>
<td>• related terms, e.g. pesticide, pest control, etc.;</td>
<td></td>
<td>□ Probably not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• acronyms, e.g. Bluetongue, BTV, etc. If an acronym or abbreviation is used, a full text term or substantial part of a full text term should also be present;</td>
<td></td>
<td>□ Definitively not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• spelling variants, e.g. behavior, behaviour; anaemia / anemia;</td>
<td></td>
<td>□ Not applicable</td>
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<tr>
<td></td>
<td>• old and new terminology, e.g. aspartame / Phenylalanine;</td>
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<td></td>
<td>• brand and generic names, e.g. imidacloprid, Gaucho;</td>
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<tr>
<td></td>
<td>• lay and scientific terminology e.g. aspartame /1-Methyl N-L-alpha-aspartyl-L-phenylalanate.</td>
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<tr>
<td></td>
<td>• common typos (e.g. mitomycin/mitomicin)</td>
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<td></td>
<td>In addition, the following aspects should be considered:</td>
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<tr>
<td></td>
<td>• translation issues, which may lead to new terms or to limitations (however language should not be a limitation of the ELS and if applied should be explained);</td>
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<tr>
<td></td>
<td>• if apparently irrelevant or excessively broad free text terms were used.</td>
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</tbody>
</table>

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13 Translation issues are mainly related to the sources of information. Usually world scientific literature of primary importance and all relevant non-English language papers are translated to give access to research. In a few cases this does not happen.
### Appraisal question

4. **Were appropriate controlled terms (subject headings) identified for each search concept and information source used (when applicable)?**

   Assessment should consider whether:
   - the subject headings/indexing terms used are relevant;
   - any subject headings/indexing terms are missing;
   - any subject headings/indexing terms are too broad or too narrow;
   - any subject headings/indexing terms were exploded where necessary and *vice versa*;\(^\text{14}\)
   - if no subject headings were used, the reason for omission was explained;
   - the use of any subheadings is helpful (i.e. not too focused);
   - the use of any floating subheadings would have been helpful and, if used, was appropriate;
   - if no text words were used, the reason for this omission was explained.

<table>
<thead>
<tr>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 3.6 of the report</td>
<td>☐ Definitively appropriate</td>
<td>No mention is made about controlled terms although at least Medline Express supports them.</td>
</tr>
<tr>
<td>☐ Probably appropriate</td>
<td></td>
<td></td>
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<tr>
<td>☐ Probably not appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Definitively not appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Not applicable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{14}\) Controlled vocabulary is not available in all bibliographic databases.

5. **Was a pilot study carried out (when applicable)?**

   The search should be pilot tested whenever the literature available is not too small. Pilot testing could be done, for example, by assessing the relevance of a subset of records retrieved and checking if records already known to be relevant were captured by the search. Pilot testing results can be used to revise the search terms or identify new ones.

<table>
<thead>
<tr>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 3.6 of the report</td>
<td>☐ Definitively appropriate</td>
<td>No mention is given about that.</td>
</tr>
<tr>
<td>☐ Probably appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Probably not appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Definitively not appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Not applicable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. **Was the appropriate spelling used?**

   Assessment should consider whether there were any spelling errors (NOTE: some spelling errors may be deliberately included – see item 3);

<table>
<thead>
<tr>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section 3.6 of the report</td>
<td>☐ Definitively appropriate</td>
<td>Assuming that the real strings are the ones reported in the report.</td>
</tr>
<tr>
<td>☐ Probably appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Probably not appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Definitively not appropriate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Not applicable</td>
<td></td>
<td></td>
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</tbody>
</table>

\(^{15}\) The use of headings and sub-headings or major terms and narrow terms is applied differently from database to database (e.g. PubMed/Mesh or CABI/CAB Thesaurus). Before performing searches it is considered best practice to verify this feature in the concerned/used database.
<table>
<thead>
<tr>
<th>#</th>
<th>Appraisal question</th>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.</td>
<td>Was the appropriate syntax used?</td>
<td>Section 3.6 of the report</td>
<td>□ Definitively appropriate</td>
<td>Assuming that the real strings are the ones reported in the report.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether there were any errors in system syntax (e.g. truncation symbols). For instance, if the search missed truncation or truncated at the wrong point. Syntax varies depending on the service provider, e.g. the most common truncation symbols is *. Other databases may use $ or ? to represent different numbers of characters so you need to check the help files. The text word terms (free text) were ORed with the relevant subject headings;</td>
<td></td>
<td>□ Probably appropriate</td>
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<td></td>
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<td>□ Probably not appropriate</td>
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<td></td>
<td>□ Definitively not appropriate</td>
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<td></td>
<td></td>
<td></td>
<td>□ Not applicable</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Were the appropriate line numbers used?</td>
<td>Section 3.6 of the report</td>
<td>□ Definitively appropriate</td>
<td>Difficult to judge but assuming that the real strings are the ones reported in the report the strategy does not seem so complex and they could also have not used line numbers.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether there were any wrong line numbers.</td>
<td></td>
<td>□ Probably appropriate</td>
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<td>□ Probably not appropriate</td>
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<td>□ Definitively not appropriate</td>
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<td></td>
<td></td>
<td></td>
<td>□ Not applicable</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Was the use of Boolean and proximity operators appropriate?</td>
<td>Section 3.6 of the report</td>
<td>□ Definitively appropriate</td>
<td>Assuming that the real strings are the ones reported in the report, the strategy seem quite simple and the Boolean operators seem to be used in an appropriate way.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether:</td>
<td></td>
<td>□ Probably appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• there were any mistakes in the use of Boolean or proximity operators.</td>
<td></td>
<td>□ Probably not appropriate</td>
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<tr>
<td></td>
<td>For instance if AND has been inadvertently replaced by OR (or vice versa);16</td>
<td></td>
<td>□ Definitively not appropriate</td>
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<tr>
<td></td>
<td>• there were any mistakes in the use of nesting with brackets;</td>
<td></td>
<td>□ Not applicable</td>
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<tr>
<td></td>
<td>if NOT is used, was there any unintended exclusion; or would another mechanism have been a more suitable alternative;17</td>
<td></td>
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<tr>
<td></td>
<td>• precision could be improved by using proximity operators (e.g. adjacent, near, within, same) instead of AND;18</td>
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<tr>
<td></td>
<td>• the width of any proximity operators is too wide or not wide enough;</td>
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<tr>
<td></td>
<td>the potential importance of word order has been accounted for.</td>
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</tbody>
</table>

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16 AND is used to combine two different concepts, e.g. (xanthomonas citri) AND (citrus fruit). AND will narrow the search: the results must include ALL stated concepts. OR is used to search for similar concepts, e.g. (xanthomonas citri) OR (citrus canker). OR will widen the search: the results will include a MINIMUM OF ONE of the named concepts.

