Annex to rbSTs evaluation to describe in detail the systematic literature search process

The update of the risk assessment on rbST was undertaken following the principles of a systematic review, with the following steps:
1. framing the questions
2. systematic search of the literature
3. identifying relevant publications
4. summarizing the evidence
5. interpreting the findings

Described here are the first 3 steps, summarizing the evidence and interpreting the findings is described in the detailed monographs.

Step 1: Framing the questions

PICO questions: rbST systematic review
Synonyms for rbST: bGH, rbGH, GH1, bovine growth hormone, somidobove, somavubove, somagrebove, sometribove, posilac, bovine somatotrophin, bovine somatotropin

- **Issue:** Possible increased hormone levels in milk and meat from animals treated with rbST
  - **Population:** Cattle, goats, sheep in a given geographic region
  - **Intervention:** rbST administration
  - **Comparator:** not administered rbST
  - **Outcome:** hormone levels in milk and/or meat of animals
  - **PICO question:** What are the hormone levels in the milk and/or meat of cattle, goats, or sheep treated with rbST versus from untreated animals?
Search terms: (rbST or synonym) AND (hormone) AND ((meat) OR (milk))
  - Hormone search terms: IGF-1, insulin, estradiol, progesterone, IGF-II, testosterone, 17β-estradiol, estrone, pregnenolone, androstenedione, hydroxyprogesterone, dehydroepiandrosterone (DHEA), dihydrotestosterone, androsterone, 17α-estradiol, estriol, cortisone, corticosterone, prolactin

- Issue: Possible increased use of antibiotics to treat mastitis in dairy animals
  - 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
  - PICO question: Are the incidences of clinically relevant mastitis different between cattle, sheep and goats treated with rbST versus untreated animals? Are there differences in antibiotic residues levels in the milk & meat products from treated vs untreated animals?
  - Search terms: (rbST & synonyms) AND (mastitis) AND (antibiotic residue)

- Issue: Possible ↑viral expression in animals treated with rbST
  - Population: Cattle, sheep and goats in a given geographic region
  - Intervention: rbST administration
  - Comparator: not administered rbST
  - Outcome: viral DNA/RNA levels in serum & milk, infectivity
  - PICO question: Are retroviral/lentiviral levels & serotype distributions different between cattle, sheep and goats treated with rbST versus untreated animals?
  - Search terms:
    - (rbST & synonyms) AND retroviral OR lentiviral infectivity
    - (rbST & synonyms) AND retrovirus OR lentivirus

- Issue: Possible ↑prion expression in cows treated with rbST
  - Population: cows in a given geographic region
  - Intervention: rbST administration
  - Comparator: not administered rbST
  - Outcome: prion levels in tissue and milk, infectivity
  - PICO question: Are prion levels in meat and milk & prion infectivity different between cows treated with rbST versus untreated animals?
  - Search terms:
    - (rbST & synonyms) AND prion OR milk OR meat
    - (rbST & synonyms) AND bovine spongiform encephalitis
    - (rbST & synonyms) AND prion AND infectivity

- Issue: Possible increased health risks of consuming milk or meat from rbST-treated animals versus untreated animals
  - Population: infants only OR human population
**Intervention**: consumption of milk or meat from rbST-treated dairy animal (goat, cattle, sheep)

**Comparator**: consumption milk from untreated animal

**Outcome**: infectious & noninfectious disease incidence

**PICO question**: 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 to the equivalent age groups consuming meat or milk from untreated animals?

**Search terms**:
- (rbST & synonyms) AND infants AND diabetes mellitus
- (rbST & synonyms) AND humans NOT infants AND health
- Cow’s milk AND infants AND diabetes mellitus
- Goat’s milk AND infants AND diabetes mellitus
- Sheep’s milk AND infants AND diabetes mellitus
- Milk-based infant formula AND diabetes mellitus
- Milk-based infant formula AND health
- Milk-based infant formula AND disease
- Milk AND cancer

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

**Literature Search – Methods Section**

A systematic search of public literature database on recombinant bovine somatotropin (rbST) was conducted in order to update JECFA’s previous review of rbST at its 50th meeting. Specifically, the systematic review was looking for material published since JECFA’s last review that addresses the issues raised during the 50th meeting, namely (time frame: 1998 to present):

- Increased hormone levels in milk and meat from animals treated with rbST, especially IGF-1 levels;
- Increased incidences of mastitis in dairy animals treated with rbST and concomitant increases in antibiotic use and residues in milk;
- Increased viral and/or prion expression in animals treated with rbST; and
- Increased health risks of consuming milk or meat from rbST-treated animals versus untreated animals, especially increased risk for NIDDM in children.