17 NOT is used to restrict the search, e.g. pig* NOT pigeon. The results will exclude ALL records containing the excluded term even those containing the term searched. From this, NOT should be used with caution because it may have a larger exclusion effect than anticipated, as it may exclude records of interest that coincidentally discuss both terms.

18 SAME or NEAR are used to combine two different concepts adding a notion of proximity, e.g. bisphenol A NEAR bottle. SAME will narrow the search: the results must include ALL your stated concepts in the same sentence (for instance).
### Appraisal question

**10. Were limits appropriately used?**

Assessment should consider whether:

- any of the limits used seem unwarranted;
- any potentially helpful limits are missing;
- if restrictions to focus (major indexing terms) are used, there is an adequate justification for their use.

*Information as reported*:
Section 3.6 of the report

*Appraisal*:

- Definitively appropriate
- **Probably appropriate**
- Probably not appropriate
- Definitively not appropriate
- Not applicable

*Rationale for the appraisal*:
Not many details are reported so it is difficult to say. The years considered seem to be appropriate as well as the language (English). Although it is difficult to say if the language was inserted also as a limit of the search or as an inclusion criteria.

---

**11. Were search filters (if used to identify study designs) appropriately used?**

Assessment should consider whether:

- any filters used are appropriate for the topic
- any helpful and relevant available filters are missing

*Information as reported*:
Section 3.6 of the report

*Appraisal*:

- Definitively appropriate
- **Probably appropriate**
- Probably not appropriate
- Definitively not appropriate
- Not applicable

*Rationale for the appraisal*:
It seems that search filters were not used.

---

**12. Was the search strategy correctly adapted for each database used?**

The searcher may adapt the search strategy for additional databases and/or interfaces. Adaptations should be provided for review.

The adaptations should be assessed to ensure that they are correct (e.g. truncation symbols, controlled vocabulary, lemmatization option, etc.)

*Information as reported*:
Section 3.6 of the report

*Appraisal*:

- Definitively appropriate
- **Probably appropriate**
- Probably not appropriate
- Definitively not appropriate
- Not applicable

*Rationale for the appraisal*:
It is difficult to say. However, the search strategy seems very simple and can be replicated in each of the databases considered.
# Appraisal question | Information as reported | Appraisal | Rationale for the appraisal
--- | --- | --- | ---
**B. Assessing the information sources searched**

13. **Assess if the search was extensive enough, i.e. assess if the right (relevant and reliable) combinations of information sources were searched.**

More than a single database should be searched. Searches of information sources for different types of publication would help to demonstrate that the search had been extensive:

- Major bibliographic databases (journals and books)
- Information sources recording:
  - Dissertations
  - Conference reports
  - Reports
  - Ongoing research/research registers

In addition, one or more of the following search techniques should be reported:

- Reference checking
- Handsearching
- Citation searches
- Checking websites of relevant organisations

Section 3.6 of the report

□ Definitively appropriate
□ **Probably appropriate**
□ Probably not appropriate
□ Definitively not appropriate
□ Not applicable

The databases searched seem to have an adequate covering of the topic.
### Appendix C – Details of the critical appraisal of St-Pierre’s Systematic Review

#### Systematic Review Critical Appraisal Tools

<table>
<thead>
<tr>
<th>#</th>
<th>Appraisal question</th>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Review question and eligibility criteria for study selection</td>
<td></td>
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</tr>
<tr>
<td>1.</td>
<td>Was the review question clearly formulated?</td>
<td>'To provide an updated evaluation of the efficacy and safety of sometribove zinc suspension (rbST-Zn), a form of recombinant bovine somatotropin, in lactating dairy cows' (pag.551)</td>
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<tr>
<td></td>
<td>Formulating the review question means properly translating the review question into 'PICO' elements (Population(s); Intervention(s); Comparator(s); Outcome(s) (including adverse effects where relevant)):</td>
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<td>• have the relevant PICO elements (and these elements are adequately characterised);</td>
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<tr>
<td></td>
<td>If the PICO elements and study design(s) are not appropriate to answer the review question, there is a risk of excluding relevant studies and/or including irrelevant studies.</td>
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<tr>
<td></td>
<td>'Formed at the request of Health Canada, the mandate for the CVMA expert panel was to determine whether the use of rbST in accordance with its label directions would increase milk production without resulting in serious health problems that could not be adequately controlled by cattle management practices implemented at that time' (pg 551)</td>
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<td></td>
<td>As for the ‘update’ they refer to the SR performed by Health Canada.</td>
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<td>Three of the four PICO elements are described:</td>
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<tr>
<td></td>
<td>P: lactating dairy cows</td>
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<tr>
<td></td>
<td>I: rbST-Zn administered in accordance with the FDA-approved label directions</td>
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<tr>
<td></td>
<td>C: not defined.</td>
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<tr>
<td></td>
<td>O: efficacy and safety of rbST-Zn</td>
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<tr>
<td></td>
<td>Efficacy and safety not adequately specified although the reference to CVMA SR might partially clarify that safety means 'animal health'. The study remains rather vague with view to the outcomes. Only in the statistical methods and data analysis sections outcomes of interest are listed. This raises questions as to if outcomes were defined a priori.</td>
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<td></td>
<td>□ Definitively appropriate</td>
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<td>□ Probably not appropriate</td>
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<td>□ Definitively not appropriate</td>
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<tr>
<td></td>
<td>□ Not applicable</td>
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<tr>
<td>#</td>
<td>Appraisal question</td>
<td>Information as reported</td>
<td>Appraisal</td>
<td>Rationale for the appraisal</td>
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</tbody>
</table>
| 2. | Were the eligibility criteria related to study characteristics appropriate and clearly defined a priori? | Appropriately defining *a priori* the study(s) that shall be selected for inclusion in the review, implies that they:  
  - have the appropriate study design to answer the review question and the study design must be adequately characterised. For instance for efficacy studies it should be stated if the reviewer(s) chose to include only randomised, double-blind, placebo controlled studies, or e.g. allocation concealment was also considered as inclusion criteria (for other study designs alternative items will be relevant). Study-specific elements should be considered such as e.g. length of follow-up. 
  
  *If the eligibility criteria for study selection are not defined a priori, there is a risk of introducing a bias due to selective inclusion/exclusion of studies influenced by the results of such studies.* | Studies must have been published in a peer-reviewed scientific journal or reviewed by a regulatory agency, included a control group, and involved the administration of the rbST-Zn formulation commercially available to US dairy producers in accordance with the FDA-approved label directions (500 mg, SC, q 14 d, beginning between 57 and 70 days postpartum). Studies that involved the administration of rbST-Zn in an extra label manner or formulations of rbST not approved by the FDA were excluded. | □ Definitely low risk of bias  
□ Probably low risk of bias  
□ Probably high risk of bias  
□ Definitely high risk of bias  
□ Not applicable | *No study characteristics have been included in the eligibility criteria. This might have led to an inclusion of observational studies. However, it was required to have a control group.*                                                                                                                                                                                                                                                                                                                                                 |
| 3. | Did the reviewers avoid the use of report characteristics as eligibility criteria? If applied, were these criteria defined a priori? | In principle all studies should be considered both from peer-reviewed studies and grey literature. If limits are applied such as publication type, language, years, geographical/political area, etc... these should be documented, justified and defined *a priori.*  
  
  *See above. No mention of report characteristics as eligibility criteria.* | | □ Definitely appropriate  
□ Probably appropriate  
□ Probably not appropriate  
□ Definitely not appropriate  
□ Not applicable | *Search terms were only in English and there is no evidence that papers in languages other than English were searched.  
Only reports reviewed by Regulatory Agencies were included from grey literature.  
No justifications have been given for the limitation to report time (1972-2012) but overall it seems acceptable since it covers a significant period of time before acceptance of the drug (1993).*                                                                                                                                                                                                                                                                                                                                                       |
### B. Search process

#### 4. Was the extensive literature search performed in an appropriate way?

Please refer to ELS CAT and give an overall appraisal of the appropriateness of the ELS done.

<table>
<thead>
<tr>
<th>Appraisal question</th>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitively not appropriate □ Not applicable</td>
<td>□ Definitively appropriate</td>
<td>The flow reports a number of studies identified in the literature (n=69) clearly not corresponding to the string. The search strategy is quite broad and overall is appropriate to meet the objectives of the SR. Spelling variants have been used, but not truncation. The combination of databases seems to cover the topic appropriately. For a detailed appraisal look at the CAT for ELS.</td>
</tr>
</tbody>
</table>

#### C. Study selection process

#### 5. Were preventative steps taken to minimise bias and errors in the study selection process?

Study selection is normally a 2-step process: (1) rapid assessment of titles and abstracts to exclude obviously irrelevant records and (2) examination of full-text documents. The assessment should be done evaluating:

- whether the selection was carried out by at least two mutually independent reviewers, in parallel. It is also acceptable to have two reviewers in the 2nd step only (i.e. examination of full-text documents), provided that in the first step an over-inclusive approach was applied;
- the presence of a clearly defined consensus procedure for disagreements (e.g. discussion or involvement of another reviewer). Ideally the eligibility criteria should be pilot-tested by the reviewers on a subset of records and re-formulated if prone to misinterpretation.

<table>
<thead>
<tr>
<th>Appraisal question</th>
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<th>Rationale for the appraisal</th>
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<tbody>
<tr>
<td>The studies on the final list were reviewed by the domain experts to verify that they were conducted in compliance with the inclusion criteria.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitively not appropriate □ Not applicable</td>
<td>□ Definitively appropriate</td>
<td>No check of excluded papers performed by experts. No mention of procedures for resolving disagreement in the reviewing process.</td>
</tr>
</tbody>
</table>

No check of excluded papers performed by experts.

No mention of procedures for resolving disagreement in the reviewing process.
6. **Were the results of the study selection process consistent with the eligibility criteria previously defined?**

   Assessment should be done evaluating the following items:
   - total number of records identified;
   - number of records and studies excluded at each stage;
   - list of excluded studies, and primary reasons for exclusion after examination of full-text documents (e.g. not relevant, duplicate study, etc.).

   Assessment is easier when the necessary information is provided in a flow diagram.

   Flow diagram provided with included/excluded study at each stage of the screening.

   In the discussion it is stated ‘In the present meta-analysis, only logSCC data derived from randomized controlled trials were evaluated.

   ‘the domain experts .... added any other studies of which they were aware that met the inclusion criteria’.

   ‘studies used by Monsanto (the company that originally marketed rbST-Zn) in its submission to Health Canada for product approval wherever possible, were located and data extracted’.

   □ Definitively appropriate
   □ Probably appropriate
   □ Probably not appropriate
   □ Definitively not appropriate
   □ Not applicable

   The study selection process is not clear and not well described.

   Studies meeting eligibility criteria added by experts after the process of screening for relevance ended.

   Although identification of relevant studies by experts is useful when scoping literature in order to refine the search strategy, it should not be done once literature search has been completed. This could have increased the risk of bias.

   The number of studies identified through literature search in Figure 1 is not plausible given the search string (69 is too small). The appraisal team tried the same search with ORs in PubMed and retrieved 7300 studies. It could be that there was a screening phase that is not reported

   The list of included studies is provided (Table 1).

   The list of excluded studies and motivation for exclusion is given only for a subset of studies (those in the Health Canada SR). Therefore it is not possible to assess the compliance of the screening process to the eligibility criteria.