The search was conducted in use of Agricola, CINAHL, Food Science Source, FSTA, EMBASE, PubMed, and GlobalHealth/CAB 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 through the present. Specifically, the terms used for each element in the Boolean combinations were as follows:

- ‘rbST’: ‘rbst' OR 'somatrem'/exp OR 'somatrem' OR 'genotonorm'/exp OR 'genotonorm' OR 'growth hormone, methionyl'/exp OR 'growth hormone, methionyl' OR 'methionyl growth hormone'
hormone'/exp OR 'methionyl growth hormone' OR 'methionyl somatotropin'/exp OR 'methionyl somatotropin' OR 'methionyl growth hormone'/exp OR 'methionyl growth hormone 20k' OR 'methionyl growth hormone 22k'/exp OR 'methionyl growth hormone 22k' OR 'methionyl somatotropin'/exp OR 'methionyl somatotropin' OR 'protropin'/exp OR 'protropin' OR 'sm 9500'/exp OR 'sm 9500' OR 'sm9500'/exp OR 'sm9500' OR 'somatohorm'/exp OR 'somatohorm' OR 'somatonorm'/exp OR 'somatonorm' OR 'somatotropin, methionyl'/exp OR 'somatotropin, methionyl' OR 'sometribove'/exp OR 'sometribove' OR 'ym 17798'/exp OR 'ym 17798' OR 'ym17798'/exp OR 'ym17798' OR 'somagrebove'/exp OR 'somagrebove' OR '1 [n2 (n methionyl alpha aspartyl) glutamine] growth hormone'/exp OR '1 [n2 (n methionyl alpha aspartyl) glutamine] growth hormone' OR 'growth hormone [1 [n2 (n methionyl alpha aspartyl) glutamine] ]'/exp OR 'growth hormone [1 [n2 (n methionyl alpha aspartyl) glutamine] ]' OR 'somavubove'/exp OR 'somavubove' OR '127 leucine growth hormone'/exp OR '127 leucine growth hormone' OR 'growth hormone [127 leucine]/exp OR 'growth hormone [127 leucine]' OR 'bovine somatotropin':ab,ti OR 'bovine somatotropin':ab,ti OR 'posilac':ab,ti OR 'sometribove':ab,ti OR 'somagrebove':ab,ti OR 'somagrebove':ab,ti OR 'somavubove':ab,ti OR 'somavubove':ab,ti OR 'bovine growth hormone':ab,ti OR 'bovine growth hormones':ab,ti OR gh1:ab,ti OR rbgh:ab,ti OR bhg:ab,ti

- **Meat**: 'beef'/exp OR 'beef' OR 'meat' OR 'meat'/exp OR meat OR beef:ab,ti OR hamburger*:ab,ti OR steak*:ab,ti OR veau:ab,ti OR bifteck:ab,ti OR cheeseburger*:ab,ti OR burger*:ab,ti OR beefsteak*:ab,ti OR veal:ab,ti OR roast:ab,ti OR roasts:ab,ti OR jerky:ab,ti OR sausage*:ab,ti OR oxtail*:ab,ti OR liver:ab,ti OR ribs:ab,ti

- **Milk**: 'milk'/exp OR 'milk' OR milking:ab,ti OR 'dairy product'/exp OR 'dairy product' OR milk:ab,ti OR dairy:ab,ti OR 'artificial milk'/exp OR 'artificial milk' OR 'infant formula':ab,ti OR 'feeding formula':ab,ti OR 'infant formulas':ab,ti OR 'feeding formulas':ab,ti OR cheese*:ab,ti OR butter*:ab,ti OR 'ice cream':ab,ti OR kefir:ab,ti OR whey:ab,ti OR yoghurt*:ab,ti OR yogurt*:ab,ti OR zabadi:ab,ti OR curds:ab,ti OR ghee:ab,ti OR smen:ab,ti OR paneer:ab,ti OR quark:ab,ti OR cream:ab,ti OR creme:ab,ti OR skyr:ab,ti OR custard*:ab,ti