   Despite not mentioned in the eligibility criteria only RCTs
### D. Data extraction from the included studies

#### 7. Was data extraction carried out appropriately and adequately?

**Was the approach defined *a priori?***

Assessment should be done evaluating:

- if data extracted are relevant for the PICO elements:
  - if relevant data on study subjects, intervention and control groups were identified and extracted;
  - if relevant study outcomes and related measures of precision were extracted (i.e. either summary data on each intervention group or effect estimates with confidence intervals) and, if not available, proxy information was used;
- if sources of ‘clinical’ heterogeneity (i.e. related to the PICO elements) across the included studies were explored and the relevant data extracted;
- if sources of methodological heterogeneity (i.e. related to the study design characteristics) across the included studies were explored and the relevant data extracted (e.g. parallel vs. cross-over, sample size, follow-up period);
- if any assumptions related to data extraction were justified;
- if data extraction was harmonised across the different studies from which the data were extracted (e.g. measurement unit, data coding).

Ideally the data extraction form should include a column containing what is reported in the study and a column containing how the data was ‘translated’ for the purposes of the review, if translation was necessary.

Ideally information on sources of funding in the individual studies should be extracted (to evaluate the risk of publication bias).

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<td></td>
<td></td>
<td></td>
<td></td>
<td>were considered for assessing potential impact of rBST on logSCC.</td>
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</tbody>
</table>

From each study included in the present meta-analysis, the data manager extracted data for treatment means, SEs of the means, and other relevant statistics for all variables and entered it into an electronic database. Domain expert cross-checked and verified all entries.

**Outcomes:**

- milk production and composition (mean actual milk, fat, and protein yields; mean fat, protein, and lactose percentages; and mean 3.5% FCM yield)
- udder health (clinical mastitis incidence rate/100 cow-days at risk and the mean logSCC),
- reproduction: i.e. proportion of cows that conceived during an LRP and ERP, mean number of days from parturition to conception, mean number of inseminations per pregnancy, proportions of cows that had fetal loss, twins (or multiple births), and developed cystic ovaries,
- body condition (BCS),
- lameness (proportion cows with clinical lameness; lameness lesions; traumatic lesions, number of affected cows/1 000 cow-days at risk)

- Definitively appropriate
- Probably appropriate
- Probably not appropriate
- Definitively not appropriate
- Not applicable

Not possible to say whether list of data to extract was defined a priori. Relevant data on study subjects, intervention and control groups were identified and extracted. Relevant study outcomes and related measures of precision extracted. Information on the sources of funding of the individual studies is not reported. It is likely that the data extraction was performed in a harmonised way since done centrally and reviewed later by experts. Details provided on how transformations were applied in order to get harmonised information.
### Appraisal question

**8. Were preventative steps taken to minimise bias and errors in the data extraction process?**

Assessment should be done evaluating:
- if data extraction was carried out by at least two mutually independent reviewers, in parallel;
- the presence of a clearly defined consensus procedure for disagreements (e.g. discussion or involvement of another reviewer);
- the presence of a procedure for obtaining and/or confirming data from researchers;
- if duplicate studies were identified and data extracted only once, with appropriate explanations.

Ideally the data extraction forms should be pilot-tested by the reviewers on a subset of records and improved if prone to misinterpretation.

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<tr>
<td>culling (culling density= number cows culled/10 000 cow-days at risk)</td>
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</table>

Each domain expert was provided with a reprint of each study relevant to his area of expertise. Experts cross-checked and verified all entries in the database for their particular domain.

- Definitively appropriate
- Probably appropriate
- Probably not appropriate
- Definitively not appropriate
- Not applicable

Process to check correctness of data extraction process in place.

### E. Assessment of the methodological quality of the studies included in the review

**9. Was the methodological quality of the studies included in the review appropriately and adequately appraised? Was the approach defined a priori?**

Assessment should be done evaluating:
- if methodological flaws and biases relevant to the study design(s) included in the SR were identified and adequately assessed;
- if quality assessment was harmonised across the different studies by the specific outcomes included in the review.

The elements to consider for appraising the methodological quality of the studies included in the review vary depending upon the study designs. Different critical appraisal tools are available and all should be considered in view of the subject of the review and, if needed, adapted.¹⁹

- Definitively appropriate
- Probably appropriate
- Probably not appropriate
- Definitively not appropriate
- Not applicable

Process to check correctness of data extraction process in place.

| No mention of any assessments of methodological quality of the studies. Methodological quality was not considered in the mathematical synthesis. |

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¹⁹ A tool for appraising RCTs in the EFSA context is the SAS Critical Appraisal Tool for RCTs (to be published in EFSA’s website).
### Appraisal question

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<th>Rationale for the appraisal</th>
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<tbody>
<tr>
<td>10.</td>
<td>Were preventative steps taken to minimise bias and errors when appraising the methodological quality of the studies included in the review?</td>
<td></td>
<td></td>
<td>No assessment of methodological quality.</td>
</tr>
</tbody>
</table>

Assessment should be done evaluating:
- if quality assessment was carried out by at least two mutually independent reviewers, in parallel;
- the presence of a clearly defined consensus procedure for disagreements (e.g. discussion or involvement of another reviewer)

Ideally the appraisal tool should be pilot-tested, also to maximise consistency of application between different reviewers.

### F. Data analysis and synthesis of results

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<tr>
<td>11.</td>
<td>Was the analysis and synthesis of the individual effect estimates properly undertaken? Was the approach defined a priori?</td>
<td>For the present meta-analysis, a random-effects model, in which the evaluated studies were assumed to be a random sample from the population of such studies, was used to allow for a broad inference space. For each outcome evaluated, the number of studies that provided data for the meta-analysis and the results of the test of heterogeneity were summarized. Pooled estimated mean and related standard errors were computed as a result of the meta-analysis.</td>
<td></td>
<td>Not possible to assess whether outcomes and methodologies for data analysis and synthesis have been defined a priori. Biological relevance of the outcomes addressed. Standard approaches for statistical analysis were used. Methodologies used to analyse and synthesise data are appropriate except for the assessment of safety outcomes where test of difference instead of test of equivalence was performed. This can be still acceptable provided that it is correctly interpreted.</td>
</tr>
</tbody>
</table>

- If a meta-analysis was carried out, it should be assessed if it was appropriate to calculate the pooled estimate;
- Meta-analysis should be appraised evaluating:
  - if the effect measure chosen was appropriate for the data type of the outcomes measured in the individual studies (e.g. for continuous variables, differences of means; for dichotomous variables, risk ratios);
  - if the appropriate pair-wise comparisons of intervention groups were made;
  - if the units of analysis were correctly identified (i.e. the analysis must take into account the level at which randomisation occurred as in most circumstances the number of observations in the analysis should match the number of ‘units’ that were randomised). Thus carefulness is required for study designs like cluster-randomised trials, cross-over trial or multiple sites trials);
  - if statistical heterogeneity was appropriately identified and quantified (e.g. Q test, I²);
  - if a consistent model was chosen and justified (i.e. Fixed Effect Model/Random Effect Model).

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20 From the Cochrane Handbook: "The comparisons addressed in the review should relate clearly and directly to the questions or hypotheses that are posed when the review is formulated" and can be translated into the following questions: - What are the experimental and control (comparator) interventions of interest? - Does the intervention have variations (e.g. dosage/intensity, mode of delivery, personnel who deliver it, frequency of delivery, duration of delivery, timing of delivery)?
Use of rBST in dairy cattle and AMR in humans

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<tr>
<td>f</td>
<td>if missing data, when existing (i.e. entire studies, outcomes, summary data for an outcome, individual participants from summary data) were properly handled and the related assumptions clarified;</td>
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<td></td>
<td>• If the results could not be pooled using meta-analysis, it should be appraised if:</td>
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<td>o a systematic approach was applied, i.e. the same elements were described for each study;</td>
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<td></td>
<td>o the studies were organised in groups or clusters, to enable identification of patterns in results (for this purpose tables and graphics - i.e. forest plots, can be provided).</td>
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<td></td>
<td>• If the approach to data analysis and synthesis was defined a priori.</td>
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<td>12</td>
<td>If there was the need or opportunity to perform additional analyses (e.g. sensitivity or subgroup analyses, meta-regression), were they performed? Was the approach defined a priori? Assessment should be done evaluating:</td>
<td>Sub-group analyses were performed in order to assess the impact of parity on the results.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitely not appropriate □ Not applicable</td>
<td>Not clear whether additional analyses planned a priori. The sub-group analysis was performed considering a factor that is biologically relevant especially for assessing the efficacy of rBST to increase milk production.</td>
</tr>
<tr>
<td></td>
<td>• if, when enough data were available for subgroup analysis or meta-regression, such analyses were performed. The reasons for performing or not performing the analyses should be explained and the results clearly discussed;</td>
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<td></td>
<td>• if sensitivity analysis was conducted to test the robustness of the results with respect to any assumptions and decisions that were made in the review (e.g. related to the search, the eligibility criteria, the approach taken to handle missing data, the methodological quality of the study, sources of funding of the individual studies, etc);</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>• if the approach (i.e. when to perform a sub-group and/or sensitivity analysis and how) was defined a priori.</td>
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</table>
### G. Reporting bias

#### 13. Was risk of publication bias addressed? Was the approach defined \textit{a priori}?

Risk of publication bias can be addressed in the review by using a combination of graphical aids (e.g. funnel plot, other available tests) and/or statistical tests (e.g. Egger regression test). The approach should be defined \textit{a priori}.

For assessing publication bias, the authors of the review could also consider the source of funding of the individual studies included in the review.

Under this appraisal item other types of reporting biases could be assessed, e.g.:

- Publication bias: the publication or non-publication of research findings, depending on the nature and direction of the results;
- Time lag bias: the rapid or delayed publication of research findings, depending on the nature and direction of the results;
- Multiple (duplicate) publication bias: the multiple or singular publication of research findings, depending on the nature and direction of the results;
- Location bias: the publication of research findings in journals with different ease of access or levels of indexing in standard databases, depending on the nature and direction of results;
- Citation bias: the citation or non-citation of research findings, depending on the nature and direction of the results;
- Language bias: the publication of research findings in a particular language, depending on the nature and direction of the results;
- Outcome reporting bias: the selective reporting of some outcomes but not others, depending on the nature and direction of the results.

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<tbody>
<tr>
<td>□ Definitively appropriate</td>
<td>□ Probably appropriate</td>
<td>Publication bias not addressed. As far as the risk of mastitis and other health and welfare outcomes are concerned the publication bias would probably result in an overestimation of the risk (studies with positive outcomes have higher probability to be published). Assuming that a conservative approach is taken from an animal health perspective, the risk of publication bias can be considered having a low impact.</td>
</tr>
</tbody>
</table>
### Interpretation of Results and Conclusions

#### 14. Did the conclusions reflect the results of the review and any limitation in the process?

Assessment should be done evaluating if the conclusions took into account the following aspects:

- the pooled and/or individual studies estimates (e.g. via appropriate forest plot);
- if performed, the results of the additional analyses;
- the results of the assessment of methodological quality of the included studies.
- any limitations in the process, e.g. limitations of the search, limited number of reviewers involved in the selection and data extraction process, etc.

If the quality of the body of evidence was evaluated in the review, this should be reflected by the conclusions.

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<tr>
<td></td>
<td>Results of the test for heterogeneity suggested that the risk of clinical mastitis in cows among the studies evaluated was dependent on factors other than rbST-Zn administration. This finding supports the concept that the risk for development of clinical mastitis associated with increasing milk production can be compensated by improved management on modern commercial dairy operations. When examined on a per unit of milk basis, increase in incidence of clinical mastitis attributable to rbST-Zn administration (0.1 cases/cow/year) is approximately four to nine times less than the increase in incidence of clinical mastitis attributable to other factors such as season, parity, stage of lactation, etc. Results of the present meta-analysis found no evidence that the rbST-Zn formulation commercially available to US producers administered to lactating dairy cows in accordance with the FDA-approved label had any unmanageable adverse effects on milk composition, udder health, reproduction, body condition, lameness, or longevity.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ [Definitively not appropriate]</td>
<td>Conclusions are drawn not only on the basis of the results of the meta-analysis but using data extracted from other sources too. It is not specified how these additional sources were identified. The interpretation of the results of the test for heterogeneity as far as the mastitis is concerned is inappropriate. Only interpretation possible is that studies exhibit lack of homogeneity in the results that could be due to different reasons (e.g. study setting, seasonality, population etc.). In no way conclusions can be drawn on dependency/independency of the mastitis risk on rbST-Zn administration. There is a fundamental issue related to the assessment of health outcomes (including mastitis incidence). Absence of evidence is not evidence of absence. Therefore test designed in order to prove that a difference exists cannot be used for assessing the complementary hypothesis (e.g. that incidence of mastitis is not different in treated and untreated cows). If a test is not able to demonstrate that a difference...</td>
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### Appraisal question