- **Child**: 'child'/exp OR 'child' OR 'children'/exp OR 'children' OR 'youth'/exp OR 'youth' OR youth*: OR newborn*: OR newborn'/exp OR 'newborn' OR 'new born' OR 'childhood disease'/exp OR 'childhood disease' OR 'baby'/exp OR 'baby' OR babies OR 'infant'/exp OR 'infant' OR infant* OR childhood* OR toddler* OR kid OR kids OR 'young patient' OR boy*: OR girl*: OR 'young age' OR pediatr*: OR paediatr*: OR 'child death'/exp OR 'child death' OR 'child health'/exp OR 'child health' OR 'child care'/exp OR 'child care' OR 'childhood mortality'/exp OR 'childhood mortality' OR 'child hospitalization'/exp OR 'child hospitalization' OR 'pediatric hospital'/exp OR 'pediatric hospital' OR child*

- **Cattle**: 'cattle'/exp OR 'cattle' OR bull:ab,ti OR bulls:ab,ti OR ox:ab,ti OR oxen:ab,ti OR bovine:ab,ti OR cattle:ab,ti OR cows:ab,ti OR cow:ab,ti OR steer:ab,ti OR steers:ab,ti OR heifer*:ab,ti OR calf:ab,ti OR calves:ab,ti AND 'abigar' OR 'abyssinian shorthorned zebu' OR 'aceh' OR 'achham' OR 'adaptaur' OR 'afghan' OR 'african boran' OR 'africanus' OR 'afrikaner' OR 'agerolese' OR 'ala tau' OR 'alambadi' OR 'albanian dwarf' OR 'alberes' OR 'alderney' OR 'alentejana' OR 'alentejana cattle' OR 'aleutian wild cattle' OR 'aliad dinka' OR 'alistana-sanabresa' OR 'allmogekor' OR 'allmogekor' OR 'alur' OR 'american angus' OR 'american beef friesian' OR 'american brown swiss' OR 'american milking devon' OR 'american white park' OR 'amerifax' OR 'amrit mahal' OR 'amsterdam island cattle' OR 'anatolian black' OR 'andalusian black' OR 'andalusian blond' OR 'andalusian grey' OR 'angeln cattle' OR 'angonie' OR 'angus cattle' OR 'ankina' OR 'ankole-watusi' OR 'aosta cabbage' OR 'apulian podolian' OR 'aracena' OR 'arado' OR
The results of each of these searches were entered into EndNote, and the databases were edited to remove duplicates and irrelevant citations. The remaining databases were as follows: ‘rbST AND child’ (125 relevant citations, 2032 total citations); ‘rbST AND meat’ (136 relevant citations, 852 total citations); ‘rbST AND cattle’ (215 relevant citations, 2793 total citations); ‘rbST AND milk’ (141 relevant citations, 1451 total citations); and ‘pre-2005 citations’ (155 relevant citations; 7038 total). Citations that were deemed irrelevant included: editorials and review articles that did not contain primary study data; news articles; and entries that did not address the issues enumerated above. Entries that were discarded as irrelevant also included the following types of studies:

- Studies conducted with dairy breeds containing genetic mutations that enhance endogenous bST production or lactation parameters;
- Studies solely examining the effects of rbST treatment on reproductive parameters, such as ovulation or reproductive performance; and
- Clinical studies concerning the effects of IGF-1 treatment and/or GH treatment in patients with abnormal growth syndromes.
The edited databases were then searched for citations relevant to the issues above using keyword searches and also by reading each abstract.

Additional searches were undertaken in PubMed to address the following:

1. Toxicological studies of rbST in laboratory animals

Search terms used: (rbST OR bovine somatotropin) AND toxicity

2. Bioavailability and bioactivity of rbST and IGF-I (Insulin like growth factor – I)

Search terms used: (rbST OR bovine somatotropin) AND bioavailability; (rbST OR bovine somatotropin) AND oral absorption; (rbST OR bovine somatotropin) AND oral AND activity; (rbST OR bovine somatotropin) AND oral AND absorption; IGF-I AND bioactivity; IGF-I AND oral AND absorption

3. Dietary IGF-I and human health effects

IGF-I AND food AND disease; IGF-I AND food AND health; IGF-I AND milk AND disease; IGF-I AND milk AND health; IGF-I AND milk AND cancer; IGF-I AND milk AND diabetes