15. If a protocol was provided, are appropriate justifications given for any described deviations from the protocol?

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<td>□ Probably appropriate</td>
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<td>□ Probably not appropriate</td>
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<td>□ Definitively not appropriate</td>
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<td></td>
<td>□ Not applicable</td>
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<th>Rationale for the appraisal</th>
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<td>exists it has to be considered inconclusive.</td>
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</table>

| No protocol provided |

### I. Additional comments

16. Add here any aspects that should be outlined and are not covered above.

- Possible Conflict of Interest: Milliken and Associates, LLC (Milliken) received funding from Eli Lilly and Co, for statistical consultation. The other authors have no conflict of interest to declare regarding the performance of this study or the information contained in this manuscript.
- Composition of the panel: The US Panel was composed by a data manager and project coordinator, a professional statistician, and six domain (milk production and composition, udder health, reproduction, body condition, lameness, and culling) experts.
### Extensive Literature Search Critical Appraisal Tools

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<tr>
<td></td>
<td><strong>A. Assessing the search strategy</strong></td>
<td>'A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.' See page 551, data sources of the report.</td>
<td></td>
<td>Only one search concept was searched (Intervention) that should result in a sensitive search.</td>
</tr>
<tr>
<td></td>
<td><strong>1. Was the review question appropriately translated into search concepts?</strong></td>
<td>'A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.' See page 551, data sources of the report.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitely not appropriate □ Not applicable</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitely not appropriate □ Not applicable</td>
</tr>
<tr>
<td></td>
<td><strong>2. Was the search string an optimal combination of the search concepts for sensitivity and precision?</strong></td>
<td>'A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.' See page 551, data sources of the report.</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitely not appropriate □ Not applicable</td>
<td>□ Definitively appropriate □ Probably appropriate □ Probably not appropriate □ Definitely not appropriate □ Not applicable</td>
</tr>
</tbody>
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21 Precision refers to the proportion of relevant records among all the records retrieved by a search strategy (relevant records retrieved/all records retrieved). Sensitivity refers to the proportion of relevant records retrieved by a search strategy (relevant records retrieved/total relevant records) (Glanville et al. 2014. Technical Manual for Performing Electronic Literature Searches in Food and Feed Safety).
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</table>
| 3. | **Were the appropriate free-text terms** (i.e., terms in the title and abstract) identified for each search concept? Assessment should consider whether, for each search element, the search included:  
- all possible synonyms (e.g., welfare, wellbeing, etc.);  
- related terms, e.g., pesticide, pest control, etc.;  
- acronyms, e.g., Bluetongue, BTV, etc. If an acronym or abbreviation is used, a full text term or substantial part of a full text term should also be present;  
- spelling variants, e.g., behavior, behaviour; anaemia/anemia;  
- old and new terminology, e.g., aspartame/Phenylalanine;  
- brand and generic names, e.g., imidacloprid, Gaucho;  
- lay and scientific terminology e.g., aspartame/\-Methyl N-L-\alpha-aspartyl-L-phenylalanate.  
- common typos (e.g., mitomycin/mitomicin)  
In addition, the following aspects should be considered:  
- translation issues,\(^2\) which may lead to new terms or to limitations (however, language should not be a limitation of the ELS and if applied should be explained);  
- if apparently irrelevant or excessively broad free text terms were used | A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.\(^*\) See page 551, data sources of the report. | □ | The terms related to the intervention seem to be appropriate. Synonyms and one brand name have been used. |

\(^2\) Translation issues are mainly related to the sources of information. Usually world scientific literature of primary importance and all relevant non-English language papers are translated to give access to research. In a few cases this does not happen.
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<td>4</td>
<td><strong>Were appropriate controlled terms (subject headings) identified for each search concept and information source used (when applicable)</strong>?</td>
<td>&quot;A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.&quot; See page 551, data sources of the paper.</td>
<td>Definitively appropriate</td>
<td>No mention is made about controlled terms although at least PubMed supports them.</td>
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<td></td>
<td>Assessment should consider whether:</td>
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<td>□ Definitively appropriate</td>
<td>□ Probably appropriate</td>
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<tr>
<td></td>
<td>• the subject headings/indexing terms used are relevant;</td>
<td></td>
<td>□ Probably not appropriate</td>
<td>□ Not applicable</td>
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<tr>
<td></td>
<td>• any subject headings/indexing terms are missing;</td>
<td></td>
<td>□ Definitively not appropriate</td>
<td>□ Not applicable</td>
</tr>
<tr>
<td></td>
<td>• any subject headings/indexing terms are too broad or too narrow;</td>
<td></td>
<td>□ Not applicable</td>
<td></td>
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<tr>
<td></td>
<td>• any subject headings/indexing terms were exploded where necessary and vice versa;</td>
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<td></td>
<td>• if no subject headings were used, the reason for omission was explained;</td>
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<td></td>
<td>• the use of any subheadings is helpful (i.e. not too focused);</td>
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<td>• the use of any floating subheadings would have been helpful and, if used, was appropriate;</td>
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<td></td>
<td>• if no text words were used, the reason for this omission was explained.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><strong>Was a pilot study carried out (when applicable)?</strong></td>
<td>&quot;A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.&quot; See page 551, data sources of the paper.</td>
<td>Definitively appropriate</td>
<td>No mention about pilot study.</td>
</tr>
<tr>
<td></td>
<td>The search should be pilot tested whenever the literature available is not too small.</td>
<td></td>
<td>□ Probably appropriate</td>
<td>□ Probably not appropriate</td>
</tr>
<tr>
<td></td>
<td>Pilot testing could be done, for example, by assessing the relevance of a subset of records retrieved and checking if records already known to be relevant were captured by the search. Pilot testing results can be used to revise the search terms or identify new ones.</td>
<td></td>
<td>□ Definitively not appropriate</td>
<td>□ Not applicable</td>
</tr>
</tbody>
</table>

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23 Controlled vocabulary is not available in all bibliographic databases.