For assays of IGF-1 and rbSTs, the search was conducted using the following databases:

- Web of Science TM Core Collection (1956-present). The core collection consists of
  - Science Citation Index Expanded (1956-present)
  - Social Sciences Citation Index (1956-present)
  - Arts & Humanities Citation Index (1975-present)
  - Conference Proceedings Citation Index- Science (1990-present)
  - Conference Proceedings Citation Index- Social Science & Humanities (1990-present)
  - Book Citation Index– Science (2005-present)
  - Book Citation Index– Social Sciences & Humanities (2005-present)
  - Current Chemical Reactions (2009-present)
  - Index Chemicus (2009-present)
- BIOSIS Citation IndexSM (2009-present)
- CABI : CAB Abstracts® and Global Health® (1910-present)
- Current Contents Connect® (1998-present)
- Data Citation IndexSM (2009-present)
- Derwent Innovations IndexSM (1963-present)
- FSTA (Food Science and Technology Abstracts) ( 1969 - present)
- MEDLINE® (1950-present)
- SciELO Citation Index (1997-present) (not used for somidobove)
- Zoological Record® (1978-present)

**Search terms used for assay of IGF-1 in serum**

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
insulin-like growth factor (IGF-1): ("igf-1" or "insulin like growth factor-1" or "igf 1" or "insulin like growth factor 1" or "igf-I" or "insulin like growth factor-I" or "igf I" or "insulin like growth factor I") – topic field
AND
Serum: serum – topic field

**Search terms used for assay of IGF-1 in milk**

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
insulin-like growth factor (IGF-1): ("igf-1" or "insulin like growth factor-1" or "igf 1" or "insulin like growth factor 1" or "igf-I" or "insulin like growth factor-I" or "igf I" or "insulin like growth factor I") – topic field
AND
Milk: (milk or milk* or dairy or dair*) – topic field

**Search terms used for assay of IGF-1 in plasma**

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
insulin-like growth factor (IGF-1): ("igf-1" or "insulin like growth factor-1" or "igf 1" or "insulin like growth factor 1" or "igf-I" or "insulin like growth factor-I" or "igf I" or "insulin like growth factor I") – topic field
AND
**Plasma:** (plasma or plasm*) - topic field

**Search terms used assay of IGF-1 in tissue**

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
insulin-like growth factor (IGF-1): ("igf-1" or "insulin like growth factor-1" or "igf 1" or "insulin like growth factor 1" or "igf-I" or "insulin like growth factor-I" or "igf I" or "insulin like growth factor I") – topic field
AND
Tissue: (tissue or tissues) – topic field

**Search terms used for assay of recombinant bovine somatotropin (rbST) in milk**

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
rbST: (“recombinant bovine somatotropin” or “rbST”) – topic field
AND
Milk: (milk or milk* or dairy or dair*) – topic field
Search terms used for **Assay of recombinant bovine somatotropin (rbST) in serum**
assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
rBST: (“recombinant bovine somatotropin” or “rbST”) – topic field
AND
Serum: serum – topic field

Search terms used assay of recombinant bovine somatotropin (rbST) in plasma

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
rBST: (“recombinant bovine somatotropin” or “rbST”) – topic field
AND
Plasma: (plasma or plasm*) - topic field

Search terms used for assay of recombinant bovine somatotropin (rbST) in tissue

assay: (assay or assays or identif* or analy* or measur*) – topic field
AND
rBST: (“recombinant bovine somatotropin” or “rbST”) – topic field
AND
Tissue: (tissue or tissues)

Search terms used for IGF-1 and absorption

insulin-like growth factor (IGF-1): (“igf-1” or "insulin like growth factor-1” or "igf 1" or "insulin like growth factor l" or "igf-I" or "insulin like growth factor-I" or "igf I" or "insulin like growth factor I") – topic field
AND
Milk: (milk or milk* or dairy or dair*) – topic field
AND
Absorption: (absorb* or absorp*) – topic field

Search terms used for somidobove

Somidobove: (somidobove or somidobovum or somidobovo ) - topic ; somidobo* - topic exp;
(somidobove or somidobovum or somidobovo ) – topic exp;

Timespan: for all searches except somidobove was 2004 – current and for somidobove 1997 – current