24 The use of headings and sub-headings or major terms and narrow terms is applied differently from database to database (e.g. pubmed/Mesh or CABI/cab Thesaurus). Before performing searches it is considered best practice to verify this feature in the concerned/used database.
<table>
<thead>
<tr>
<th>#</th>
<th>Appraisal question</th>
<th>Information as reported</th>
<th>Appraisal</th>
<th>Rationale for the appraisal</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td><strong>Was the appropriate spelling used?</strong></td>
<td>‘A literature search was conducted...: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.’ See page 551, data sources of the report.</td>
<td>□ Definitively appropriate</td>
<td>Assuming that the real strings are the ones reported in the paper.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether there were any spelling errors (NOTE: some spelling errors may be deliberately included – see item 3);</td>
<td></td>
<td>□ Probably appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Probably not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Definitively not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Not applicable</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><strong>Was the appropriate syntax used?</strong></td>
<td>‘A literature search was conducted...: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.’ See page 551, data sources of the report.</td>
<td>□ Definitively appropriate</td>
<td>Assuming that the real strings are the ones reported in the paper, the search was simple and no subject headings were used.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether there were any errors in system syntax (e.g. truncation symbols). For instance, if the search missed truncation or truncated at the wrong point. Syntax varies depending on the service provider, e.g. the most common truncation symbols is *. Other databases may use $ or? to represent different numbers of characters so you need to check the help files. the text word terms (free text) were ORed with the relevant subject headings;</td>
<td></td>
<td>□ Probably appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Probably not appropriate</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>□ Definitively not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Not applicable</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><strong>Were the appropriate line numbers used?</strong></td>
<td>‘A literature search was conducted...: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.’ See page 551, data sources of the report.</td>
<td>□ Definitively appropriate</td>
<td>Assuming that the real strings are the ones reported in the paper, the search was simple and the use of line numbers was not requested.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether there were any wrong line numbers.</td>
<td></td>
<td>□ Probably not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Definitively not appropriate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>□ Not applicable</td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Appraisal question</td>
<td>Information as reported</td>
<td>Appraisal</td>
<td>Rationale for the appraisal</td>
</tr>
<tr>
<td>----</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>9.</td>
<td>Was the use of Boolean and proximity operators appropriate?</td>
<td>'A literature search was conducted...: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.' See page 551, data sources of the paper and Figure 1 page 552.</td>
<td>□</td>
<td>There are no details on how this search was implemented. It is assumed that 'OR' is used as Boolean operator.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether:</td>
<td></td>
<td>DEFINITIVELY APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- there were any mistakes in the use of Boolean or proximity operators.</td>
<td></td>
<td>PROBABLY APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- there were any mistakes in the use of nesting with brackets;</td>
<td></td>
<td>PROBABLY NOT APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- if NOT is used, was there any unintended exclusion; or would another mechanism have been a more suitable alternative;</td>
<td></td>
<td>DEFINITIVELY NOT APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- precision could be improved by using proximity operators (e.g. adjacent, near, within, same) instead of AND;</td>
<td></td>
<td>NOT APPLICABLE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- the width of any proximity operators is too wide or not wide enough;</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>- the potential importance of word order has been accounted for.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Were limits appropriately used?</td>
<td>'A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 see page 551, data sources of the paper.</td>
<td>□</td>
<td>No details are reported so it is difficult to say. Years from 1975 to 2012 were considered and this is virtually meaning all available years. Not mention is given to language. It is assumed that the search was limited to English.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether:</td>
<td></td>
<td>DEFINITIVELY APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- any of the limits used seem unwarranted;</td>
<td></td>
<td>PROBABLY APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- any potentially helpful limits are missing;</td>
<td></td>
<td>PROBABLY NOT APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- if restrictions to focus (major indexing terms) are used, there is an adequate justification for their use.</td>
<td></td>
<td>DEFINITIVELY NOT APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NOT APPLICABLE</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Were search filters (if used to identify study designs) appropriately used?</td>
<td>No information is provided</td>
<td>□</td>
<td>No information is provided. However, given the simplicity of the search it can be assumed that search filters were not used.</td>
</tr>
<tr>
<td></td>
<td>Assessment should consider whether:</td>
<td></td>
<td>DEFINITIVELY APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- any filters used are appropriate for the topic</td>
<td></td>
<td>PROBABLY APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- any helpful and relevant available filters are missing</td>
<td></td>
<td>PROBABLY NOT APPROPRIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DEFINITIVELY NOT APPROPRIATE</td>
<td></td>
</tr>
</tbody>
</table>

25 AND is used to combine two different concepts, e.g. (xanthomonas citri) AND (citrus fruit). AND will narrow the search: the results must include ALL stated concepts. OR is used to search for similar concepts, e.g. (Xanthomonas citri) OR (citrus canker). OR will widen the search: the results will include a MINIMUM OF ONE of the named concepts.

26 NOT is used to restrict the search, e.g. pig* NOT pigeon. The results will exclude ALL records containing the excluded term even those containing the term searched. From this, NOT should be used with caution because it may have a larger exclusion effect than anticipated, as it may exclude records of interest that coincidentally discuss both terms.

27 SAME or NEAR are used to combine two different concepts adding a notion of proximity, e.g. bisphenol A NEAR bottle. SAME will narrow the search: the results must include ALL your stated concepts in the same sentence (for instance).
### Use of rBST in dairy cattle and AMR in humans

#### Appraisal question

12. **Was the search strategy correctly adapted for each database used?**

The searcher may adapt the search strategy for additional databases and/or interfaces. Adaptations should be provided for review. The adaptations should be assessed to ensure that they are correct (e.g. truncation symbols, controlled vocabulary, lemmatization option, etc.)

#### Information as reported

*A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between 1975 and 2012 by use of the following search terms: bST, rbST, sometribove, sometribove zinc, Posilac, bovine somatotropin, and bovine growth hormone.* See page 551, data sources of the paper.

#### Appraisal

<table>
<thead>
<tr>
<th></th>
<th>Definitively appropriate</th>
<th>Probably appropriate</th>
<th>Probably not appropriate</th>
<th>Definitively not appropriate</th>
<th>Not applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Rationale for the appraisal

It is difficult to say. However, the search strategy seems very simple and can be replicated in each of the databases considered.

---

#### Assessing the information sources searched

13. **Assess if the search was extensive enough, i.e. assess if the right (relevant and reliable) combinations of information sources were searched.**

More than a single database should be searched. Searches of information sources for different types of publication would help to demonstrate that the search had been extensive:

- A. Major bibliographic databases (journals and books)
- B. Information sources recording:
  - Dissertations
  - Conference reports
  - Reports
  - Ongoing research/research registers

In addition, one or more of the following search techniques should be reported:

- Reference checking
- Handsearching
- Citation searches
- Checking websites of relevant organisations

#### Information as reported

*A literature search was conducted of studies indexed in PubMed, Agricola, Web of Science, and CAB Direct between ...* See page 551, data sources of the paper.

#### Appraisal

<table>
<thead>
<tr>
<th></th>
<th>Definitively appropriate</th>
<th>Probably appropriate</th>
<th>Probably not appropriate</th>
<th>Definitively not appropriate</th>
<th>Not applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Rationale for the appraisal

The databases searched seem to have an adequate covering of the topic.
Appendix D — Methodological approach for appraising the two systematic reviews

The methodological approach that has been followed to perform the appraisal of the two SRs consisted in the following steps.

- **Reviewer team:**

| EFSA’s Animal health and welfare (AHAW) team | Two reviewers |
| EFSA’s Assessment and methodological support (AMU) unit | Three reviewers (one reviewed only extended literature search) |

**Decisions taken by the reviewer team before starting the appraisal:**

- The team will proceed in steps:
  - Assessment of the methodological quality of the two SRs with respect to their own review question;
  - Identification of the reasons (if any) that justify discrepancies in the results;
  - Assessment of the capability of the existing SRs to address our question that is defined as follows: ‘Have any use (including any formulation, doses/dosing frequencies, vehicle and administration form) of BST/rBST (irrespective of the compliance with producer instructions) in dairy cows an effect on prevalence and incidence of clinical and subclinical mastitis as compared to the levels observed in not exposed comparable dairy cows (i.e. same genetics, same management practices, same bedding and stable cleaning conditions) observed in the same climate conditions and at the same period of the year?’

  *Identify elements considered/not considered in the two SRs that could support/weaken the strength of the answer to our question if addressed using the two available SRs*

- First meeting: to agree on how to appraise items and how to share responsibilities
- Second meeting: to discuss inconsistencies and agree on the conclusions to be summarised in the report that will contain three sections (see bullet-points above).

**As for the assessment of the existing systematic reviews (SRs), including the Extensive Literature Searches (ELS) that were developed:**

- The team will ask for additional information to the corresponding authors of the two SRs in order to get any protocol or more detailed reports if available;
- The existing EFSA CAT for the appraisal of SR and ELS will be used;
- Each reviewer will assess the two SRs in parallel. The split of the items between AMU and AHAW is provided in Table E1 and E2 (item number refers to the numbering in the CAT).

**Table D1: SR CAT**

<table>
<thead>
<tr>
<th>Item</th>
<th>Assessor</th>
<th>How</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All</td>
<td>Review question should allow not ambiguous identification of PICO elements</td>
</tr>
<tr>
<td>2</td>
<td>All</td>
<td>Two elements to be assessed Whether study designs to be included • have been defined <em>a priori</em> • are appropriate to address the Review</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Item</th>
<th>Assessor</th>
<th>How</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>All</td>
<td>Two elements to be assessed&lt;br&gt;Whether criteria based on report characteristics (if any)&lt;br&gt;- have been defined <em>a priori</em>&lt;br&gt;- are appropriate to address the Review question (e.g. Japanese papers not considered for a topic crucial for Japan)</td>
</tr>
<tr>
<td>4</td>
<td>All</td>
<td>Go to ELS CAT</td>
</tr>
<tr>
<td>5</td>
<td>AMU</td>
<td>Assessment of the process</td>
</tr>
<tr>
<td>6</td>
<td>All</td>
<td>Assess whether the study selection process is:&lt;br&gt;- fully traceable&lt;br&gt;- meets the eligibility criteria</td>
</tr>
<tr>
<td>7</td>
<td>All</td>
<td>Data extraction:&lt;br&gt;See instructions on CAT</td>
</tr>
<tr>
<td>8</td>
<td>AMU</td>
<td>Assessment of the process</td>
</tr>
<tr>
<td>9</td>
<td>AMU</td>
<td>Two elements to be assessed&lt;br&gt;- Whether methodological quality has been appraised: identifying items <em>a priori</em>&lt;br&gt;- Looking at appropriate items&lt;br&gt;Use existing EFSA CATs (for the design for which they are available) as a sort of benchmark</td>
</tr>
<tr>
<td>10</td>
<td>AMU</td>
<td>Assessment of the process</td>
</tr>
<tr>
<td>11</td>
<td>All</td>
<td>Two elements to be considered:&lt;br&gt;- Biological relevance of the summarised evidence&lt;br&gt;- Appropriateness of the statistical synthesis</td>
</tr>
<tr>
<td>12</td>
<td>All</td>
<td>Two elements to be considered:&lt;br&gt;- <em>A priori</em> plan for additional analysis&lt;br&gt;- Appropriateness of the analysis</td>
</tr>
<tr>
<td>13</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>14</td>
<td>All</td>
<td>Assess whether author conclusions: reflect results of the analysis are biologically relevant</td>
</tr>
<tr>
<td>15</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>All</td>
<td>Example of a comment could be that the author could have a Conflict of interest</td>
</tr>
</tbody>
</table>

**Table D2: ELS CAT**

<table>
<thead>
<tr>
<th>Item</th>
<th>Assessor</th>
<th>How</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AMU in consultation with AHAW</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>All</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>5</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>6</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>7</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>8</td>
<td>AMU</td>
<td>Technical issue</td>
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<tr>
<td>9</td>
<td>AMU</td>
<td>Technical issue</td>
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<tr>
<td>10</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>11</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>12</td>
<td>AMU</td>
<td>Technical issue</td>
</tr>
<tr>
<td>13</td>
<td>AMU in consultation with AHAW</td>
<td></td>
</tr>
</tbody>
</table>